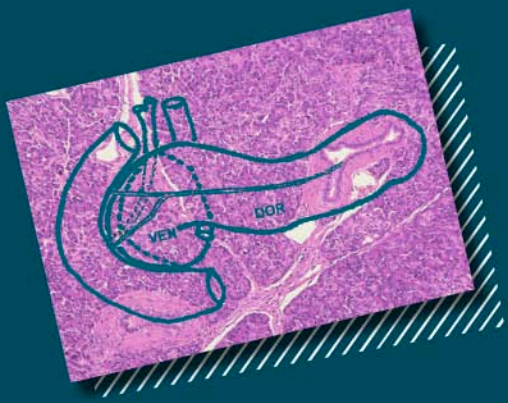
Pancreas

Pathological Practice and Research

Editor
K. Suda



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Editor

Koichi Suda *Tokyo*

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Contents

Preface

The pancreas lies deep in the body. It is a calm, silent organ located behind the stomach, with much hope and possibilities for solving the physiological and pathological problems of its behavior. Because the pancreas is a complicated organ, it is important in an anatomical and embryological sense, and because of its frequent age-related lesions. It develops from two buds fused into a single organ with a ductal system, close to the biliary tract and duodenum. Both mucous-cell hyperplasia, which corresponds to PanIN-1, and cystic dilatation of the branch pancreatic duct, relevant to branch-duct-type intraductal papillary-mucinous tumors, frequently occur in elderly persons, resulting in the modification of the tissue surrounding it, i.e. atrophy. Moreover, pathological changes in the pancreas are focal or patchy in nature (i.e. normal tissue is found adjacent to the affected foci), especially in non-tumorous lesions, but not homogeneous and diffuse in the case in the liver.

Nowadays, many different imaging methods and approaches allow the form of the pancreas and its parenchyma to be seen in detail and in repetition or sequence, while the problems posed by biopsy specimens, apart from the risk involved in obtaining the sample, are of sampling error and small sample size because of unequally distributed foci or sparing neighboring areas in the whole organ, as mentioned above.

When doing a pathological study of the pancreas, my colleagues and I appreciate not only the pancreas itself, in a morphological sense, but also its relationship with its neighboring organs such as the duodenum, biliary tract (especially in the pancreatico-choledocho-ductal junction) and liver, and its developmental and anatomical characteristics.

Here, my colleagues and I describe a number of pathological changes in the behavior of the pancreas based on our experience and knowledge. Our opinions may include those which differ from established ones. They are to stimulate discussion resulting from detailed histopathological or clinicopathological observations.

I offer my deep appreciation of my fellow department members as well as my publisher Karger for their kind assistance and consideration in publishing this book.

My hope is that this book will be a useful reference source for all those who wish to investigate and practice research in pancreatology.

Koichi Suda, Tokyo

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Development of the Pancreas with Relation to Its Paired Ventral Anlagen

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Abstract

In order to understand the anatomical variations and congenital anomalies of the pancreas, many of which have practical surgical implications, it is important to realize that this organ originates from two separate embryonic anlagen: a ventral and a dorsal primordium. An annular pancreas is a rare malformation, and it is generally accepted that the ring formation originates from a single ventral pancreas, as suggested by Lecco. However, an annular pancreas may also originate from paired ventral pancreata, thus supporting Baldwin's hypothesis. Here, we attempt to clarify the pathogenesis of the annular pancreas.

An annular pancreas is a rare malformation in which a band of pancreatic tissue surrounds the descending portion of the duodenum, either completely or incompletely, and is in continuity with the head of the pancreas. The anomaly is often discovered incidentally and/or at autopsy. Some patients with this anomaly develop duodenal stenosis, obstructive jaundice and pancreatitis; however, many remain asymptomatic and the anomaly is only discovered accidentally in adulthood.

Many infants with this anomaly also have various other congenital anomalies such as Down's syndrome, malrotation, esophageal atresia, duodenal atresia, duodenal diverticulum, pancreas divisum, imperforate anus and congenital heart disease. The diagnosis is usually obtained by endoscopic retrograde cholangiopancreatography (ERCP) and/or histological analyses. Although several theories have been proposed to explain the origins of annular pancreas, the pathogenesis is still controversial [1–4]. It is generally accepted that the ring formation originates from the ventral pancreas, as suggested by Lecco and Baldwin [1, 2]. The difference between Lecco's and Baldwin's hypotheses is

whether the ventral pancreatic anlage is single or paired. On the basis of embryologic analyses, many gastroenterologists and pathologists have come to believe that the ventral pancreatic anlage is initially paired, with the left lobe normally disappearing during development, as described by Odgers [5]. However, the histogenesis of the ventral pancreatic anlage is also controversial because most of the resected and/or autopsied annular pancreata that have been investigated histopathologically support Lecco's hypothesis. We present an annular pancreas that was investigated histopathologically and immunohistochemically, which supports Baldwin's hypothesis with reference to the histogenesis of the ventral pancreatic anlage [6].

Embryological Development

In order to understand the anatomical variations and congenital anomalies of the pancreas, many of which have practical surgical implications, it is important to realize that this organ originates from two separate embryonic anlagen: a ventral and a dorsal primordium. On or about the 24th day of gestation, the diverticulum begins to bud from the ventral surface of that part of the primitive digestive tube which is destined to later become the duodenum. This hepatic anlage invades the ventral mesentery and later develops into the liver, bile ducts, and gallbladder. Some two days later (26th day of gestation), a similar diverticulum emanates from the dorsal surface of the digestive tube. In normal pancreatic development, the pancreas arises from the dorsal and ventral anlagen in the 4-week embryo (fig. 1). The ventral anlage consists of two buds, a right and left lobe, and they arise on each side of the common bile duct, as described by Odgers [5]. The left lobe of the ventral pancreatic anlage disappears rapidly. This develops into the dorsal anlage of the pancreas, growing rapidly within the dorsal mesentery. The smaller ventral pancreatic anlage buds a little later from the hepatic diverticulum on the 32nd day (fig. 2) [7].

A series of rapid development changes (elongation of the hepatic anlage to from the bile duct, disappearance of the ventral mesentery, rapid growth of the left wall of the duodenum) leads to a rotation of the common bile duct, together with the ventral pancreatic anlage, into a dorsal position behind the primitive superior mesenteric vessels.

Thus, the dorsal and ventral portions of the pancreas come into close contact by the 37th day of gestation. While these two portions and their drawing ducts begin to amalgamate, the right leaf of the dorsal mesentery fuses with the posterior abdominal wall, thus determining the retroperitoneal position of the pancreas and three-quarters of the duodenum. This avascular plane, the fascia of Treitz, separates the posterior aspect of the pancreas from the abdominal

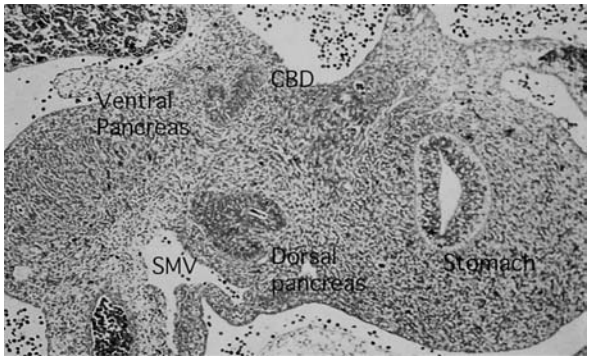


Fig. 1. Fifth-week embryo. Both a ventral and dorsal pancreatic anlage were observed. The dorsal pancreatic anlage was already lobed. HE. CBD = Common bile duct; SMV = superior mesenteric artery.

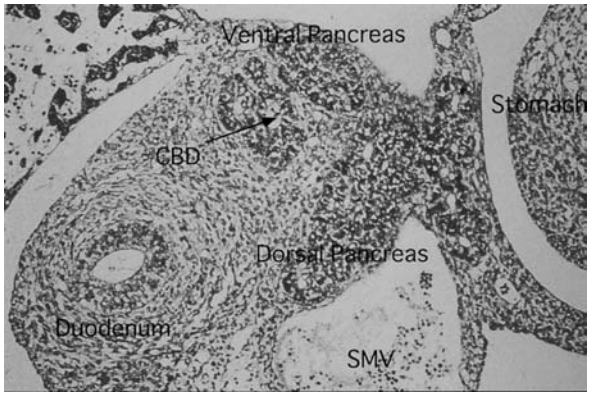


Fig. 2. Sixth-week embryo. Both the ventral and dorsal pancreatic anlage were lobed and partially fused. A series of rapid development changes leads to a rotation of the common bile duct, together with the ventral pancreatic anlae, into a dorsal position behind the primitive superior mesenteric vessels. HE. CBD = Common bile duct; SMV = superior mesenteric artery.

wall. It is this plane that facilitates the mobilization maneuver described by Kocher.

By the end of the 7th week of gestation, with the embryo only about 13 mm long, gross morphological development of the pancreas is largely complete. The ventral anlage now comprises the uncinate process and most of the pancreatic head. Its duct (the duct of Wirsung) fuses with the duct of the dorsal anlage and drains into the duodenum together with the common bile duct.

The dorsal anlage constitutes the body and tail of the pancreas and the cranial part of the head. The distal part of its duct joins that draining the ventral anlage, although its proximal portion (the duct of Santorini) either drains into the duodenum through a minor papilla or drains retrogradely into the the duct of Wirsung; in some cases it degenerates completely.

The functional development of the pancreas into an exocrine and endocrine gland occurs much later. Secretory acini first appear at the ends of ducts in the third gestational month. Trypsin is formed at about 22 weeks, but full exocrine function is not achieved until six months after birth [8].

Primary islet cells, which probably originate from the neural crest (as do other cells of the APUD system) appear in the 8th week, but are gradually replaced by secondary islets from the third gestational month onwards. Insulin may be detected from the end of the third month, but full endocrine function is not established until after birth.

Paired Ventral Pancreatic Anlage and Annular Pancreas

An annular pancreas is a rare malformation and its pathogenesis is still controversial. In the normal course of development between the 8- and 12-mm stages (sixth week), the common duct and the right portion of the ventral primordium are carried dorsally around the circumference of the duodenum to lie adjacent to the dorsal pancreas. This rotation is the result of duodenal growth, during which all enlargement is from the ventral side only. The duct of the longer, dorsal pancreas anastomoses with that of the ventral pancreas to form the main pancreatic duct (duct of Wirsung), which opens into the common duct. If the proximal portion of the dorsal primordium duct persists, it forms an accessory duct (duct of Santorini). How this normal pattern is altered to produce an annular pancreas is not clear, and a number of explanations have been proposed. Tieken's theory suggests that hypertrophy of both lobes occurs, and that these eventually coalesce to form a ring; Lecco's theory proposes adhesion of the distal tip of the ventral primordium to the duodenal wall prior to its migration; Baldwin's theory is based on persistence of a hypothetical left lobe assuming that the ventral lobe is originally a paired structure; while Erimoglu's theory involves the formation of a ring by fusion of aberrant pancreatic tissue from the duodenum [1–4]. It is now generally accepted that the ring formation originates from the ventral pancreas, as suggested by Lecco (fig. 3) [2]. However, on the basis of clinicopathological analyses of pancreata with pancreaticobiliary maljunctions, many gastroenterologists and pathologists have come to believe that the ventral pancreatic anlage is initially paired, and that the left lobe normally disappears over time, as shown by Odgers [5, 9, 10].

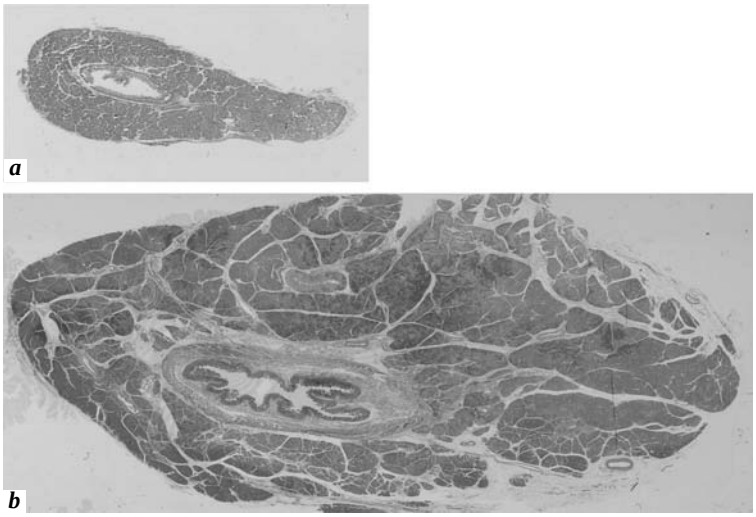
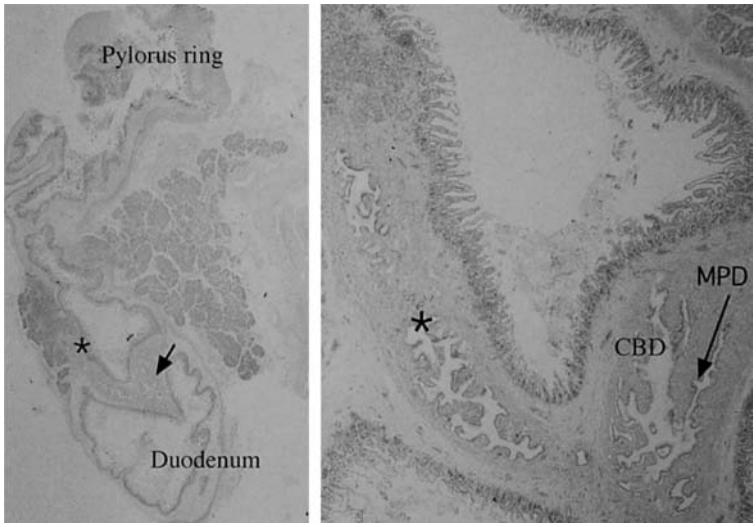


Fig. 3. Sections of annular pancreata, as suggested by Lecco. An infantile (***a***) and an adult annular pancreas (***b***). HE.

With improvements in imaging techniques such as computed tomography, ERCP and magnetic resonance cholangiopancreatography (MRCP), annular pancreata are being recognized with increasing frequency. Most cases have been diagnosed by ERCP and/or MRCP, although some have been discovered incidentally during surgery or autopsy [11–13]. At present, if patients with an annular pancreas have no symptoms or related complications such as weight loss due to pyloric stenosis, severe abdominal pain, obstructive jaundice or a pancreaticobiliary maljunction, etc., they are followed up conservatively. Therefore, annular pancreas case reports with a histological analysis are still rare. As most resected and/or autopsied annular pancreata that have been investigated histopathologically support Lecco's hypothesis [14, 15], there is a discrepancy between histopathological analyses of the annular pancreata and clinicopathological analyses of pancreata with a pancreaticobiliary maljunction, i.e. the former support Lecco's hypothesis of a single ventral pancreas, while the latter support Baldwin's hypothesis of paired ventral pancreata [3, 6, 14, 15]. Whether the ventral pancreatic anlage is single or paired is the most basic and important embryological point in understanding the pathogenesis of annular pancreas [16–20], because many researchers have come to believe that if the left lobe of the ventral pancreatic anlage does not disappear a pancreaticobiliary maljunction occurs [21, 22]. However, Nobukawa and colleagues and Muraoka and colleagues reported a case with an annular pancreas with



a *b*

Fig. 4. ***a*** Low-power view of a pancreatic polypeptide-stained section. The normal main pancreatic duct (arrow), the common bile duct, and an unusually large pancreatic duct (asterisk) from the annular pancreas were found. ***b*** High-power view of an HE-stained section. The unusually large pancreatic duct (asterisk) from the annular pancreatic tissue on the opposite side of the normal pancreatic head flows into the major papilla.

persistence of the left lobe of the ventral pancreatic anlage which did not cause a pancreatobiliary maljunction [6, 23, 24]. The histogenesis of the ventral pancreatic anlage has not yet been clarified, and not even in the most recent textbooks of embryology [25–27].

Our evaluations revealed that the ring formation originated from the left lobe of paired ventral pancreata (fig. 4), thus supporting Baldwin's hypothesis. It was proved that persistence of the left lobe of paired ventral pancreata was not associated with occurrence of a pancreatobiliary maljunction.

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Vascular Anatomy of the Pancreas

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Abstract

Recently, various reduction resections of the pancreas have been performed. The vascular anatomy of the pancreas is unique and complicated, it is therefore especially important for surgeons to understand it.

The vascular anatomy of the pancreas is unique, and complicated compared with other organs. Only surgeons with sufficient knowledge pf the vascular anatomy of the pancreas should perform reduction surgery. In this paper, the vascular anatomy of the pancreas together with its embryological and anatomical development and implications for reduction surgery is documented.

The Blood Supply of the Pancreas

Arteries

The pancreas, in particular its head, has an abundant blood supply basically derived from the celiac axis and the superior mesenteric artery (SMA). In fact, the collateral pathways between these two arteries are so efficient that the cut surface of the pancreas removed en bloc using the Whipple procedure will often continue to bleed until the very last jejunal branch (and the proximal jejunal artery itself) has been divided. The general pattern of the arterial blood supply and anatomy of the pancreas is shown in figure 1.

The pancreatic head and uncinate process receive arterial blood from two pairs of pancreatoduodenal (PD) arcades. The superior PD arteries, the anterior and posterior, arise from the gastroduodenal artery (GDA) (either separately or

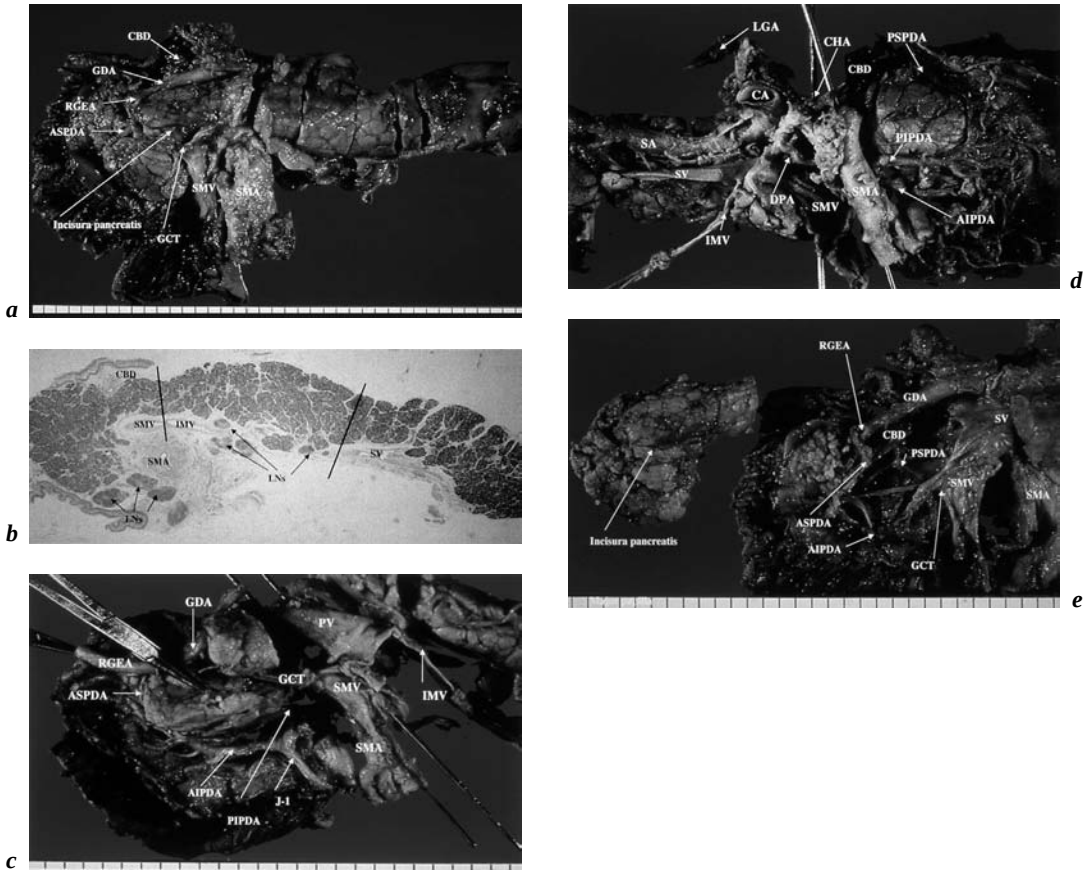

Fig. 1. Vascular anatomy (autopsied pancreas). ***a*** Gross appearance. ***b*** Horizontal section of the entire infantile pancreas on pancreatic polypeptide staining. The lines indicate the boundaries between the head, body, and tail. ***c*** Anterior PD arcade, SMA, and SMV are shown on the front of the pancreas. ***d*** Posterior PD arcade, SMA, SMV, SA, and SV are shown on the back of the pancreas. ***e*** The appearance of the vascular anatomy after removal of the pancreatic head. AIPDA = Anterior inferior pancreatoduodenal artery; ASPDA = anterior superior pancreatoduodenal artery; CA = celiac artery; CBD = common bile duct; CHA = common hepatic artery; DPA = dorsal pancreatic artery; GCT = gastrocolic trunk; GDA = gastroduodenal artery; IMV = inferior mesenteric vein; J-1 = first branch of jejunal artery; LGA = left gastric artery; LNs = lymph nodes; PIPDA = posterior inferior pancreatoduodenal artery; PSPDA = posterior superior pancreatoduodenal artery; PV = portal vein; RGEA = right gastro-epiploic artery; SA = splenic artery; SMA = superior mesenteric artery; SMV = superior mesenteric vein; SV = splenic vein.

from a common trunk). The inferior pair of PD arteries arises from the SMA, either separately or together with one of the proximal jejunal arteries. If the latter is accidentally ligated with an inferior PD artery, proximal segment jejunum ischemia may result and necessitate removal of more jejunum (e.g. in the course of Whipple's procedure) than is normally the case.

Both pairs of arcades supply the pancreatic head as well as the duodenal wall, and communicate freely with one another. Whereas the anterior PD arcade runs close to the inner curve of the duodenum, the posterior arcade passes posterior to the intrapancreatic portion of the common bile duct, maintaining a greater distance from the duodenum.

The rule, stated in most textbooks, that the close inter-relationship of the duodenum and pancreas regarding blood supply prevents removal of one without the other has been largely refuted in actual practice. Thus, duodenum-preserving total pancreatectomy has been performed successfully, providing that the duodenal branch of the GDA supplying the first portion of the duodenum and the first 3 cm of the anterior inferior pancreatoduodenal artery (AIPDA) supplying the fourth part of the duodenum are preserved [1]. However, the tenuous blood supply to the remaining duodenum in some cases, and oncological requirements in most other situations, make this procedure the exception that proves the rule.

Branches of the splenic artery (SA) supply the body and tail of the pancreas. These include multiple small branches to the upper border of the pancreas and the dorsal pancreatic artery. The latter arises from the proximal 2 cm of the SA, but it may also originate from the GDA or from an aberrant right hepatic artery. Apart from providing branches to the head and uncinate process, this artery sends off a large, but variable, inferior or transverse pancreatic artery to supply the body and tail of the pancreas from below. Its branches usually communicate with those pancreatic arteries giving off some epiploic branches to the greater omentum, including the left colic artery.

Veins

The veins draining the pancreas largely run parallel to the arteries. They drain into the portal vein (PV) or its two main tributaries, the superior mesenteric vein (SMV) and splenic vein (SV). The anterior superior pancreatoduodenal vein (ASPDV) drains into the right gastro-epiploic veins. The posterior superior pancreatoduodenal vein (PSPDV) is a constant tributary entering the PV from the right, just behind the upper border of the pancreas. As mentioned before, tributaries entering the anterior surface of the SMV or PV are very rare, but even so dissection between the pancreatic neck and the great veins must be done carefully. The inferior PD veins usually terminate as a common trunk draining into the SMV. This trunk is short and, in passing under what appears to

be just one anterior vein, the posterior branch is easily stopped by pressure from behind. The inferior mesenteric vein (IMV) enters the SV in 38% of subjects [2], in another one-third it drains into the splenomesenteric confluence, and in the remainder it terminates in the SMV. The left gastric or coronary vein enters the PV in one-quarter of subjects [3]. In total pancreatectomy this vein must be preserved, since here it is the only vessel remaining to drain the proximal gastric segment. There are a number of rare abnormalities of the PV. It may run in front of the duodenum and it may drain into the superior vena cava. Total anomalous pulmonary venous drainage may occur into the PV and present as a congenital cardiac defect [4]. Finally, congenital strictures of the PV can suggest tumor infiltration in patients whose tumors are not really inoperable.

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Anomalous Lesions of the Pancreatic Head and Vaterian System, Related to Their Structures

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Abstract

There are a variety of anomalous lesions that can arise in the pancreaticobiliary system. Pancreaticobiliary maljunction (PBM), in which the junction of the bile duct and the pancreatic duct is external to the muscularis propria of the duodenum, is a factor contributing to choledochal cyst and biliary tract carcinoma. The pancreas consists embryologically of the ventral and dorsal anlagen, and is divided into two pancreata by the distribution of pancreatic polypeptide (PP)-islets. Anomalies based on two pancreata are pancreas divisum and annular pancreas.

Pancreaticobiliary Maljunction

Pancreaticobiliary Maljunction and Variations of the Pancreatico-Choledocho-Ductal Junction

Pancreaticobiliary maljunction (PBM) is a form of congenital anomaly in which the junction of the pancreatic and biliary ducts is located outside the duodenal wall. The configuration of the junction varies and is occasionally complex. This type of anomaly is almost always seen in patients with choledochal cyst or congenital biliary dilatation [1], and is also sometimes found in patients with congenital biliary atresia [2]. PBM, however, may occur independently of any other developmental changes in the common bile duct.

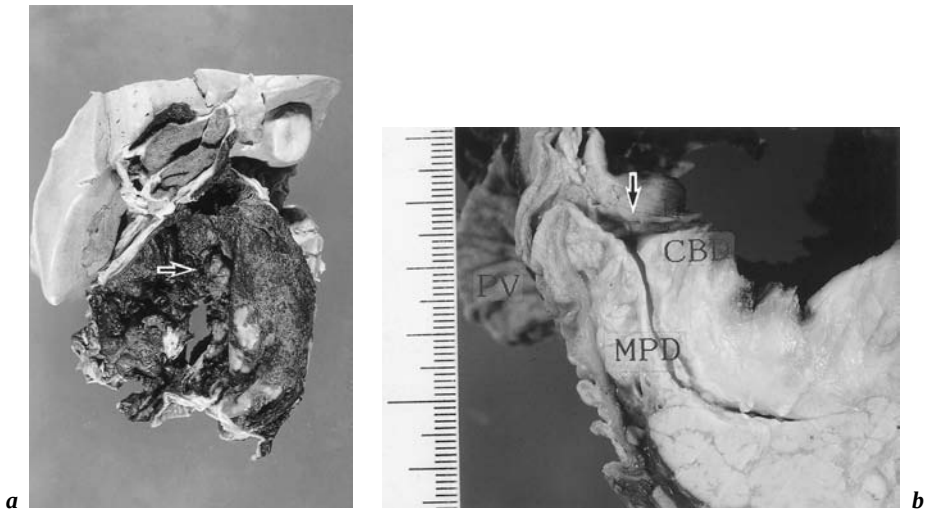


Fig. 1. Autopsy findings of choledochal cyst. ***a*** A huge choledochal cyst in an autopsy case (25-year-old man) with a polypoid carcinoma (arrow) in the posterior cyst wall and a metastatic nodule in the liver. ***b*** Pancreatico-choledochal-ductal junction (arrow) situated external to the propria muscularis of the duodenum. CBD = Common bile duct; MPD = main pancreatic duct; PV = papilla of Vater. From [30] with permission.

In the presence of PBM, pancreatic juice may flow freely into the extrahepatic bile duct and also the gallbladder, because the intraductal pressure of the pancreatic duct is usually higher than that of the biliary tract [3, 4]. Babbitt [1] postulated therefore that this influx of pancreatic juice into the common bile duct may be a factor causing cystic dilatation. As previously indicated, however, maljunction is not always associated with cystic dilatation, and the role of maljunction in the development of congenital cystic dilatation of the bile duct remains controversial.

Patients with PBM frequently develop neoplastic changes in the biliary tract [5, 6], regardless of cystic dilatation of the bile duct. The percentage of concomitant malignancy in the biliary tract is reported to be significantly high [7–9].

Figure 1 shows a huge choledochal cyst, 15 cm in diameter, in an autopsy case (25-year-old man), associated with a polypoid carcinoma, which had arisen in the posterior cyst wall with metastasis to the liver. The junction of the main pancreatic duct (MPD) and the common bile duct (CBD) was situated external to the muscularis propria of the duodenum, a condition which is referred to as PBM, thus forming an extended common channel [10, 11].

Tokuyama [12] classified the manner in which the CBD and MPD open into the duodenum into three types as follows: Type I: separate openings, Type II: one opening without a common channel, and Type III: common channel

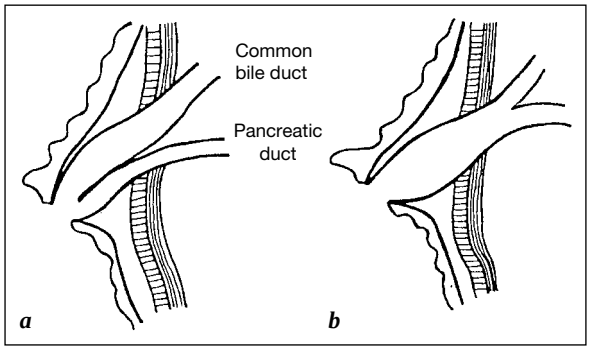


Fig. 2. Common channel formation type (Type III). ***a*** Junction in the submucosal layer (Type IIIa). ***b*** Junction below the propria muscularis (Type IIIb). From [2] with permission.

formation. Type III was subdivided into two variations: the junction in the submucosal layer (a) and the junction below, or external to, the muscularis propria of the duodenum (b), designated PBM as described above, according to our previous study [2] as shown in figure 2. PBM was found in 18 (13.8%) of 130 autopsy and surgical cases of biliary tract carcinoma [10], including the case shown in figure 1, but in none of 199 control cases.

Mechanism of Pancreatic Juice Reflux into the Biliary Tract in PBM

The reason why PBM is abnormal is possibly explained more clearly by our reconstruction study [2], as shown in figure 3. In the controls, the CBD and MPD penetrate the muscularis propria of the duodenum obliquely and parallel to each other, and form a junction in the submucosal layer just before opening into the duodenum. The angle of the ductal junction is therefore very sharp. The sphincter of Oddi, which surrounds both ducts and the common channel, normally consists of three sections: the sphincter choledochus, the sphincter pancreaticus and the sphincter ampullae [13]. Of these, the sphincter muscle at the distal end of the choledochus (sphincter choledochus) is the best-developed. It regulates the outflow of bile and prevents free communication between the bile and pancreatic ducts.

In cases of PBM, however, the junction of the ducts is situated external to the muscularis propria of the duodenum, thus forming an extended common channel [10, 11], as described above. The angle of the ductal junction is less sharp in these patients than in control cases. The well-developed sphincter muscle is situated in the submucosal layer, as in the control, but it mainly surrounds the common channel (sphincter ampullae), and the sphincter choledochus is extremely hypoplastic. These anatomical findings suggest the

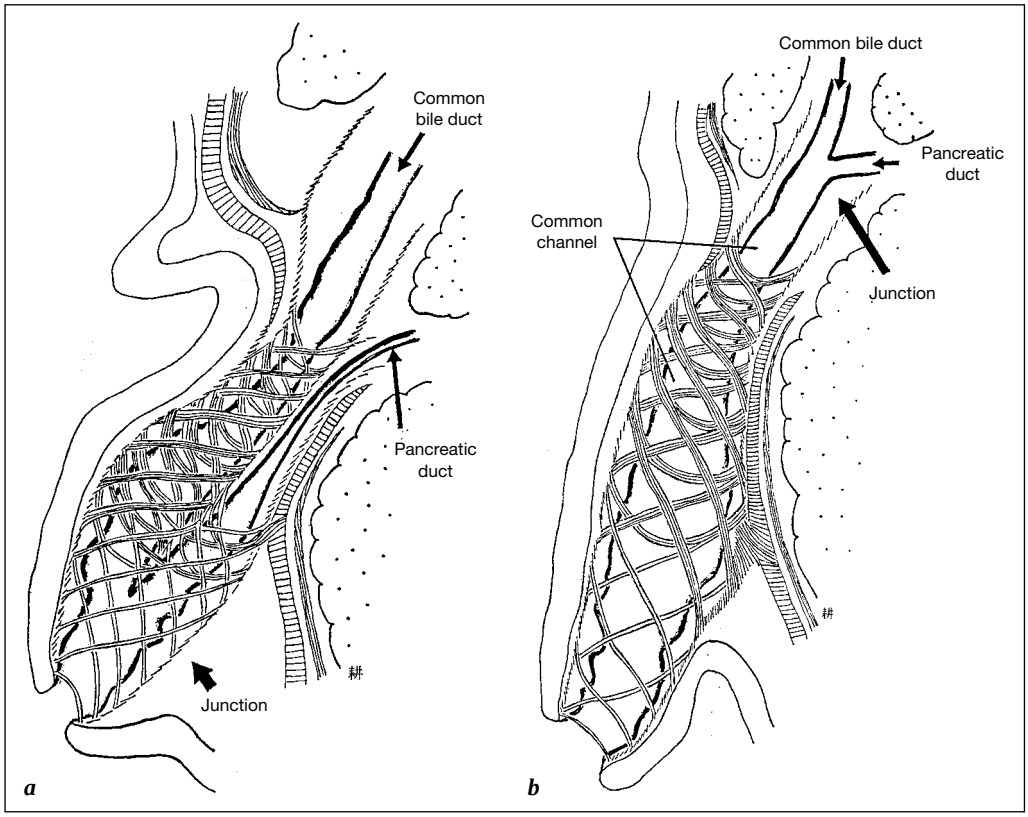
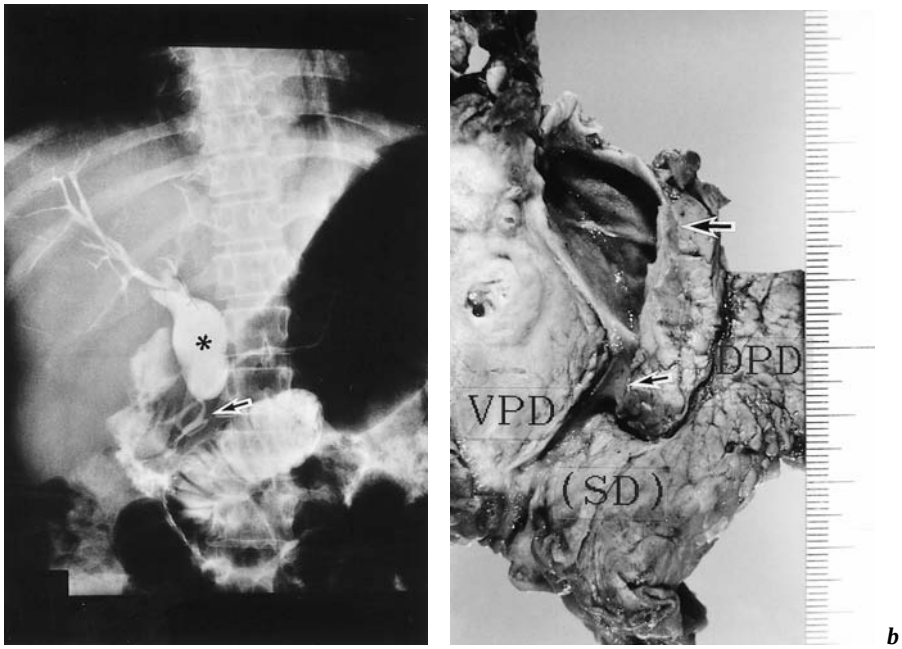


Fig. 3. Sphincter muscle in PBM. A diagram showing the sphincter muscle at the end of the common bile duct and the MPD in controls (***a***) and in patients with PBM (***b***). From [2] with permission.

possibility of free communication between the ducts in cases of PBM. As the intraductal pressure of the pancreatic duct is normally higher than that of the bile duct [3, 4], reflux of pancreatic juice may occur into the bile duct and could lead to non-suppurative chronic inflammation of the bile duct.

Location of Junction of Pancreatic Duct and Terminal Bile Duct in PBM

Suda et al. [14] reported that in two specimens with a 'narrowed duct segment' distal to the cyst in patients with choledochal cysts a minute orifice was found macroscopically in the segment and was identified microscopically as a small duct from the pancreatic parenchyma, and that these small pancreatic



a ***b***

Fig. 4. One of the anatomical locations of junction of pancreatic duct and terminal bile duct in a case of PBM with congenital choledochal dilatation and carcinoma of the gallbladder (a 50-year-old woman). ***a*** ERCP showing a choledochal cyst (asterisk) and maljunction (arrow). Note narrow segment of the common bile duct between cyst and maljunction. ***b*** Postoperative preparation of the case with PBM (small arrow) and a choledochal cyst (large arrow). DPD = Dorsal pancreatic duct; SD = duct of Santorini; VPD = ventral pancreatic duct. From [14] with permission.

ducts were derived from the ventral pancreas, based on the distribution of islets with pancreatic polypeptide cells (PP islets) [14], as shown in figure 4.

From anatomical and radiological analyses of the junction of the pancreatic duct with the bile duct, there are variations in the location of the union of the terminal bile duct with ventral pancreatic duct system [15], as shown in figure 5.

Distribution after Fusion of Ventral and Dorsal Anlagen, Branch Duct Fusion of the Pancreatic Duct and Annular Pancreas

Identification of the Originating Primordium

After fusion, the ‘ventral’ and ‘dorsal’ pancreata can be distinguished [16] by the distribution of the PP islets [17, 18], which are distributed selectively in

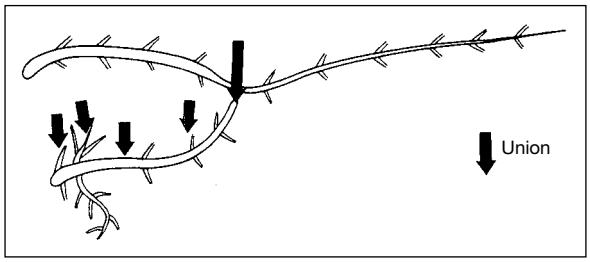


Fig. 5. Schematic drawing of various junctions/sites between the terminal bile duct and ventral pancreatic duct system in patients with PBM. From [15] with permission.

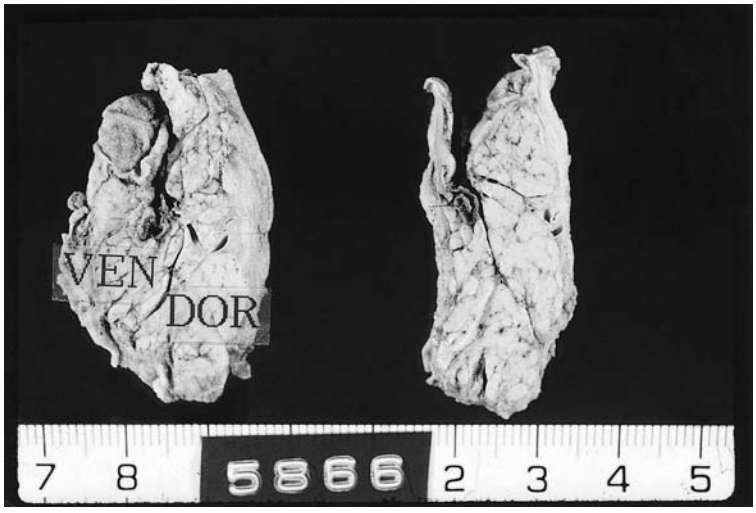


Fig. 6. Macroscopic appearences of coronary sections of ventral (VEN) and dorsal pancreas (DOR).

the ventral pancreas. In some cases both pancreata can be identified macroscopically (fig. 6) There are two further distinct characteristic differences. One is the shape of the islets: those in the ventral pancreas, which include abundant PP cells, are irregular, in contrast to the neatly round or oval islets found in the dorsal pancreas (fig. 7). The other is the distribution of fatty infiltration in the pancreas: there is more fat in the dorsal pancreas than in the ventral pancreas [16]. The ventral primordium forms the posterior part of the head of the pancreas, completely or partially surrounding the CBD (fig. 8) and the uncinate

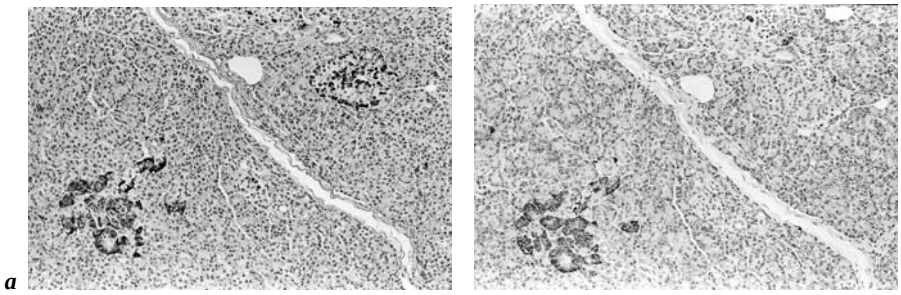


Fig. 7. Irregularly shaped and round or oval islets. ***a*** An irregularly shaped islet and oval islet were positively stained by the Grimelius silver method. ***b*** An irregularly shaped islet included abundant PP cells immunohistochemically, whereas an oval islet contained few PP cells, The border between both islets was identified as a fusion line of the ventral and dorsal pancreata. From [18] with permission.

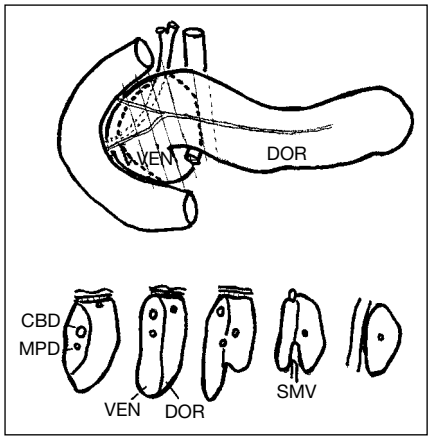


Fig. 8. Distribution of ventral and dorsal pancreas after fusion. CBD = Common bile duct; DOR = Dorsal pancreas; MPD = main pancreatic duct; SMV = superior mesenteric vein; VEN = ventral pancreas.

process. However, the dorsal bud forms the remaining ventral parts of the head, the isthmus, the body, and the tail of the pancreas.

The fusion line between both pancreata has no defined border, but it is the so-called 'locus minoris resistantiae' and it is the easiest 'pathway' for a duodenal diverticulum to penetrate the pancreas [19], as shown in figure 9.

Pancreas Divisum

The term pancreas divisum originally signified a very rare congenital anomaly in which the parenchyma of the ventral and dorsal pancreas are separated as a double pancreas. Recently, however, the term has been widely used to describe

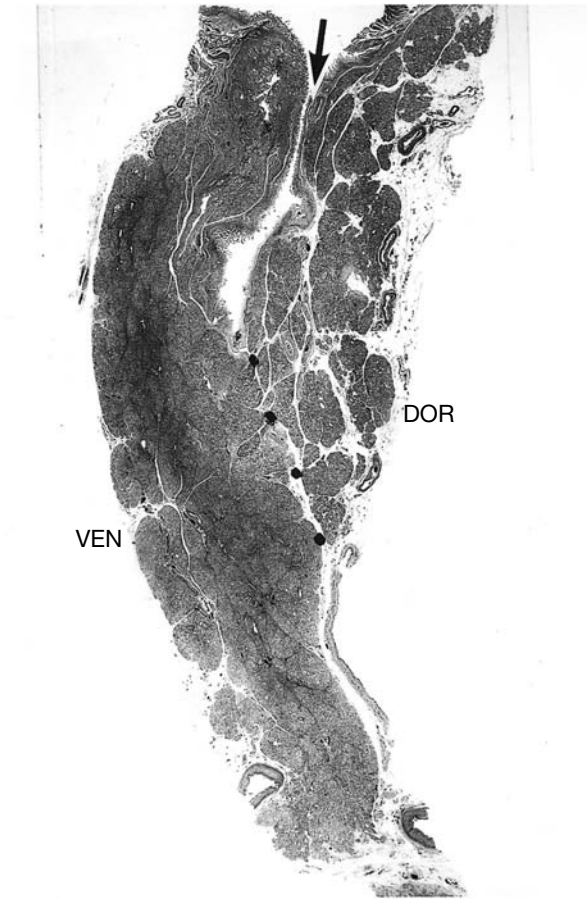


Fig. 9. Duodenal diverticulum (arrow) penetrating into a fusion line, signified by the dotted line, of the ventral (VEN) and dorsal (DOR) pancreata. From [19] with permission.

two ductal systems that do not unite or communicate and separately drain to the two duodenal papillae [20, 21]. In this condition, pancreatic juice from the dominant dorsal moiety flows out only through the minor papilla, in which the outlet is notably small in most cases. This raises the question of whether this variation plays a role in the development of pancreatic pain or pancreatitis. The clinical relevance of pancreas divisum has been argued repeatedly [20]. Figure 10 shows an example of isolated dorsal pancreatitis associated with pancreas divisum. This condition strongly suggests inadequate drainage from the minor papilla.

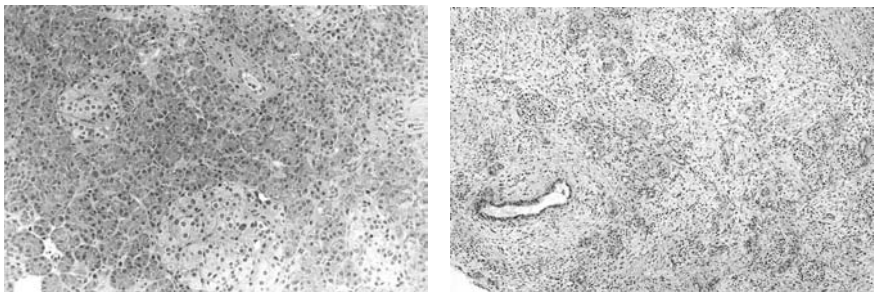


Fig. 10. So-called dorsal pancreatitis in patients with pancreas divisum. Tissue of the ventral pancreas did not show any abnormal findings (***a***), whereas the dorsal pancreas tissue demonstrated marked atrophy or disappearance of acinar cells and inter- and intralobular fibrosis (***b***). HE stain. ×100 (***a***), ×100 (***b***).

Branch Duct Fusion of the Ventral and Dorsal Pancreatic Duct

A case of fusion via two so-called inferior branches between the ventral and dorsal pancreatic ducts was studied both macroscopically and immunohistochemically, based on the organogenesis of the pancreas [22], as shown in figure 11. Radiologically, branch fusion seemed to be composed of an inferior branch of the ventral pancreatic duct and an inferior branch of the dorsal pancreatic duct. By mapping PP islets in the material obtained by pancreatoduodenectomy, however, the branch was identified as a branch of the dorsal pancreatic duct. Thus, fusion between two inferior branches was not established, but was found to consist of an inferior branch of the dorsal pancreatic duct connected with the ventral pancreatic duct. We therefore challenge the concept of the ansa pancreatica [23].

Annular Pancreas

An annular pancreas consists of a collar or ring of pancreatic tissue surrounding the second part of the duodenum and continuing into the head of the pancreas on either side. The gut lumen is usually narrowed.

According to Suda [24], the anomalous phenomenon of annular pancreas can be explained clearly as the result of two fusions between the PP-rich and PP-poor areas (fig. 12). The posterior fusion is considered to be part of the normal developmental process because of the arrangement of the duct system. The fusion of the anterior portion is thought to be anomalous.

Annular pancreatic tissue was thus demonstrated to arise from the ventral primordium, which supports Lecco's theory [25], the most reliable one, that the free end of the ventral anlage is fixed.

Nobukawa et al. [26] describe an annular pancreas originating from paired ventral pancreata, which supported Baldwin's hypothesis [27], and attempted to

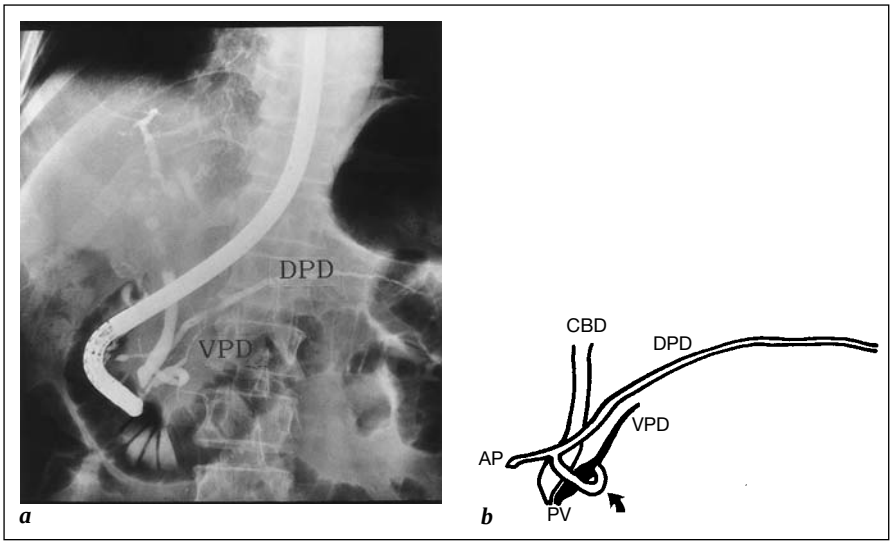


Fig. 11. Branch duct fusion of the ventral and dorsal pancreatic duct. ERCP. ***a*** The ventral (VPD) and dorsal (DPD) pancreatic ducts fuse via the 'two inferior branches'. ***b*** From the distribution of the PP islets, the branch fusion is shown to consist of a side-to-end fusion between the ventral pancreatic duct and the inferior branch (arrow) of the dorsal pancreatic duct. AP = Accessory papilla; CBD = common bile duct; PV = papilla of Vater. From [22] with permission.

clarify the pathogenesis. The patient was a 1-day-old Japanese male newborn, born after 32 weeks of pregnancy. He died the next day from respiratory failure due to esophageal atresia. Autopsy incidentally demonstrated an annular pancreas that was examined histologically. An unusually large pancreatic duct encircled by pancreatic tissue passed around the duodenum, and the duct was confirmed to connect with the major papilla after joining with the common channel (fig. 13), as indicated later. The islets of the encircling pancreas were positive for pancreatic polypeptide. The normal main and accessory pancreatic duct were also identified. The former and the CBD formed the common channel. Histologic and immunohistochemical evaluation demonstrated that the ring formation originated from the left lobe of the paired ventral pancreata.

Absence of Pancreatic Body and Tail

Congenital aplasia of the body and tail of the pancreas is an extremely rare anomaly. Ghon and Roman [28] reported the case of a 14-year-old boy in whom the head of the pancreas was flat and disk shaped, but neither the body nor tail

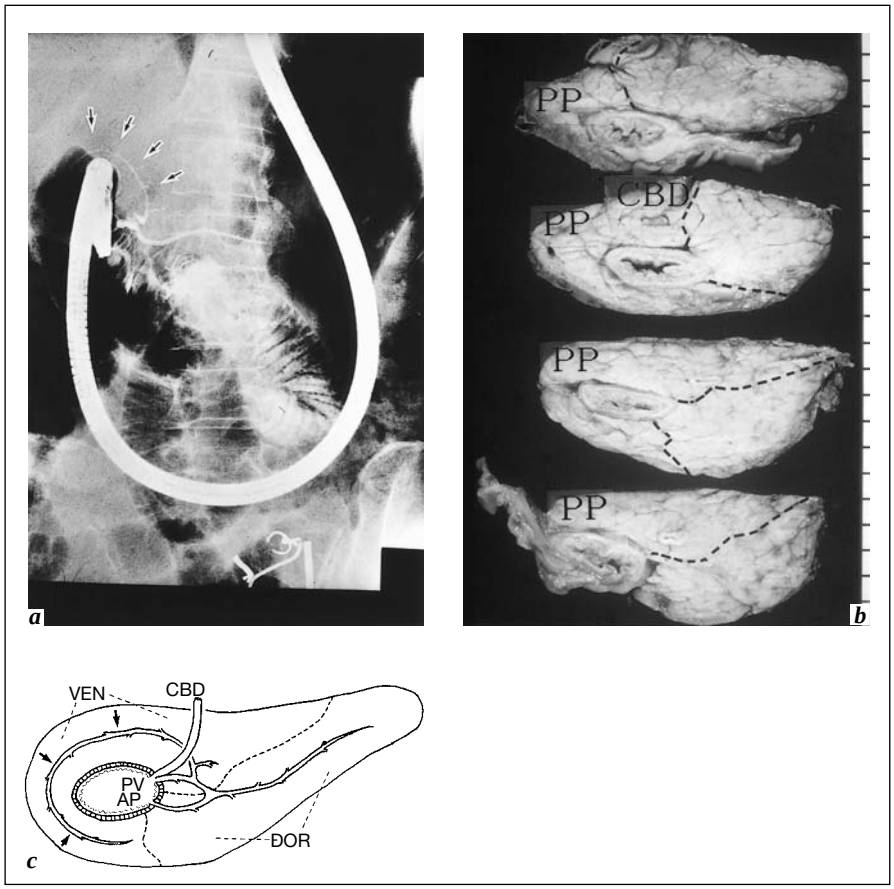


Fig. 12. Annular pancreas derived according to Lecco's theory. ***a*** Endoscopic pancreatogram in a case of annular pancreas (a 64-year-old woman). An additional ring-like pancreatic duct (arrows) is observed surrounding the second portion of the duodenum. From [31] with permission. ***b*** Macroscopic appearance of the serial cut-surfaces of the resected specimen. The patient had carcinoma in the middle part of the extrahepatic bile duct, and pancreaticoduodenectomy was performed. The dotted line signifies the fusion line between the ventral and dorsal pancreata. From [24] with permission. ***c*** A scheme of the pancreatic duct of the case in (***b***). The ventral (VEN) and dorsal pancreas (DOR) were determined by the procedure demonstrated in figure 7. A pancreatic duct (arrows) started from the anterior portion of the annular tissue to the lateral and posterior portions, finally connecting to the MPD. From [24] with permission. AP = Accessory papilla; CBD = common bile duct; MPD = main pancreatic duct; PP = pancreatic polypeptide rich area; PV = papilla of Vater.

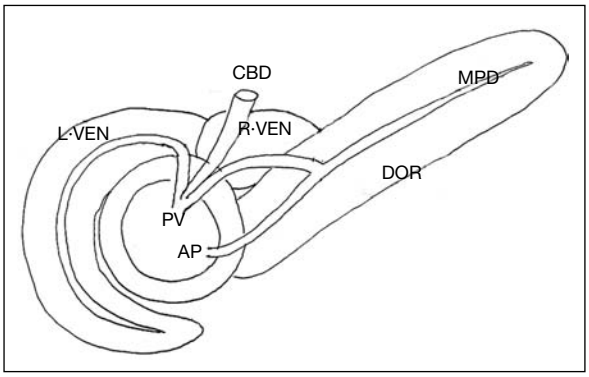


Fig. 13. Annular pancreas, supporting Baldwin's hypothesis. Ring formation originated from the left lobe (L·VEN) of the paired ventral pancreata. AP = Accessory papilla; CBD = common bile duct; DOR = dorsal pancreas; MPD = main pancreatic duct; PV = papilla of Vater; R·VEN = right lobe of ventral pancreas.

of the pancreas nor the minor papilla was observed. Based on the distribution of the ventral and dorsal pancreas after fusion of both anlagen, the term 'aplasia of the body and tail of the pancreas' should be reserved for conditions such as those reported by Ghon and Roman [28]. Therefore, this anomaly is not present when both the duct of Santorini and the minor papilla are present. Congenital aplasia of the body and tail of the pancreas derives from a defect of the dorsal pancreatic anlage and should not be considered a type of acquired atrophy [29].

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Normal Structure/Shape and Distended Glands of Papilla of Vater

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Abstract

The papilla of Vater is a cylindrical protuberance that houses a common channel or the terminations of the common bile duct and main pancreatic duct. In cases of pancreaticobiliary maljunction, the papilla of Vater, especially the plica longitudinalis, is slightly raised and has a longer slope, because it houses only the long common channel. The projecting papilla is covered with a circumscribed zone of mucous membrane that differs sharply from that of the surrounding mucosa. Glands near the surface of the papilla may be distended, are described as distended glands and also termed adenomatoid hyperplasia. Distended glands of the ampullary mucosa were frequently found, replacing the surrounding duodenal mucosa, and measured on average 1,532 μm. The distended glands are found on Oddi's sphincter muscle and not on the muscularis mucosa of the duodenum. Immunohistochemically, the distended glands were negative or weakly positive for CA19–9, and showed 19.6 ± 21.0% on average for Ki67LI, while the level was 9.0 ± 11.0% in the intrapapillary glands, without a significant difference. Therefore, such glands might be not only related to malignant changes, but also a kind of adaptive phenomenon against bile and pancreatic juice flow.

Structure and Shape of the Papilla of Vater

The papilla of Vater, also called the major papilla, which is usually situated medially at the midportion of the second part of the duodenum, is a cylindrical protuberance that houses a common channel or the terminations of the common bile duct (CBD) and main pancreatic duct (MPD) (fig. 1). In most cases, especially among Japanese, both ducts join within the duodenal wall [1] and have a short common chamber, named an ampulla or the common channel (fig. 2).

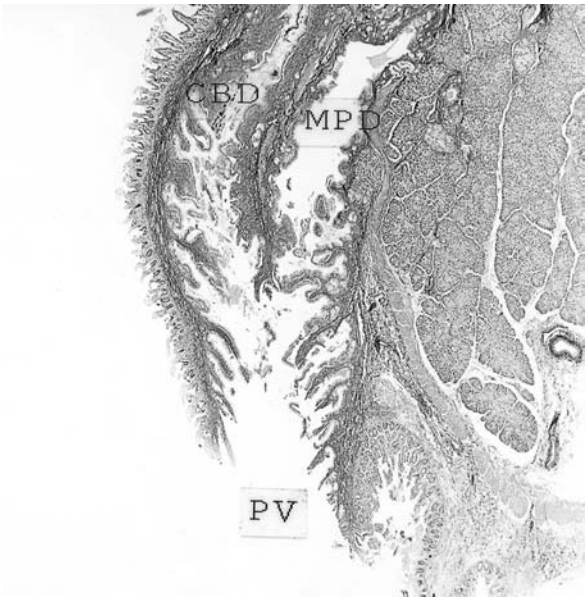

Fig. 1. Longitudinal section of the PV (3 days, F). The CBD and the MPD merge and formed a common channel. HE. Low magnification. CBD = Common bile duct; MPD = main pancreatic duct; PV = papilla of Vater. (Reproduced with permission from [7].)

Histologically, the papilla of Vater is covered by a triangular fold of the duodenal mucosa along the outer surface including the plica longitudinalis. The duodenal mucosa in the region immediately surrounding the outlet of the common channel, where CBD and MPD join, is covered by a sheath of epithelium with small villi. In contrast, the ampullary or intrapapillary mucosa is present with numerous papillary processes that are much larger than those of the duodenal villi, and form valvules at the orifice. The transition from the mucosa of the ampulla or the pancreaticobiliary duct to the duodenal mucosa is more abrupt and occurs just at the outlet of the ampulla or the outer edge of the papilla of Vater, and such areas of transition are inherently unstable [2]. Hence, the region surrounding the outlet of the common channel/pancreaticobiliary duct is simply covered by ampullary and duodenal mucosae. Therefore, the 'papillary mucosa' is morphologically and anatomically equal to the ampullary mucosa, but not to the duodenal mucosa.

Normally, the plica longitudinalis at the papilla of Vater is prominently protuberated, and reflects a transition between the termination of the CBD/MPD and the common channel, as mentioned above (figs. 1–3). In cases with

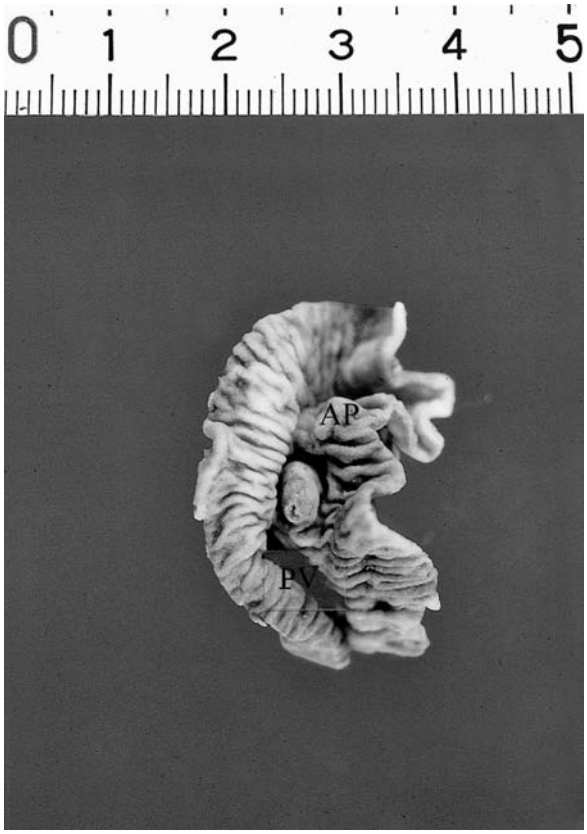

Fig. 2. PV, control (6 days, M) on outer view. The plica longitudinalis shows a cylindrical protuberance. AP = Accessory papilla; PV = papilla of Vater.

pancreaticobiliary maljunction (PBM), however, the plica longitudinalis is gently raised and has a longer slope, because it only houses the long common channel (fig. 4). In some post-cholecystectomized patients, the plica longitudinaris is rather flat and not raised, because of unused atrophic sphincter muscles of Oddi. If the gallbladder is absent, as in the rat and horse, the muscular ring of the Oddian zone is poorly developed and strictly duodenal in nature [3]. Hence, in some post-cholecystectomized patients the Oddi sphincter muscle is atrophied similar to that of the gallbladder that is absent animals. Therefore, the shape of papilla of Vater, especially in its protuberation, is based on housing ducts and thickening of the sphincter muscle.

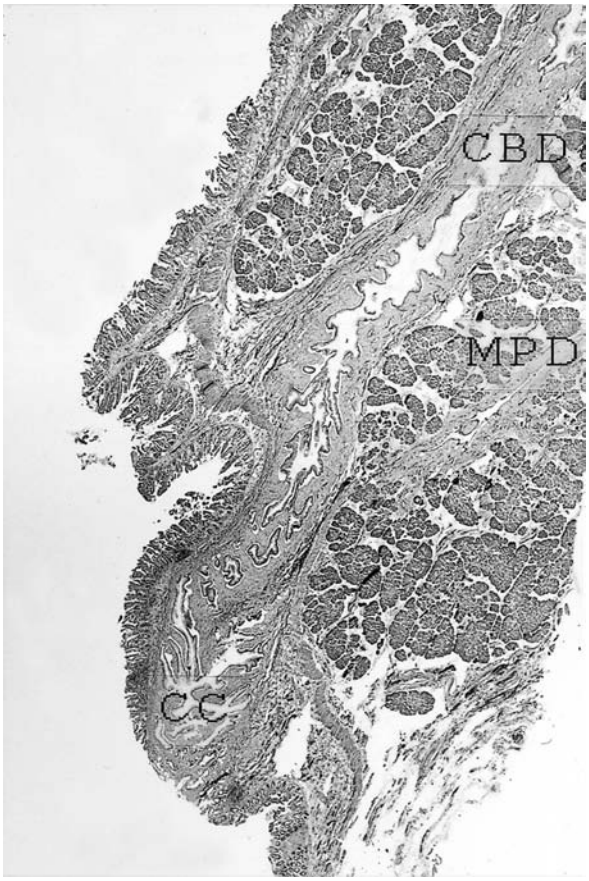

Fig. 3. Histology of the plica longitudinalis. The protuberance portion consists of the terminations of the CBD and MPD and CC. CBD = Common bile duct; CC = common channel; MPD = main pancreatic duct. (Reproduced with permission from [11].)

Distended Glands of the Papilla of Vater

The projecting papilla of the duodenum is covered with a circumscribed zone of mucous membrane that differs sharply from that of the surrounding duodenum. The mucosa of the papilla has few or no villi, and often the mucosal glands are large and appear hyperplastic. Goblet cells are conspicuous, and the cells lining the glands are larger and longer than in the epithelium of the adjacent duodenal glands. Frierson Jr [4] noted that glands near the surface of the papilla may be distended with mucus, and described them as distended glands. Such a change has also been termed 'adenomatoid hyperplasia' [5], and a similar

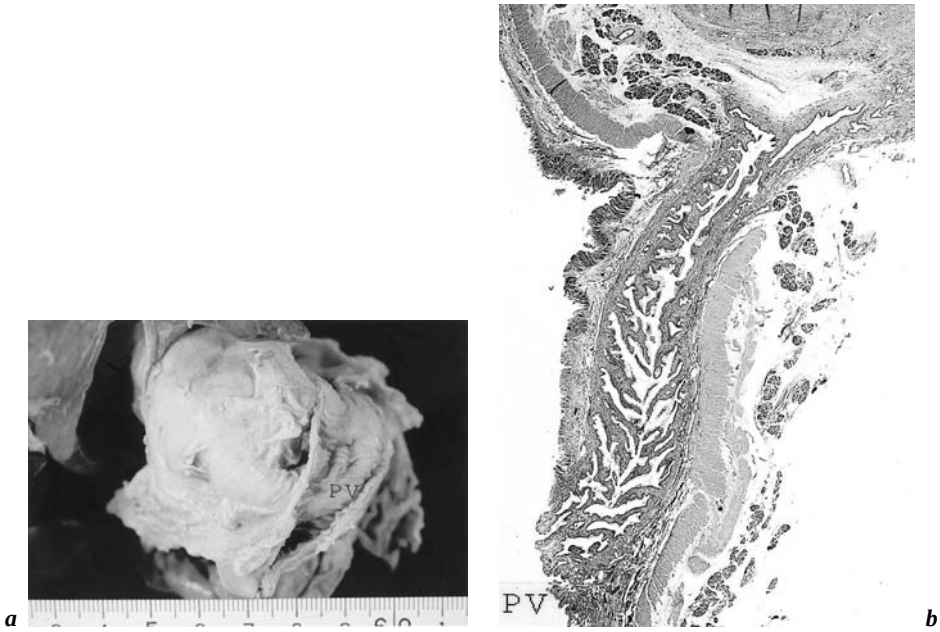

Fig. 4. PV in a patient (40 days, F) with a choledochal cyst. ***a*** The plica longitudinalis is gently raised and has a longer slope on outer view because it only houses the common channel. (***b***) HE. Low magnification. PV = Papilla of Vater. (Reproduced with permission from [11].)

change with excess mucus secretion, even in the full term fetus, has been noted [6]. This type of mucous membrane ends abruptly at the base of the papilla.

The distended glands of the ampullary mucosa are frequently found along the outer surface of the papilla of Vater at incidences of 59% in the resected materials of pancreatobiliary diseases, 22% in infants and 77% in adult autopsies, as shown in table 1. Normally, such glands replace a portion of the surrounding duodenal mucosa and measure on average 1,532 μm in length (fig. 5a) [7]. There is no particular border between these distended glands and the ampullary and duodenal mucosae.

The distended glands are to be found situated on the Oddi's sphincter muscle and not on the muscularis mucosae of the duodenum as follows: under normal circumstances, with no distended glands, the Oddi's sphincter muscle and the muscularis mucosae of the duodenum merge in an end-to-end, acute-angled manner on the outer edge of the papilla of Vater. However, in cases with distended glands the muscularis mucosae of the duodenum is joined with the Oddi's sphincter muscle in an end-to-side, less acute, rather right-angled' manner at the latter side portion, away from the outer edge (fig. 6). Thus, the

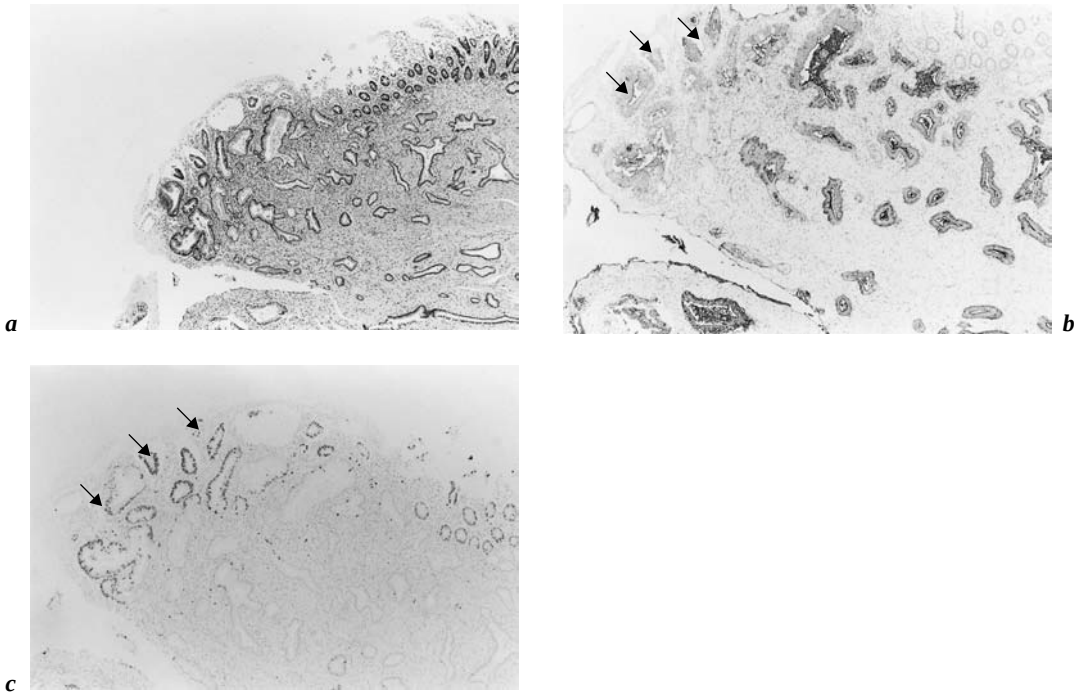

Fig. 5. Histology of the distended glands. The distended glands with intrapapillary adenomyomatous hyperplasia consisted of larger or hyperplastic and dilatated glands (***a***), were weakly immunostained for CA19–9 (arrows) (***b***) and showed high proliferation activity (arrows) (**c**). ***a*** HE. ×50. ***b*** Immunostaining for CA19–9. ×80. ***c*** Immunostaining for Ki67. ×80. (Reproduced with permission from [7].)

Table 1. Distended glands, or overreplacement, of ampullary mucosa

Source	Cases	Distended glands	Normal
Pancreatoduodenectomy	29	17	12
Autopsy (infants)	18	4	14
Autopsy (adults)	26	20	6

distended glands or ‘adenomatoid hyperplasia’ of the ampullary mucosa only grow on the extension of the Oddi’s sphincter muscle [7].

Immunohistochemically, the distended glands are negative or weakly positive for CA19–9, while the intrapapillary mucosa and valvules are positive, as shown in figure 5b and table 1. According to Sonoue [8] et al., the distended

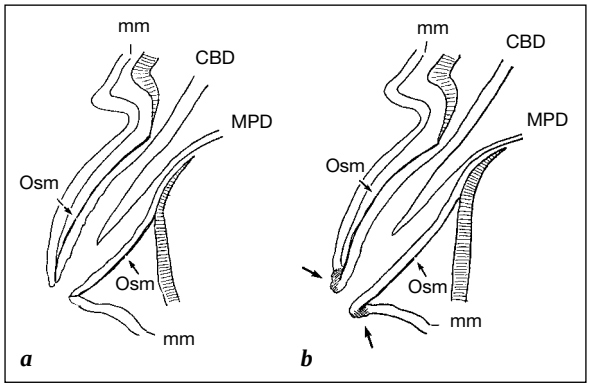

Fig. 6. Schematic drawing of the overreplacement of the ampullary mucosa. The muscularis mucosae of the duodenum (mm) and Oddi's sphincter muscle (Osm) normally merge at the edge of the papilla (***a***), while in the case of overreplacement, the muscularis mucosae is in contact with a side of the Oddi's sphincter muscle, resulting in distended glands or so-called overreplacement (arrows) (***b***). CBD = Common bile duct; MPD = main pancreatic duct. (Reproduced with permission from [7].)

glands can be immunostained with cytokeratin 7 and MUC5AC, markers of pancreaticobiliary phenotype and gastric surface mucin, respectively (fig. 7). Hence, such glands maintain the pancreaticobiliary phenotype, although they are situated on inside or toward the duodenal lumen, and acquire a selectively gastric surface phenotype. The average Ki67LI of the distended glands is 19.6 ± 21.0%, while it is 9.0 ± 11.0% in the intrapapillary glands, a difference which is not significant ($p = 0.064$) as shown in table 2. However, in 2 cases the distended glands showed a high proliferation index (fig. 5c) and were identified with adenomatous or adenoma-like structures admixed with or without similar proliferation in the tips of the valvules.

Pathogenesis and Significance of Distended Glands

The pathogenesis and significance of the distended glands of the ampullary mucosa have not been clarified. One possible explanation that, however, does not apply to all cases is as follows: intrapapillary adenomyomatous or adenomatous/myomatous hyperplasia might oppress the duodenal mucosa, resulting in atrophy and desquamation of the latter near a portion of the outlet. Finally, the end-to-side, less acute and right-angled formation occurs and the distended glands begin to grow on the extension of Oddi's sphincter muscle. According to

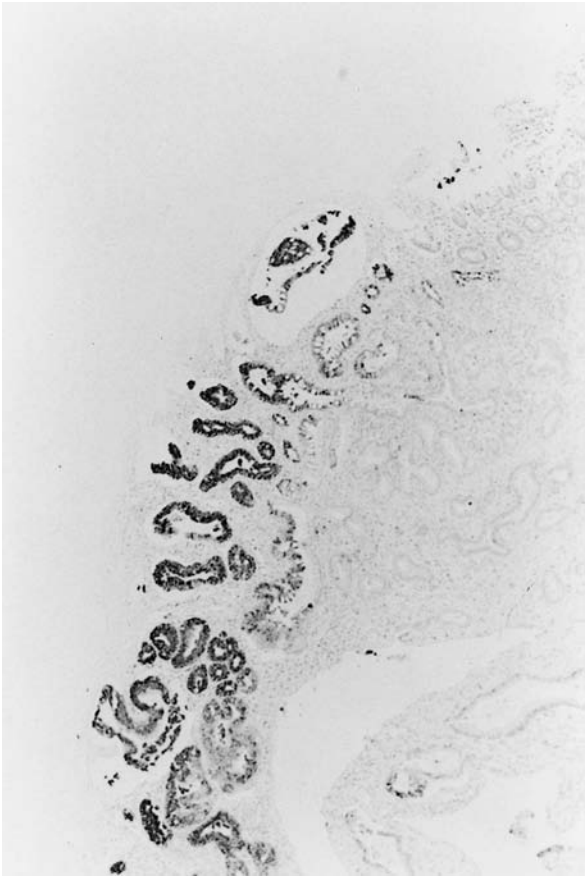

Fig. 7. MUC5AC staining of the distended glands, the same case as in figure 5. The distended glands were selectively immunopositive for MUC5AC. ×64. (Reproduced with permission from [8].)

Albores-Saavedra et al. [2], the ampullary region contains a transition from pancreaticobiliary to intestinal epithelium, and such areas of transition are inherently unstable. Although the evidence indicates that most ampullary carcinomas arise from adenomas, it is probable that some arise de novo or from nonpolypoid precursors. Extremely small invasive carcinomas have been found with no evidence of preexisting adenoma [2]. According to Klöppel [9], the ampulla of Vater harbors exophytic adenomas composed of tubular and/or villous epithelial proliferations that show an intestinal phenotype. Tumors revealing pancreaticobiliary differentiation are usually invasive and may be a carcinoma from the onset. Features of an intestinal origin are seen in the lesion arising in the ampullary region, at the interface between pancreaticobiliary epithelium and intestinal epithelium.

Table 2. Mucosal differences of the papilla of Vater

Item	Common channel valvules	Distended glands	Duodenum
Mucosal structure	papillary	dilated/glandular	glandular/tubulo-villous
CK7	+	+	−
CK20	−	−	+
CA19-9	+	−∼±	−
MUC5AC	−[1]	+	−
Ki67LI	about 10%	about 20%	about 80%[2]
	Not significant		

[1]Mostly negative.
[2]So-called proliferative zone of the duodenal mucosa.

Furthermore, on autopsy, epithelial hyperplasia bordering on severe dysplasia has been observed in the ampullary epithelium in the absence of architectural features of adenoma [10]. Immunoreactivity against anti-CA19–9 in the distended glands is mostly different from that of the intrapapillary portion of the ampullary mucosa, especially in cases with a high proliferation index as mentioned above. However, it is similar or close to that for the duodenal mucosa, as shown in table 2. The distended glands are immunopositive for CK7 and selectively positive for MUC5AC, as mentioned above. Hence, the distended glands might not only maintain a pancreaticobiliary phenotype but also acquire a gastric surface phenotype, resulting in a difference in character that leads to malignant change. Moreover, although most distended glands or so-called overreplacement are acquired, some can be found in infancy, similar to the findings of Kirk [6]. Therefore, such an occurrence might be related not only to malignant changes, but also to a kind of adaptive phenomenon against bile and pancreatic juice flow based on chronic inflammation.

Biopsy of the Papilla of Vater

Eighty-nine endoscopic biopsies of the papilla of Vater, ranging from 1 to 5 pieces with an average of 2.5 per case, were obtained from 55 patients with an average age of 59.6 (25–81) years during the study period, excluding pathologically diagnosed cases of ampullary carcinoma. The majority of patients were referred for the evaluation of suspected ampullary carcinoma.

Among the biopsied specimens, the ampullary mucosa, which consists of larger or hyperplastic and dilated glands with conspicuous goblet cells adjacent to

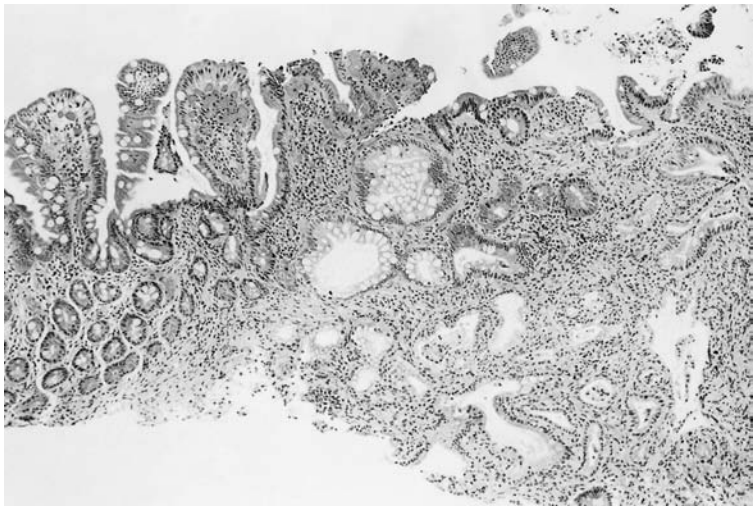

Fig. 8. A biopsy specimen of ampullary mucosa (71 years, M). The ampullary mucosa on the right side of the figure and duodenal mucosa on the left side were taken together and were in contact without border. Note that the ampullary mucosa shows larger, hyperplastic and dilated glands, but not a papillary pattern. HE. ×100. (Reproduced with permission from [7].)

the duodenal glands, was included in 43 out of the 55 cases without border along the duodenal mucosa (fig. 8) [7]. In the remaining 12 cases, only the duodenal mucosa was removed. When endoscopists try to remove specimens from the papilla of Vater, they may obtain pieces of tissue from the distended glands or so-called overreplacement mucosa and not from the intrapapillary portion. Hence, when only duodenal mucosa is removed by ampullary biopsy, no distended glands might be found or the endoscopist may have missed the papilla of Vater.

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Pancreatic Ischemic Lesions

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Abstract

There have been relatively few reports describing pancreatic ischemia, because of the rarity of pancreatic ischemia and the inaccessibility of the pancreas itself. The rarity of pancreatic ischemia may be attributed to the richly arterial supply with numerous vascular anastomoses. Nonetheless, pancreatic ischemia exists as a separate entity, and should be differentiated from acute pancreatitis or fat necrosis. This article describes the distinctive morphologic features of pancreatic ischemia, representing the coagulative necrosis of pancreatic acinar cells, and discusses the subsequent features.

Pancreatic ischemic necrosis or infarct is rare [1–3]. This rarity could be attributable to the rich arterial blood supply with numerous vascular anastomoses from the ramifications of two separate branches of the abdominal aorta [1, 2, 4]. Moreover, the inaccessibility of the pancreas may frequently interrupt the histological recognition of the ischemic changes because of the rapid onset of pancreatic autolysis in the postmortem period or after excision [4]. Furthermore, pancreatic ischemia may promptly induce other conditions, making its histological detection difficult. In fact, the two conditions, pancreatic infarct and acute hemorrhagic pancreatitis, may coincide, and the dividing line between them is clinically and histologically quite indefinite [2]. Some experimental studies also have concluded that vascular disturbances alone might actually initiate acute hemorrhagic pancreatitis [2]. Hence, there have been relatively few opportunities for pathologists to recognize the histopathological features of ischemic lesions. Nonetheless, McKay et al. noted that pancreatic infarct undoubtedly exists as a separate entity [3]. We also believe that detailed histological examination can detect ischemic lesions [5, 6]. This article

describes and discusses the pathological characteristics of pancreatic ischemic lesions.

Cause and Incidence of Pancreatic Ischemia

Some authors have described the various causes of pancreatic ischemia or infarct, such as periarteritis nodosa, malignant essential hypertension, embolism, and splenic or superior mesenteric arterial thrombosis [2]. Recently our investigations revealed pancreatic ischemia caused by disseminated intravascular coagulation (DIC) and cholesterol emboli [5, 6]. McKay et al. suggested that cardiac failure with hypotension or shock can cause pancreatic ischemia [3]. The incidence of pancreatic ischemia is unclear. McKay described that pancreatic infarcts were found in only 0.19% of 21,481 consecutive necropsies at the Mayo Clinic [3]. Our study revealed that fresh ischemic lesions were found in 20% (7/35) of cases of DIC and in 12% (2/17) of cases of cholesterol emboli [5, 6].

Pathological Findings

Macroscopic Findings

Macroscopic recognition of pancreatic ischemia depends on the severity or size of ischemia, and the presence of complicated lesions, such as hemorrhages or fat necrosis. Some authors have described that the pancreatic necrotic area can be pale and yellowish with red congested margins [2]. In our experienced case, examination by the naked eye was not able to reveal significant changes in pancreatic ischemia caused by cholesterol, but on histological examination geographic or lobular ischemic necrosis was confirmed.

Histological Findings

The representative histological features of fresh ischemia are coagulative necrosis of the pancreatic tissues [2, 7]. The size of the necrosis can vary from case to case depending on the severity of the ischemia, and extensive pancreatic necrosis can occur. Pancreatic ischemia associated with DIC is characterized by well-demarcated patchy or geographic foci composed of degenerating cells with deeply eosinophilic cytoplasm and pyknotic or disappearing nuclei, indicating coagulative necrosis of affected acinar cells (figs. 1 and 2) [5]. These patchy lesions are an early or localized form of pancreatic infarct, and are referred to as primary acinar necrosis. Similar lesions can be recognized in pancreata with cholesterol emboli [6]. Rarely, cancerous venous permeation causes

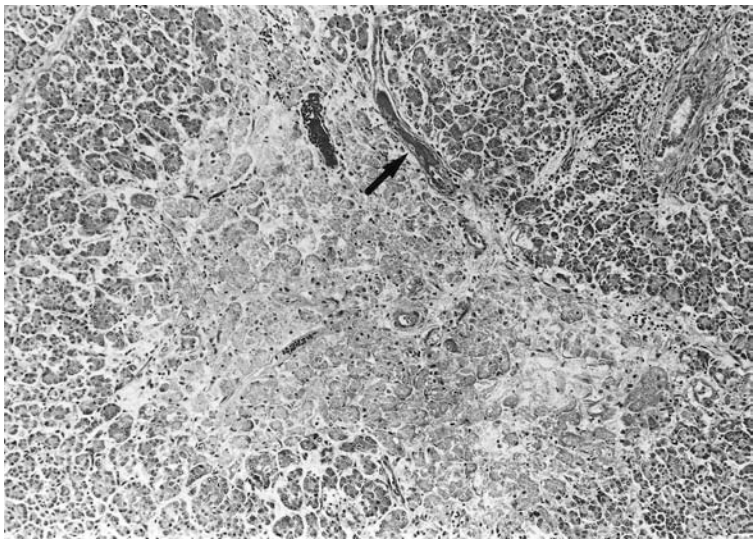


Fig. 1. Patchy ischemic lesion composed of primary acinar necrosis, associated with DIC. Fibrin thrombus is present in the interlobular artery close to the ischemic lesion (arrow) (original magnification ×70).

focal ischemia of the pancreas (fig. 3). There are relatively clearly defined borders between the ischemic lesions and surrounding normal acinar cells. The presence of these clear borders could be useful for distinguishing ischemia from postmortem autolysis.

On the basis of the pathogenetic findings, ischemic lesions can be distinguished from fat necrosis [1]. Fat necrosis is tryptic or enzymic necrosis, resulting from autodigestion of pancreatic tissues caused by extravasated pancreatic juice containing various activated enzymes [1, 8]. Therefore, fat necrosis is secondary necrosis, and fundamentally differs from the ischemic necrosis referred to as primary acinar necrosis. However, peripheral or large ischemic necrosis may induce fat necrosis and/or hemorrhage (fig. 4) [2]. There is hence a correlation between pancreatic ischemia and acute pancreatitis [2, 7].

Some authors have noted selective ischemic changes of the islets of Langerhans in newborns or infants with various forms of shock [9, 10]. These findings may indicate the vulnerability of the islets to shock-related injury in newborns or infants [10]. The patchy ischemic lesions caused by DIC or cholesterol emboli infrequently involve the islets [5, 6]. Rarely, however, focal pancreatic ischemia focusing on the islets is found in DIC cases [5]. Figure 5 shows focal and segmental ischemia of the pancreatic islets, and figure 6 shows focal necrosis involving islets and surrounding acinar cells. This

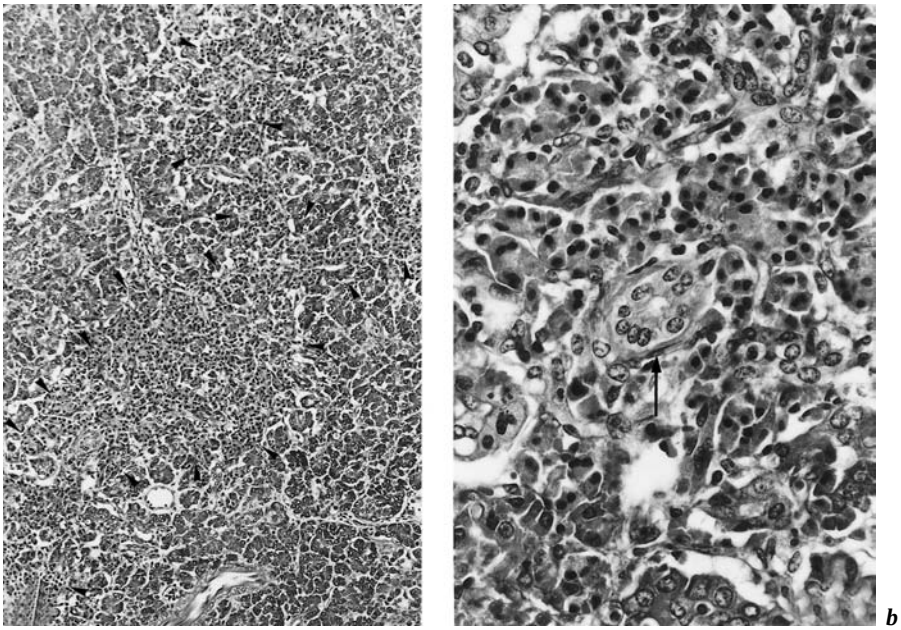


Fig. 2. Fresh ischemic lesion. ***a*** Low magnification of patchy ischemic lesions composed of degenerating acinar cells (arrow heads) (original magnification ×70). ***b*** High magnification of patchy ischemic lesions showing degenerating acinar cells with deeply eosinophilic cytoplasm and pyknotic nuclei. Intralobular ductules which have avoided the ischemic changes are present (arrow) (original magnification ×400).

morphological variety of ischemic lesions could be attributed to the size and/or location of the arteriolar lesions causing the ischemia. Using a correlation with the form of the pancreatic microvasculature, Takahashi et al. established three types of pancreatic ischemic injuries: (1) 'peripheral necrosis', chiefly occurring in the peripheral areas of the pancreatic lobules and not involving the islets; (2) 'central necrosis', representing a microinfarction of acinar cells and the islets, and (3) 'peripheral atrophy', characterized by the thinner and more degranulated pattern of acinar cells in the peripheral areas of the pancreatic lobules [11, 12]. According to this classification, selective ischemia of the islets could be referred to as central necrosis, and patchy acinar necrosis as peripheral necrosis. In fact, detailed examination revealed minute thrombi in the dilated vessels of the islets in cases showing central necrosis, and multifocal thrombi in the interlobular arteries of cases of peripheral necrosis [5].

Another interesting feature of the patchy ischemic lesions, as a localized or early form of pancreatic ischemia, is that intralobular ductular cells are apt to

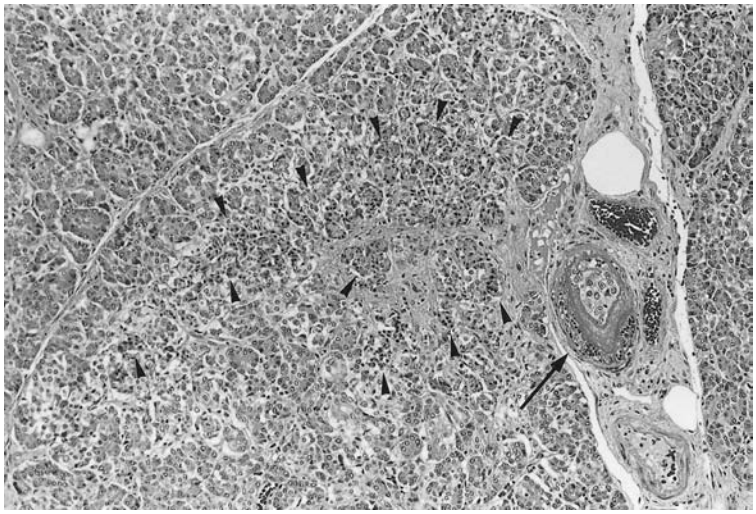


Fig. 3. Small ischemic lesions (arrow heads) associated with cancerous emboli (arrow) (original magnification ×70).

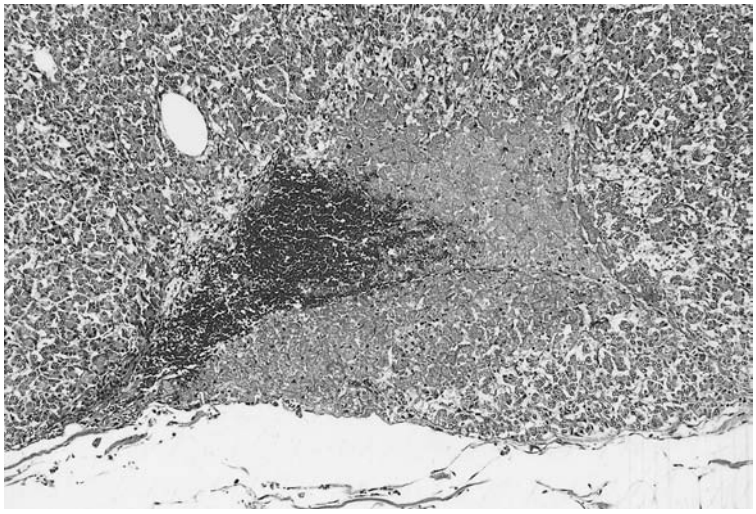


Fig. 4. Patchy ischemic lesions located on the peripheral portion of the pancreatic lobule, accompanying hemorrhage. Surrounded lobules showing interstitial edema (original magnification ×70).

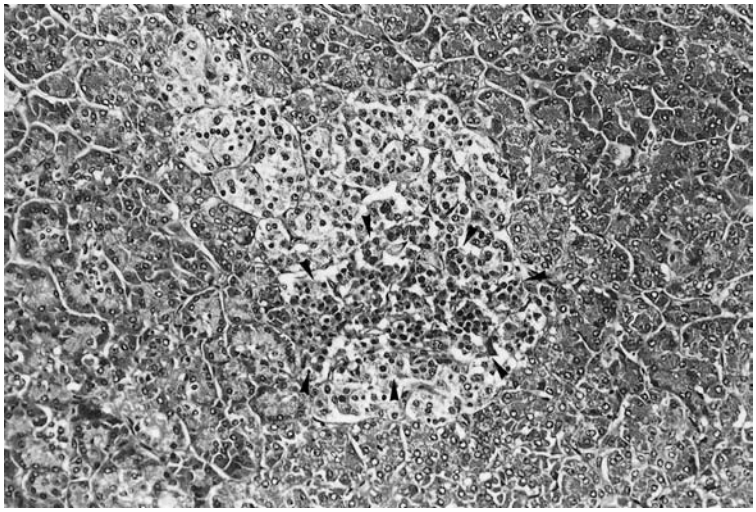


Fig. 5. Segmental ischemic lesions involving the islets of Langerhans (arrow heads) (original magnification ×200).

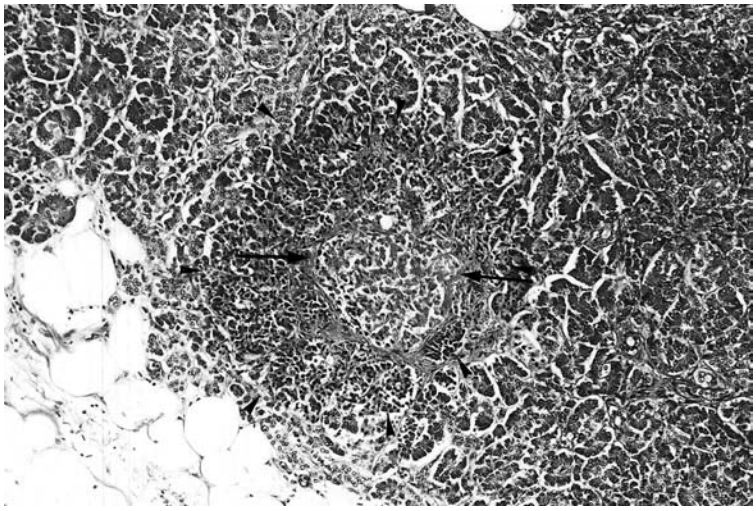
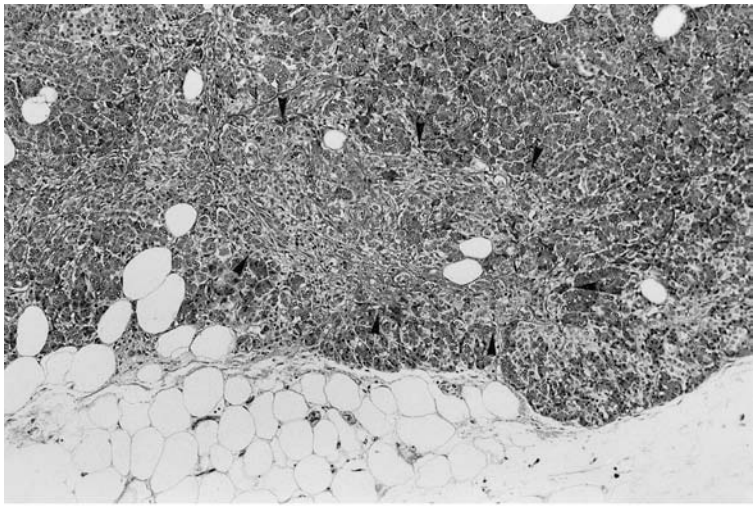


Fig. 6. Focal pancreatic ischemia (arrow heads) centrally involving the islets of Langerhans (arrows). These findings correspond to those of central necrosis (original magnification ×100).



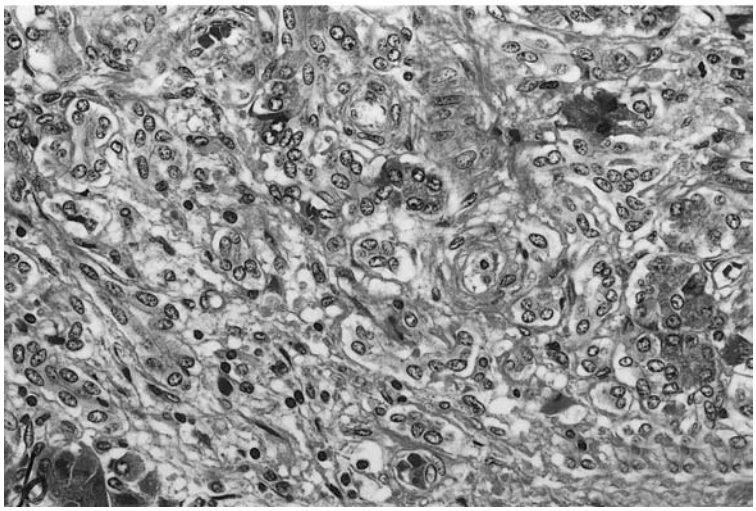


Fig. 7. Patchy fibrotic foci corresponding to the subsequent features of pancreatic ischemic lesions. ***a*** Patchy fibrotic focus with slightly retracted appearance in the pancreatic lobule (original magnification ×70). ***b*** High magnification of patchy fibrotic focus containing numerous small ductules. These ductules coud be intralobular ductules that avoided previous ischemic damage (original magnification ×400).

escape the ischemic damage (fig. 2b). These findings suggest that intralobular ductular cells are more resistant to ischemic damage than acinar cells [6], the latter being the most differentiated and most vulnerable cells in pancreatic tissues. Moreover, these findings may be the diagnostic clue to identifying the

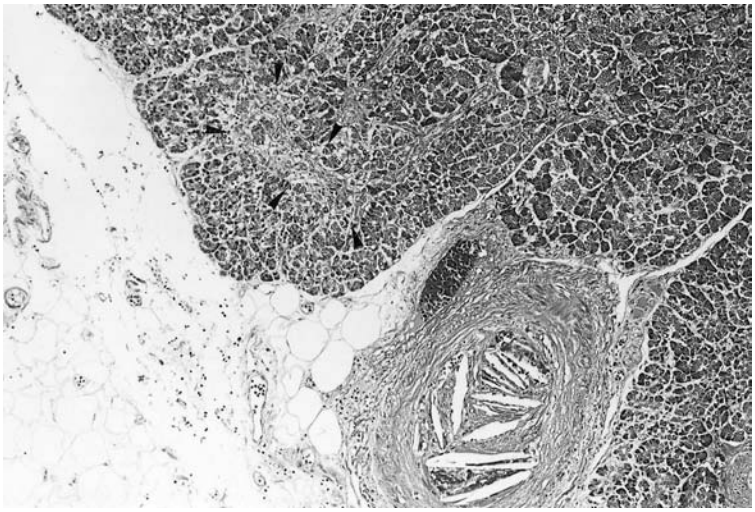


Fig. 8. Cholesterol embolus and associated patchy fibrotic focus (original magnification ×70).

subsequent or chronic lesions of the pancreatic ischemia; the presence of the undamaged intralobular ductules could be the histological hallmark of chronic ischemia, differentiating it from non-specific fibrosis [6]. Our study proposed putative sequential changes of the small pancreatic ischemia [6]. After the earliest ischemic change, the ischemic area may show interstitial edema, mild neutrophilic infiltration, and phagocytic removal of the necrotic acinar cells. Intralobular ductules are usually inconspicuous in normal pancreatic tissue, but become apparent in ischemic lesions. Patchy edematous foci with remnant ductules, acinar cell depletion and mild neutrophilic infiltrates can be referred to as 'subacute ischemic lesions'. These foci can be replaced by fibrosis and show relatively retracted features (fig. 7). Moreover, remnant ductules can be found in these fibrotic lesions. In our investigation, such lesions, suggesting chronic ischemic lesions, were found in five of the 17 postmortem pancreata with cholesterol emboli [6] (fig. 8). Unfortunately, however, the subsequent histological features of extensively involved ischemic lesions have not been elucidated.

Clinical Significance of Pancreatic Ischemia

A large infarct can induce hemorrhagic pancreatitis, the most severe form of acute pancreatitis, characterized by extensive proteolytic destruction of

pancreatic parenchyma, fat necrosis, and hemorrhages [2, 7, 8]. This disorder alone can be a life-threatening illness [7]. On the other hand, small ischemic changes can be healed, with little or no significant clinical disorders. Our previous study also suggested that life-threatening pancreatic disorders induced by cholesterol emboli are rare, although histological examination can detect patchy old lesions of pancreatic ischemia [6]. Therefore, the size of ischemic necrosis is correlated with the patient's clinical course. Whether pancreatic ischemia induces severe pancreatitis may be a significant prognostic factor. However, many experimental studies have indicated that there is marked variation in the sensitivity of individuals to reactions of pancreatic necrosis or pancreatitis [2].

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Repair/Reparative Change in Acute Pancreatitis and the Role of Fat Necrosis

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Abstract

In patients with acute pancreatitis, reparative changes of frank parenchymal necrosis, divided into granulation tissue, fibro-granulation tissue and fibrosis, occur depending on the duration and severity of the illness. Fibrosis in the apparently uninvolved areas in patients with acute pancreatitis develops in relation to frank parenchymal necrosis, and may consist of type I collagen in patients surviving longer. The role of fat necrosis in acute pancreatitis could be explained as follows: when extensive fibrin thrombi in the fat necrosis are resolved before the reparative change is accomplished, hemorrhage may extend into the surrounding tissue.

Acute hemorrhagic pancreatitis or acute pancreatic necrosis is a fatal disease and is caused by the destructive effects of pancreatic enzymes. The dominant characteristics of acute pancreatic necrosis are parenchymal necrosis, hemorrhage and fat necrosis. If patients survive, a variety of sequelae, including reparative changes, follow.

Most patients with acute pancreatitis have considerable areas of intact parenchyma, even at death after an illness of only a few days [1]. Necrotizing pancreatitis is often accompanied by interstitial pancreatitis some distance from areas of necrosis. When these cases consequently become chronic or heal, interlobular fibrosis is prominent.

Fat necrosis is thought to be the initial event of acute pancreatitis. However, causal relationships between fat necrosis and advancement to fatal pancreatitis have not yet been fully explained.

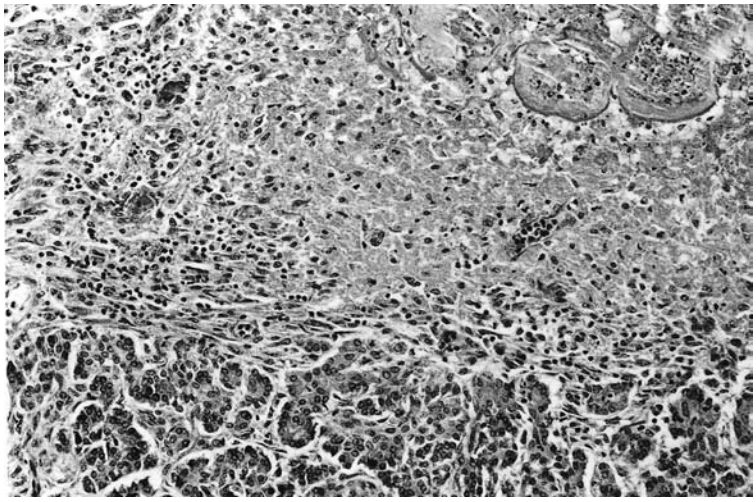


Fig. 1. Reparative changes in frank parenchymal necrosis. Parenchymal necrosis is adjacent to the exocrine tissue via granulation in a patient with an illness of 10 days. HE. ×100.

In this article, we describe in patients with acute pancreatitis both reparative changes in frank necrosis and the occurrence of fibrosis in apparently uninvolved areas away from the areas of necrosis, and discuss the role of fat necrosis in acute pancreatitis.

Reparative Changes in Frank Parenchymal and Fat Necroses in Patients with Acute Pancreatitis

Reparative changes in frank parenchymal necrosis in patients with acute pancreatitis are divided into granulation tissue, mixed or fibro-granulation tissue, and fibrosis (fig. 1). According to our previous study on 24 patients with various durations of acute pancreatitis [2], the appearance and increase in reparative changes correlates with the duration of disease as follows: (1) no reparative changes with disease of less than 6 days' duration (fig. 2); (2) granulation tissue mostly between 7 and 25 days; (3) fibro-granulation tissue between 28 and 42 days, and (4) fibrosis mainly after 8 weeks or longer, as shown in table 1.

Reparative changes in parenchymal and fat necroses are not present in the early stage of acute pancreatitis, as only necrosis is seen, and granulation tissue develops later. Based on the description of the marginal reaction against frank parenchymal necrosis by Baggenstoss [1], in patients with illness of less than

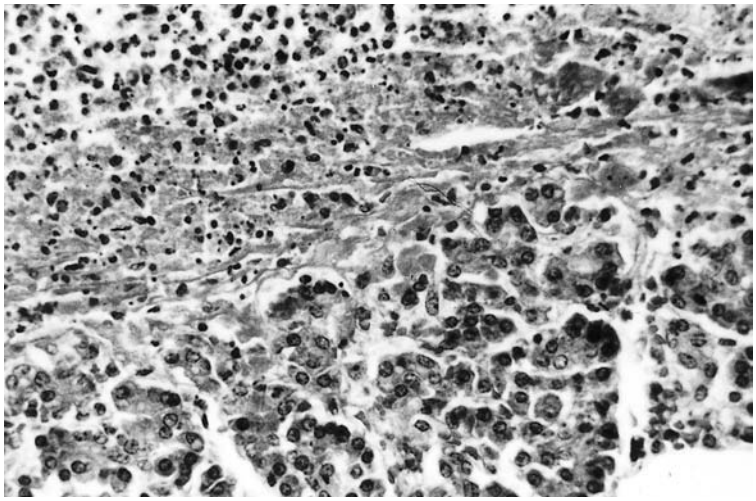


Fig. 2. No reparative change. Parenchymal necrosis directly adjacent to the exocrine tissue in a patient with an illness of 2 days. HE. ×200.

Table 1. Relationship between reparative changes in frank parenchymal necrosis and duration of illness in patients with acute pancreatitis [2]

Duration of illness, days	Cases	Reparative change
2	4	none
7–10	8	granulation tissue
13	1	granulation tissue
25	1	granulation tissue
28	1	granulation tissue/fibro-granulation
42	2	granulation tissue
42	2	fibro-granulation tissue
>60	3	fibro-granulation tissue/fibrosis
>60	2	fibrosis

one week's duration, zonal reaction features can be roughly identified after staining with hematoxylin and eosin. These are (1) an acidophilic structureless zone of necrosis; (2) a narrow basophilic zone of karyolysis and karyorrhexis; (3) an acidophilic zone containing coagulation necrosis of the cytoplasm and pyknotic nuclei, and (4) a zone of interacinar edema and a mild leukocytic reaction. From our observations, fat necrosis in the agonal stage is either accompanied by foamy histiocytes or shows no reparative reaction. Hence, reparative changes are related to the duration and severity of the illness.

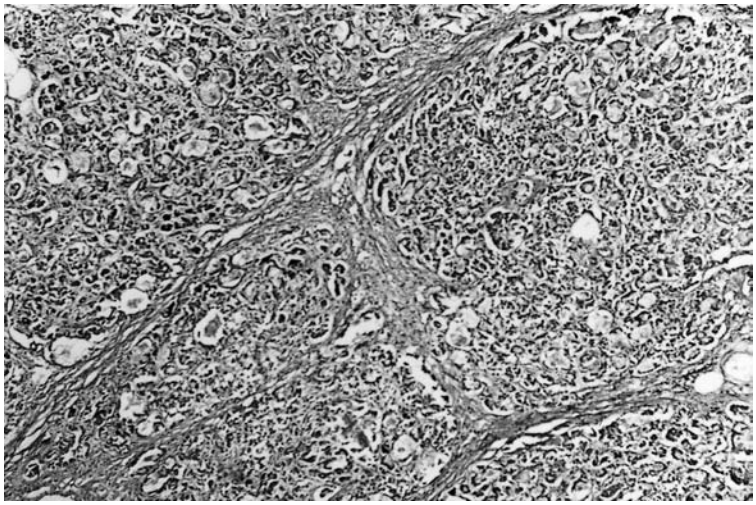


Fig. 3. Interstitial fibrosis in a patient with an illness of 30 days with acute pancreatitis. Somewhat linear fibrosis is found in the interlobular area. HE. ×40.

Fibrosis in Apparently Uninvolved Areas in Patients with Acute Pancreatitis

Necrotizing pancreatitis is often accompanied by interstitial pancreatitis some distance from the areas of necrosis (fig. 3). Several authors have noted that acute pancreatitis may cause fibrosis [3, 4]. However, interlobular fibrosis is a characteristic finding in chronic pancreatitis [5], and it is unclear whether the fibrosis in apparently uninvolved areas occurs before or after acute pancreatitis.

Our previous study revealed apparently uninvolved areas that were distant from frank parenchymal necrosis in all patients with acute pancreatitis; these areas are frequently accompanied by interlobular fibrosis [6]. The appearance and increase in interlobular fibrosis also correlates with the duration of disease, as shown in table 2, and as follows: (1) no fibrosis with illness of less than 5 days' duration (fig. 4); (2) linear fibrosis with positive immunoreactivity against anti-collagen type III between 8 days and a month (fig. 5), and (3) broad fibrosis with positive immunoreactivity against both types I and III anti-collagen with illness of 2 months or longer (fig. 6). The interlobular fibrosis is often accompanied by hemosiderin deposition, which is assumed to be due to previous inflammation with hemorrhage. Hence, fibrosis in apparently univolved areas appears to develop in relation to acute pancreatitis. This finding is supported by the interlobular distribution of hemorrhage in two patients without

Table 2. Fibrosis in apparently uninvolved areas and fat necrosis in cases of acute pancreatitis

Case	Age/ Sex	Duration of illness, days	Cause	Interlobular spaces			Fat necrosis			
				Fibrosis*	Hemosiderin	Types I/III collagen	Fibrin thrombi	Hemorrhage/ hemosiderin	Reparative change	Venous thrombus
1	57/F	4	gallstones	(inflammation with hemorrhage)			+	+/+	–	+
2	47/M	5	alcohol	(inflammation with hemorrhage)			+	+/+	–	
3	51/F	8	unknown	linear	+	–/+	+	+/+	+/–	
4	72/M	28	gallstones, after ERCP and EST	linear	+	–/+	+	+/+	G	+
5	36/M	30	alcohol	linear	–	–/+	+	–/+	G	
6	50/M	34	alcohol	linear and broad	+	+/+	+	–/+	G	+
7	37/M	70	alcohol	broad	+	+/+	+	+/+	G	
8	70/M	91	alcohol	broad	+	+/+	+	+/+	G	+
9	69/M	95	alcohol, after ERCP	linear and broad	+	+/+	+	+/+	G	

*Interlobular fibrosis, except for the thin connective tissue septa. ERCP = Endoscopic retrograde choledochopancreatography; EST = endoscopic sphincterotomy; G = granulation tissue.

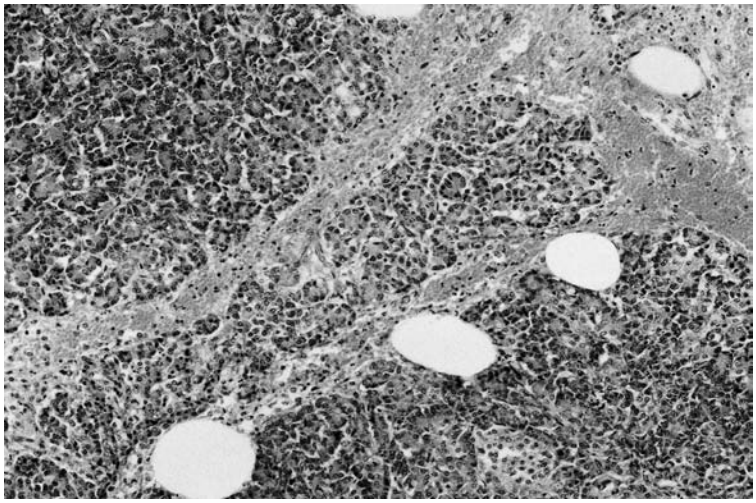


Fig. 4. Interlobular distribution of hemorrhage in a patient with an illness of 4 days. HE. ×100. From [6] with permission.

fibrosis. It is also known that pancreatic lobules appear to be more resistant to the digestive process than the interlobular spaces.

As 6 of the 9 patients had been heavy drinkers, one may consider such interlobular fibrosis as a finding in chronic pancreatitis due to chronic alcohol abuse [7]. However, one patient (case 2) with an illness of 5 days showed no interlobular fibrosis. Moreover, our previous study [5] showed no chronic pancreatitis/fibrosis in 33 of 53 cases of chronic alcoholism and in 13 of 46 cases of alcohol-dependence syndrome. Therefore, we consider such interlobular fibrosis to occur after acute pancreatitis.

Fibrosis in pancreatic tissue is one of the most characteristic findings in chronic pancreatitis [8]. Sarles et al. report chronic and acute pancreatitis to be different disease entities [9]. However, other authors [3, 4] have noted that acute pancreatitis may cause fibrosis, as mentioned above.

Immunoreactivity against anti-collagen types I and III was positive in 4 patients with an illness of 34 days to two months or longer, whereas anti-collagen type I was negative in the other 3 patients with an illness of 8 days to a month. More type III than type I collagen is present in newly formed connective tissue. So although fibrosis after acute pancreatitis may contain reversible type III collagen as in that of WBN/Kob rats [10], we emphasize that fibrosis is ultimately followed by irreversible type I collagen, corresponding to the duration of disease.

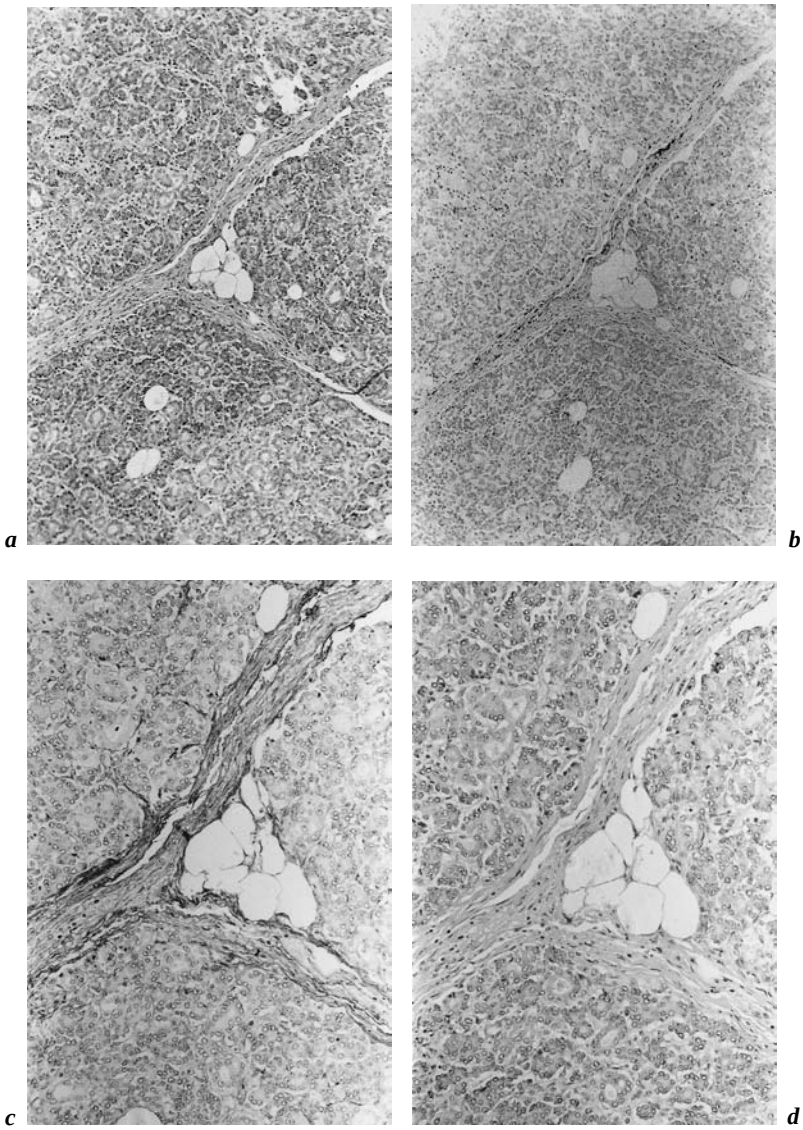


Fig. 5. Interlobular fibrosis in a patient with an illness of 28 days. ***a*** The fibrosis shows a linear type. HE. ×100. ***b*** The fibrosis was accompanied by hemosiderin deposition. Berlin's blue. ×100. ***c*** The fibrosis stained positively for anti-collagen type III. Immunostaining for anti-collagen type III. ×100. ***d*** The fibrosis stained negatively for anti-collagen type I. Immunostaining for anti-collagen type I. ×100. From [6] with permission.

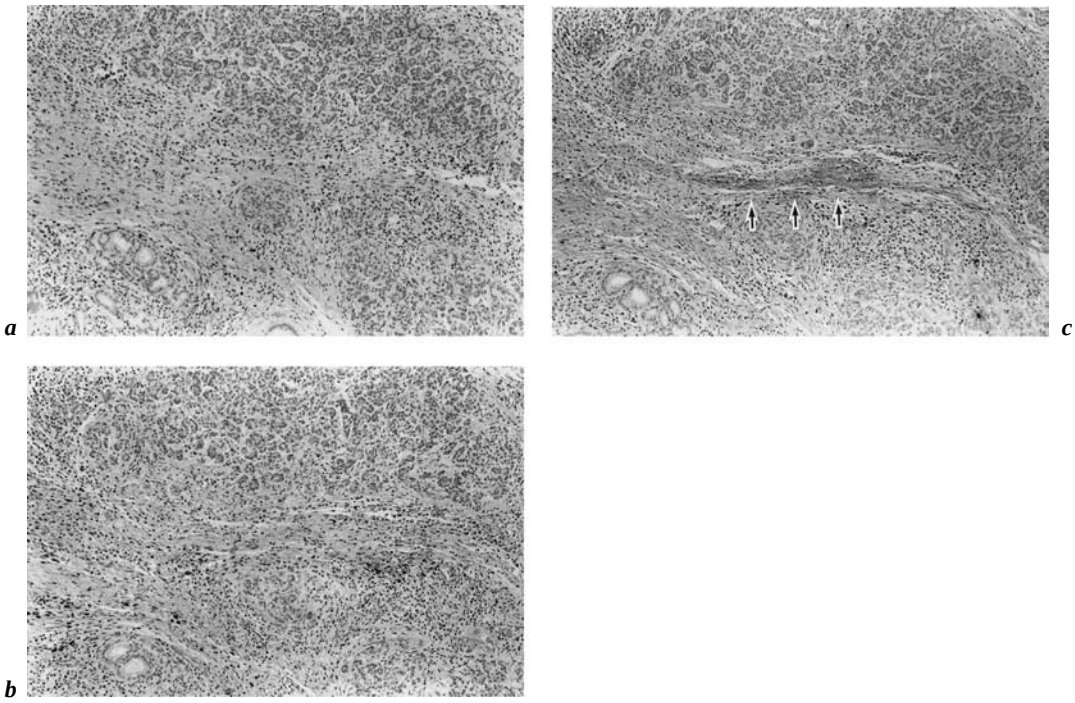


Fig. 6. Interlobular fibrosis in a patient with an illness of 2 months and 10 days. ***a*** The fibrosis shows a broad type. HE. ×50. ***b*** The fibrosis was accompanied by hemosiderin deposition. Berlin's blue. ×50. ***c*** The fibrosis stained positively for anti-collagen type I (arrows). Immunostaining for anti-collagen type I. ×50. From [6] with permission.

We therefore consider that fibrosis in apparently uninvolved areas develops in relation to acute pancreatitis, and it may consist of type I collagen in patients surviving longer.

Role of Fibrin Thrombi in Fat Necrosis

With regard to the pathogenesis of pancreatitis, Schmitz-Moormann [11] reported that acute pancreatitis begins with fat necrosis around and within the pancreas. In the second step, acinar cell necrosis, as well as vascular destruction and thrombosis, arises in the immediate vicinity of the fat necrosis. Klöppel et al. [12] also noted that the earliest autodigestive lesions are found in the peripancreatic fatty tissue. If the initial fat necrosis is extensive and effusion of

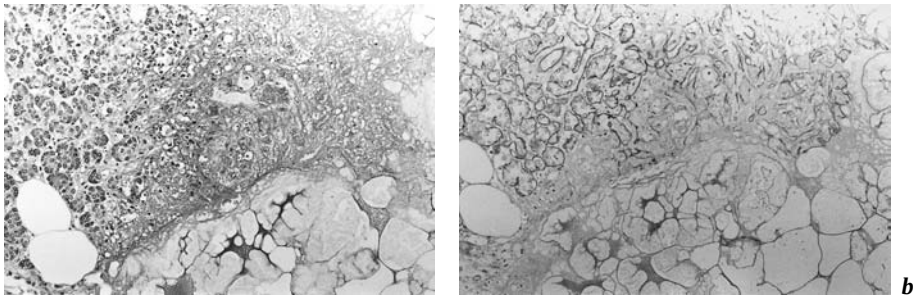


Fig. 7. Fat necrosis encroached on the acini in the intimate vicinity of the necrosis (***a***) with dissolution of the basement membrane (***b***). ***a*** HE. ×200. ***b*** Immunostaining for type IV collagen. ×200.

enzymes from the neighboring acinar cells continues, the full spectrum of fatal pancreatitis will develop. Hence, fat necrosis is most likely the lesion which definitively marks the beginning of acute pancreatitis. However, causal relationships between fat necrosis and advancement to the next stage of fatal pancreatitis, such as parenchymal necrosis and hemorrhage, have not been fully explained. We found that fat necrosis was present in all 9 patients with acute pancreatitis, as shown in table 2. Fat necrosis in the agonal stage often encroaches on the acini in the intimate vicinity of the necrosis (fig. 7). This is similar to the findings of acute pancreatitis described by Schmitz-Moormann [11] and Klöppel et al. [12]. However, fat necrosis is accompanied by either hemorrhage or hemosiderin deposition in all cases of acute pancreatitis, whereas it is seldom seen in those in the agonal stage. These findings suggest that hemorrhage occurs in the fat necrosis in the course of the illness.

Escape of elastase from the ductal system causes dissolution of blood vessel elastic fibers and probably causes hemorrhage [13, 14]. However, the cause of the hemorrhage in fat necrosis is thought to be as follows: fibrin thrombi are frequently found in fat necrosis either at the agonal stage (fig. 8) or in patients with acute pancreatitis. Hemorrhage or hemosiderin deposition in fat necrosis are seldom observed in that of the agonal stage, whereas they are found in all patients with acute pancreatitis as mentioned above (fig. 9). In a certain type of fat necrosis lesion which is accompanied by simultaneous hemorrhage and fibrin thrombi, fibrin thrombi are found in the necrosis except for areas of hemorrhagic foci. Hence, the fibrin thrombi in fat necrosis are formed shortly after occurrence of the fat necrosis, and they are then probably resolved by the action of plasmin resulting eventually in hemorrhage. Therefore, we emphasize that hemorrhage appears to occur after the resolution of fibrin thrombi in fat necrosis due to a reperfusion-injury mechanism [15].

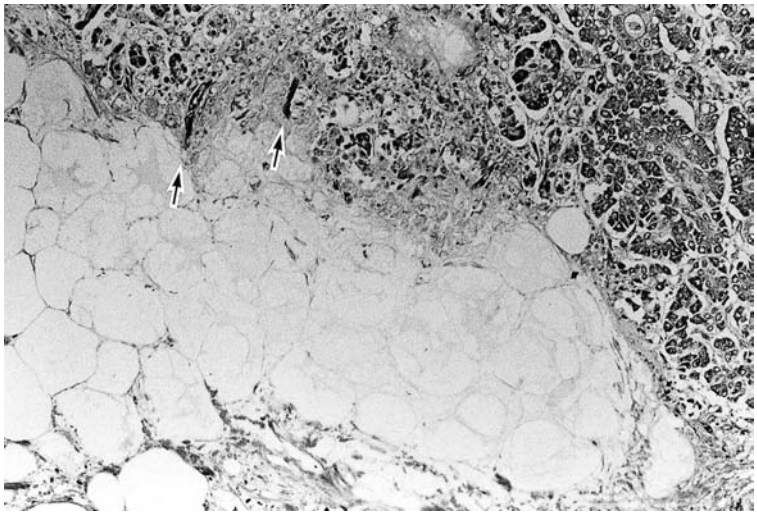
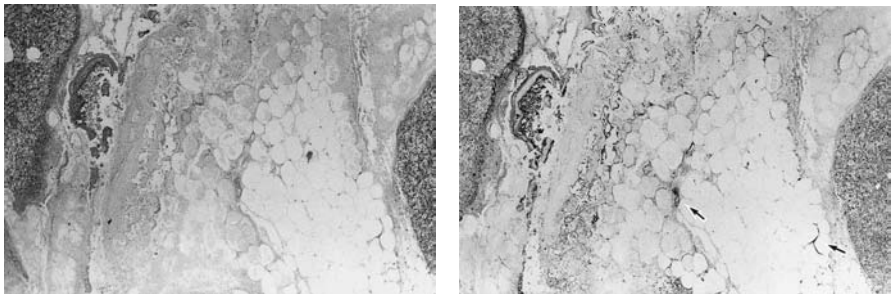


Fig. 8. Fat necrosis was accompanied by fibrin thrombi (arrows) in the capillaries. PTAH. ×100.



a ***b***

Fig. 9. Fat necrosis with hemorrhage in a patient with acute pancreatitis of 4 days' duration. Hemorrhage is found among fat necrosis at left (***a***) and fibrin thrombi (arrows) were found except in areas of hemorrhage (***b***). ***a*** HE. ×32. ***b*** PTAH. ×32.

In conclusion, the role of fat necrosis may be explained as follows: when extensive fibrin thrombi in fat necrosis are resolved before the reparative change is complete, hemorrhage might extend into the surrounding tissue.

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Pancreatic Ductal Myofibroblasts

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Abstract

Myofibroblasts in the periacinar area of the pancreas have been thought to mediate fibrogenesis in pancreatic fibrosis. We elucidated, by immunohistochemical and ultrastructural studies on surgically resected pancreatic tissue, the presence of myofibroblasts in the pancreatic duct wall in normal and various chronically damaged states of the pancreas. α-Smooth muscle actin (α-SMA)-positive cells were detected in the duct wall, but not in the periacinar space, in normal tissue. In cases with focal pancreatitis, which showed focal stenosis of the main pancreatic duct and localized acinar atrophy with scanty inflammatory cell infiltration, proliferation of myofibroblasts was observed in the subepithelial space of the duct wall at the stenotic portion. At the same time, no proliferation of myofibroblasts was detected in the stenotic portion of the main pancreatic duct wall invaded by carcinoma cells. The myofibroblast distribution in pancreatic tissue of patients with ampullary carcinoma was classified into five patterns: (1) no proliferation, same as normal tissue; (2) proliferation only in the duct wall; (3) proliferation in the duct wall and periductal area; (4) proliferation in interlobular and periacinar area in addition to the duct wall, and (5) diffuse proliferation in the parenchyma. In all cases, the distribution of myofibroblasts coincided with the distribution of fibrosis. In bile pancreatitis, fibrosis was localized in both the periductal and interlobular areas, but myofibroblasts were detected only in the periductal area. In cases with chronic alcoholic pancreatitis and alcoholic liver cirrhosis, myofibroblasts proliferated in the area of characteristic interlobular and intralobular fibrosis. Our results suggest that proliferation of myofibroblasts in the duct wall might represent a wound-healing process of the duct wall or serve to prevent elevation of intraductal pressure, while myofibroblast proliferation in the parenchyma might be related to an inflammatory process in the parenchyma.

Majno et al. introduced myofibroblasts as cells with features of both smooth muscle cells and fibroblasts in 1971 [1]. These cells have been shown to be present in normal and pathologic situations of various organs, such as the

liver, the kidney, and the heart [2–17]. Several reports have shown that cells with features of myofibroblasts participate in the fibrogenic process of the pancreas parenchyma [18–22]. These cells are termed pancreatic stellate cells, because of their morphological and functional similarity to hepatic stellate cells. We previously showed the existence of myofibroblasts in the pancreatic duct wall in normal and pathologic conditions by immunohistochemical and ultrastructural methods [23, 24].

Morphologic Features

Myofibroblasts appear in the light microscope as spindle-shaped cells with acidophilic cytoplasm [1]. In ultrastructural microscopy these cells show features of both fibroblasts and smooth muscle cells. Because the differences between smooth muscle cells and myofibroblasts are subtle, a definition of myofibroblasts is needed to identify some features by ultrastructural methods, such as peripheral bundles of microfilaments with dense bodies, a well-developed rough endoplasmic reticulum, a notched nucleus, fibronectin fibrils on the surface, and fibronexus junctions connected with the surrounding extracellular matrix [25–28]. Because these cells are immunohistochemically positive for α-smooth muscle actin (α-SMA), α-SMA is a useful marker of myofibroblasts [2, 29]. Desmin is not a useful marker of myofibroblasts because these cells are negative for desmin in the human pancreas, although positive in rats [30]. We take account of these staining patterns in diagnosis of the myofibroblast in human pancreas specimens [23].

Role in Fibrosis

These cells have been shown to be present in normal and pathologic situations of various organs, such as the liver, the kidney, and the heart [2–17, 29]. Ito cells, which are also called hepatic stellate cells or fat-storing cells, and which store vitamin A, play a central role in fibrogenesis of the liver [31]. These cells contain fat droplets which are rich in vitamin A in their cytoplasm. They activate and transdifferentiate into myofibroblast-like cells, and synthesize collagens during liver cirrhosis [32]. Recent reports have revealed that periacinar myofibroblasts, which are similar to Ito cells in morphology and functions, play a important role in pancreas fibrosis [18–22, 33, 34]. However, the detailed mechanisms of myofibroblast activation in pancreatic diseases are not known.

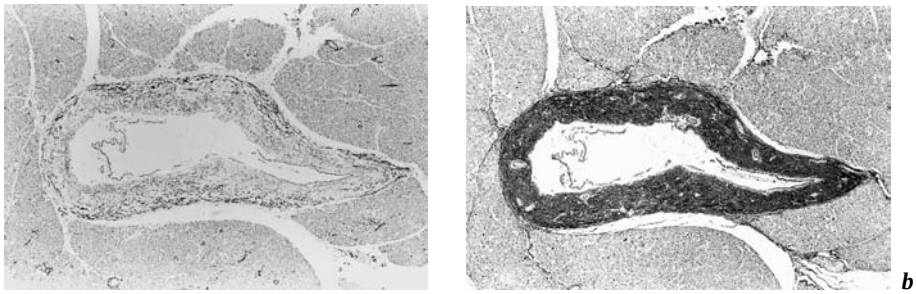

Fig. 1. The normal main pancreatic duct. ***a*** α-SMA-positive cells are found mainly in the outer region of the wall. Immunostain for α-SMA. ×60. ***b*** Elastic fibers distributed throughout the wall. Elastica van Gieson. ×60. Reproduced with permission [23].

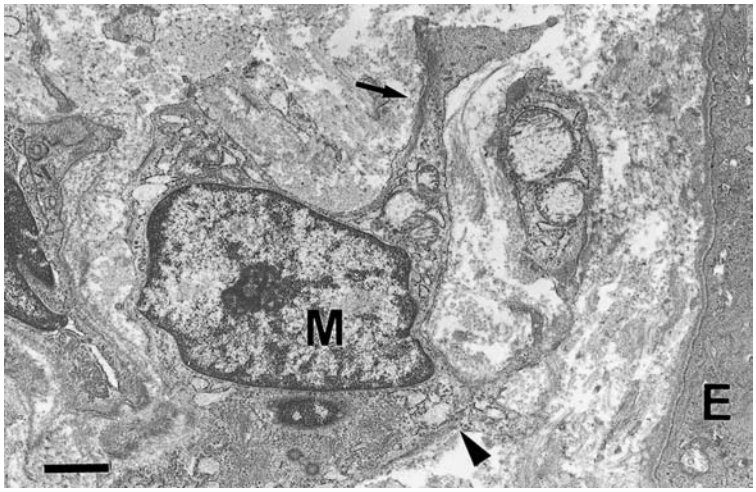

Fig. 2. Transmission electron micrograph illustrating a typical myofibroblast (M) in the normal pancreatic duct. This cell has a triangular shape, a large nucleus, and dilated cisternae of the rough endoplasmic reticulum (arrow head). Bundles of microfilaments are observed throughout the cytoplasm (arrow). E = Ductal epithelium. Scale bar = 1 μm. Reproduced with permission [23].

Distribution under Normal Conditions

Myofibroblasts positive for α-SMA and negative for desmin are scattered and distributed mainly in the outer layer of the wall of the main pancreatic duct consisting of collagen fibers and elastic fibers (figs. 1, 2). Under normal conditions, the role of these cells may play a role in the regulation of flow of pancreatic juice.

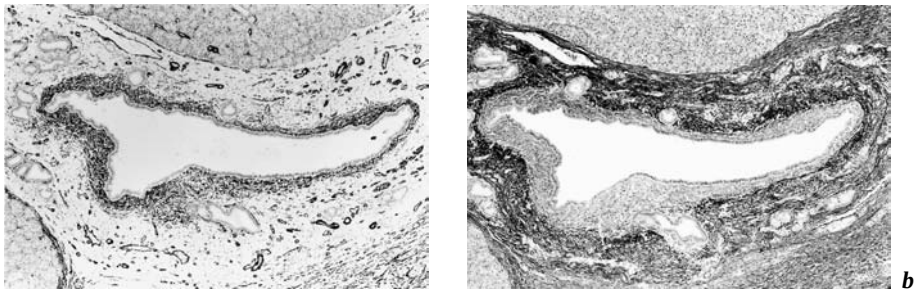

Fig. 3. Stenotic portion of the main pancreatic duct in a patient with FP. ***a*** Massive proliferation of myofibroblasts is observed in the subepithelium. Immunostain for α-SMA. ×60. ***b*** Elastic fibers are dissociated from the ductal epithelium. Elastica van Gieson. ×60. Reproduced with permission [23].

Distribution in Pathologic States

Focal Pancreatitis

Cases categorized as focal pancreatitis (FP) showed localized stenosis of the main pancreatic duct on endoscopic retrograde pancreatography and histological findings of localized acinar atrophy with massive fibrosis with scanty inflammatory cell infiltration. All cases with FP showed fibrous thickening of the wall of the main pancreatic duct without atypical epithelium at the stenotic portion. In 6 cases with FP, α-SMA-positive and desmin-negative cells, i.e. myofibroblasts, proliferated between the duct epithelium and preexisting elastic fibers in the duct wall (fig. 3). The other cases showed no proliferation of myofibroblasts, but showed collagen-fiber accumulation in the subepithelium space, resulting in dissociation of elastic fibers from the duct epithelium [24]. Because myofibroblasts play an important role in wound healing [1, 16], the same phenomena might occur at the duct wall with localized inflammation or ulceration by any causes. In 4 cases without myofibroblast proliferation, apoptosis observed to be negative for α-SMA might occur after collagen fiber synthesis in the late phase of wound healing [17] or cytoskeletal transformation

Chronic Pancreatitis

In our study, the distribution of interlobular fibrosis, one characteristic finding in alcoholic chronic pancreatitis (CP) [35] showed good agreement with the distribution of proliferating myofibroblasts (fig. 4). However, no proliferation of myofibroblasts was found in stenosed duct walls with inflammatory cell infiltration. Many studies have attempted to elucidate the mechanism of CP [36–39]. Functional disorder of acinar cells and ductal epithelial cells in the early stages

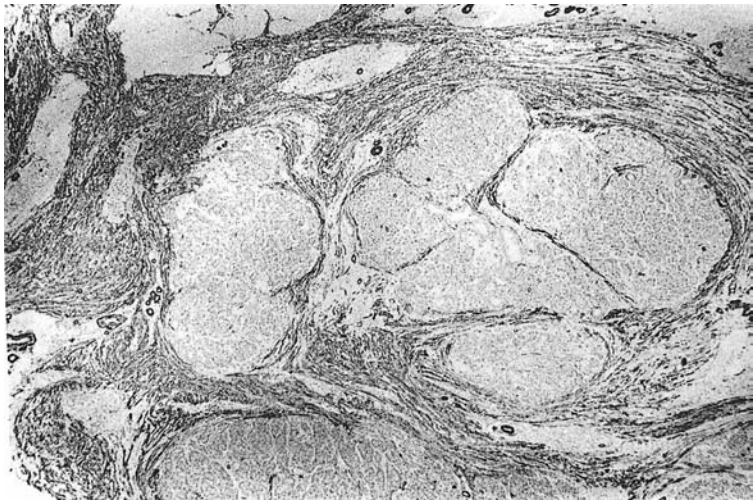

Fig. 4. Myofibroblasts, α-SMA-positive cells, proliferating in the interlobular area of a patient with chronic alcoholic pancreatitis. Immunostain for α-SMA. ×12.5. Reproduced with permission [23].

of CP have been reported. Formation of protein plugs and stones in the pancreatic duct system due to change of character of pancreatic juice, abnormality of the microcirculation [40, 41], and formation of autocrine and paracrine loops of cytokines such as transforming growth factor-β1 [40, 42–49, 50–52], and other factors have been thought to be related to progression of CP [53, 54]. Particularly the activation of cells producing several kinds of extracellular matrixes is an important factor in pancreatic fibrosis [18, 20, 21, 55]. Casini et al. reported acceleration of lipid peroxidation in acinar cells adjacent to interlobular fibrosis and acceleration of collagen fiber production in pancreatic stellate cells adjacent to them in human pancreas tissue with CP [56].

Alcoholic Liver Cirrhosis

We did not identify myofibroblast proliferation in the duct walls of the pancreas of alcoholic liver cirrhosis patients without CP. Myofibroblasts uniformly proliferated in the periacinar space (fig. 5), where fibrosis was observed, in pancreas tissues obtained from autopsy cases with alcoholic liver cirrhosis [57]. These cells are thought to be derived from pancreatic stellate cells similar to Ito cells in the liver. Moroboshi et al. reported that periacinar fibrosis accompanied with no inflammatory changes were observed in the pancreata of hard drinkers, and suggested the existence of not only secondary fibrosis due to parenchymal inflammation but also primary fibrosis [22]. In the livers of cases

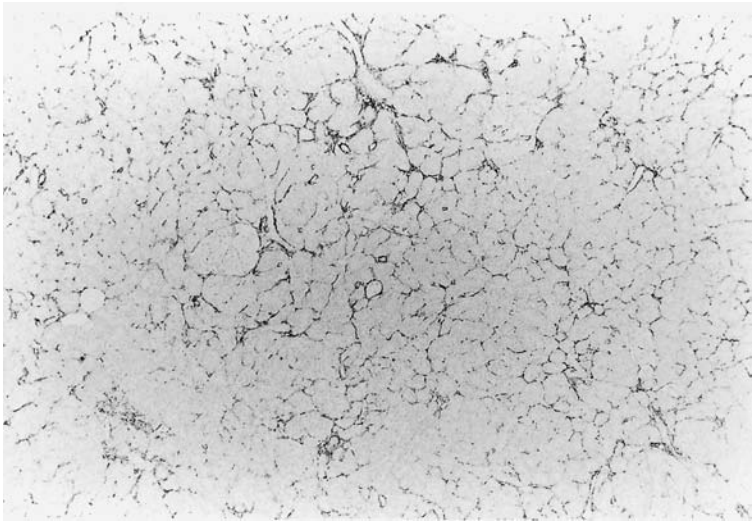

Fig. 5. Myofibroblasts, α-SMA-positive cells, proliferating in the periacinar area of a patient with alcoholic liver cirrhosis. Immunostain for α-SMA. ×200. Reproduced with permission [23].

with alcoholism, similar fibrosis in absence of hepatitis, which is called pericellular fibrosis, occurs by activation of Ito cells [58]. Several investigators have explained the mechanism of periacinar fibrosis. Portal hypertension due to liver cirrhosis may be a cause of myofibroblast activation [59], as congestive heart failure can induce Ito cells to transform into myofibroblasts [60]. Kuroda et al. suggested a contribution of the intracellular transport blockage of protein, as represented by abnormalities of zymogen granules, endoplasmic reticulum and lysosomes, to the development of acinar collagenization in patients with chronic alcoholism [61]. Recently, Apte et al. showed that rat pancreatic stellate cells were directly activated by exposure to ethanol or acetaldehyde, and generation of oxidant stress within the cells [62].

Carcinoma of the Papilla of Vater

The pancreatic tissue of patients with carcinoma of the papilla of Vater (VPCa) showed various degrees of inter- and intralobular fibrosis with acinar atrophy that correspond to obstructive CP [63, 64]. The 56 specimens of VPCa were classified into five patterns of myofibroblast distribution, as follows: (1) no proliferation, same as normal tissue (n = 6); (2) proliferation only in the duct wall (n = 10); (3) proliferation in the duct wall and periductal area (n = 15) (fig. 6); (4) proliferation in the interlobular and periacinar areas in addition to the duct wall (n = 15), and (5) diffuse proliferation in the

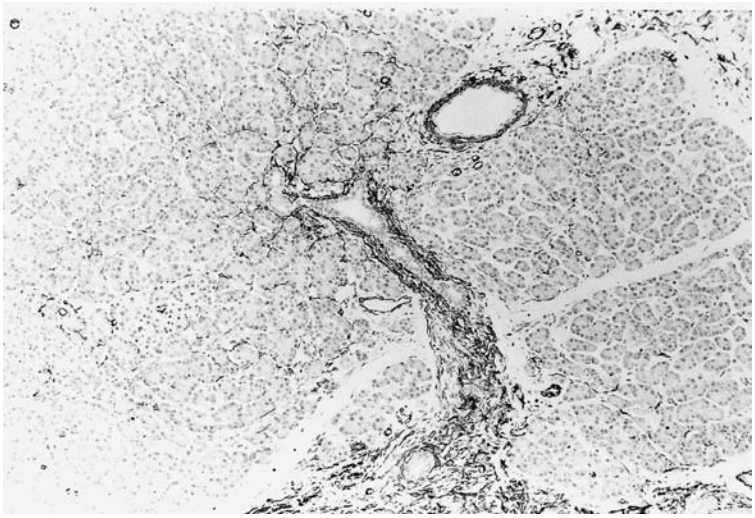

Fig. 6. Myofibroblasts, α-SMA-positive cells, proliferating in the duct wall and the periductal area of a patient with ampullary carcinoma. Immunostain for α-SMA. ×40. Reproduced with permission [23].

parenchyma (n = 10). Myofibroblasts proliferating in the duct wall were distributed mainly in the subepithelial space, resulting in dissociation of elastic fibers from the duct epithelium as seen in cases with FP. The distribution of myofibroblasts coinicided with that of fibrosis in each case, but 10 cases with myofibroblast proliferation only in the duct wall showed no fibrosis in the interlobular and periacinar areas. Myofibroblasts that contain stress fibers in their cytoplasm and connect to the extracellular matrix with microtendons participate in tissue contraction [1, 7, 65]. In VPCa cases, myofibroblasts might proliferate in the duct wall against the increase in intraductal pressure due to disturbance of the flow of pancreatic juice. When intraductal pressure rises beyond the limitation of compensation by the contractile force of proliferating myofibroblasts, pancreatitis may occur [24].

Pancreatic Carcinoma

At the stenotic portion of the main pancreatic duct, where carcinoma cells invaded into the duct wall, no myofibroblast proliferation was observed in any cases, whereas myofibroblasts proliferated in duct walls upstream from the stenotic portions, accompanied by inter- and intralobular fibrosis and acinar atrophy in various degrees.

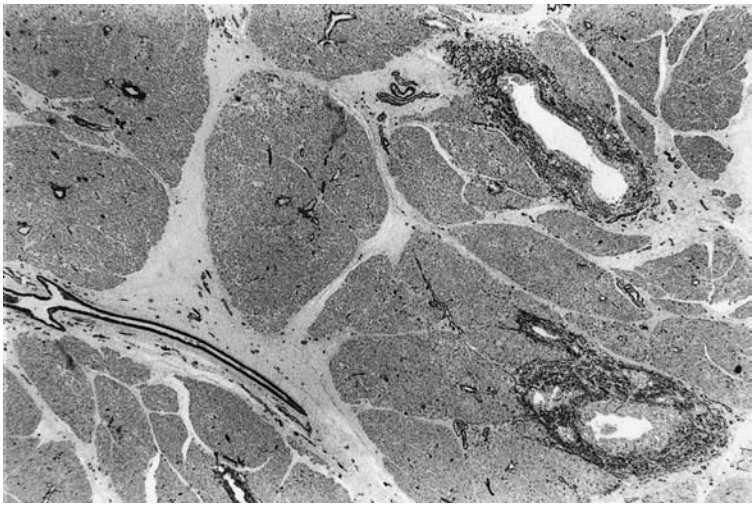

Fig. 7. Myofibroblasts, α-SMA-positive cells, proliferating in the duct wall and the periductal area, but not in the interlobular area, of a patient with pancreaticobiliary maljunction. Immunostain for α-SMA. ×12.5. Reproduced with permission [23].

Bile Pancreatitis

Degeneration and disappearance of the pancreatic ductal epithelium, intraluminal aggregation of bacilli, and diffuse interlobular fibrosis were observed in the pancreas of some cases with pancreaticobiliary maljunction [66]. Therefore, direct and/or indirect activation of pancreatic enzymes by infected bile is considered to be the mechanism of damage to acinar cells [67, 68]. In our cases with pancreaticobiliary maljunction, fibrosis was observed in the interlobular and periductal spaces, while myofibroblast proliferation was observed in the duct wall and periductal space (fig. 7). Our results indicate that apoptosis [17]or transformation into α-SMA-negative cells might occur in proliferating myofibroblasts in the interlobular space, but on the other hand fibrosis might persist in the periductal space in bile pancreatitis due to pancreaticobiliary maljunction.

Concluding Remarks

Many studies on myofibroblasts in the pancreas have clarified that these cells play important roles in the pathogenesis of pancreatitis. Analysis of myofibroblast proliferation patterns in the pancreas can help our understanding of the mechanisms of various pancreatic diseases. Our results suggest that proliferation of myofibroblasts in the duct wall might represent a wound healing process of

ductal injury or serve to prevent the elevation of intraductal pressure, while their proliferation in the parenchyma might be related to the inflammatory process of the parenchyma in various pathologic situations. Finally, how myofibroblasts activate and become to be inactive cells remains to be clarified.

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Distribution, Pathogenesis and Progression of Human Pancreatic Fibrosis

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Abstract

Fibrosis of the pancreas is one of the representative histopathologic findings in cases of chronic pancreatitis. Pancreatic fibrosis is classified into interlobular and intralobular types. In chronic alcoholic pancreatitis cases, fibrosis is mainly found in the interlobular or perilobular areas in the form of nodular pancreatitis or cirrhosis-like pancreatitis. As for the mechanism of interlobular fibrosis, incomplete obstruction of the pancreatic duct and the appearance of cells expressing α-smooth muscle actin, which is a marker for myofibroblasts, play an important role. On the other hand, intralobular fibrosis should be considered or designated as so-called pancreatic fibrosis, but not as chronic pancreatitis. In cases of chronic alcohol abuse, alcohol intake is shown to have an effect in the initial stage of periacinar collagenization through the activation of myofibroblasts and severe damage to acinar cells. Progression of fibrosis occurs due to both pancreatic duct obstruction and interlobular fibrosis admixed with myofibroblast proliferation. Therefore, myofibroblasts play an important role in both the mechanism and progression of pancreatic fibrosis.

Fibrosis of the pancreas is one of the representative histopathologic findings in cases of chronic pancreatitis. Pancreatic fibrosis is attributable to various causes, such as alcohol abuse [1], pancreatic duct obstruction [2], biliary diseases [3], acute pancreatitis [4], liver cirrhosis [5], hemochromatosis [6], ischemia [7] and unknown etiology, and presents in various degrees and distribution patterns.

In this chapter, the distribution of pancreatic fibrosis and its pathogenesis and progression are described.

Table 1. Distribution of pancreatic fibrosis corresponds with individual causes

Cause	Distribution of pancreatic fibrosis	Disease
Alcohol	interlobular or perilobular area	CAP
	intralobular or periacinar area	PS, ADS
Duct obstruction	inter- and intralobular area	COP, AIP, DP
Biliary disease	interlobular and periductal areas	BP
Acute pancreatitis	surrounding area of necrosis and interlobular area	Acute pancreatitis
Hemosiderin	intralobular and periinsular areas	Hemochromatosis
Ischemia	focal intralobular area	DIC

ADS = Alcoholic dependence syndrome; AIP = autoimmune pancreatitis; BP = biliary pancreatitis; CAP = chronic alcoholic pancreatitis; COP = chronic obstructive pancreatitis; DIC = disseminated intravascular coagulation; DP = dorsal pancreatitis in patients with pancreas divisum; PS = so-called pancreatic fibrosis.

Distribution of Pancreatic Fibrosis

The distribution of fibrosis mostly corresponds to or is based on individual causes, as shown in table 1. Pancreatic fibrosis or chronic pancreatitis due to pancreatic duct obstruction is known as chronic obstructive pancreatitis (COP), which is characterized by both inter- and intralobular fibrosis and lobular/acinar atrophy. Autoimmune pancreatitis (AIP), recently a focus of attention, shows a similar fibrosis pattern to COP, except that it is accompanied by marked lymphoplasmacytic infiltration [8]. Fibrosis due to biliary diseases such as gallstones or choledochal cysts is distributed in the interlobular and periductal areas [9]. Fibrosis after acute pancreatitis includes surrounding areas of both necrosis and lobules; the latter is called perilobular fibrosis [4]. Fibrosis in hemochromatosis or severe hemosiderosis is found in diffuse intralobular and periinsular areas [6]. However, as for chronic alcohol abuse, which is the most common cause of pancreatic fibrosis, it remains unclear, in terms of the fibrosis pattern, whether all types of pancreatic fibrosis in chronic alcohol abuse patients can be categorized as chronic alcoholic pancreatitis. According to Martin [10], there are at least three types of fibroatrophic states found in the pancreas: (1) Predominantly intralobular sclerosis (IS), which is always homogenous and diffuse (fig. 1); (2) predominantly perilobular sclerosis (PS), which presents with a 'cirrhosis-like' appearance but is irregular and sometimes patchy (fig. 2), and (3) mixed IS and PS (MS), which is often homogeneously distributed in the pancreas (fig. 3). According to our previous study [1], the pancreatic fibrosis associated with alcohol abuse can show any of Martin's

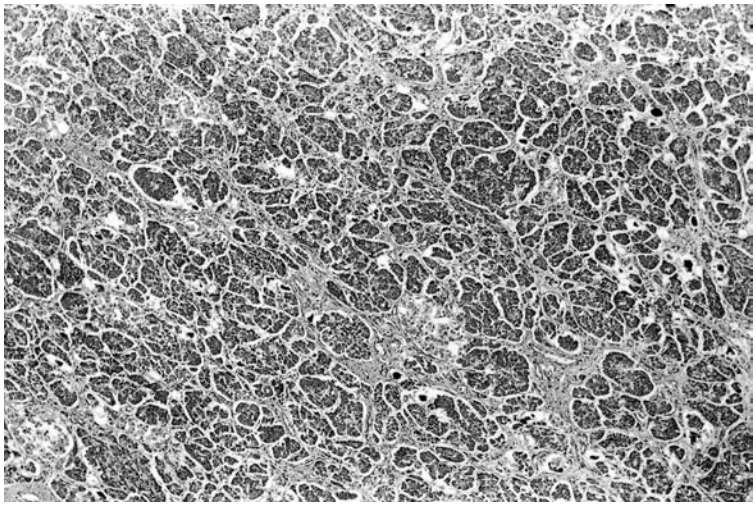

Fig. 1. Intralobular sclerosis. Moderately and diffusely distributed intralobular fibrosis in a 53-year-old man with alcoholic dependence syndrome. HE. ×40.

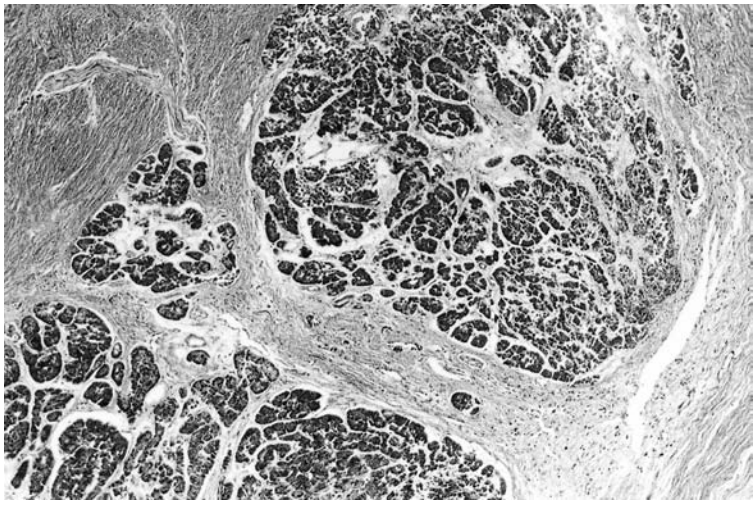

Fig. 2. Perilobular sclerosis. Marked and irregular fibrosis distributed mainly in the perilobular or interlobular areas in a 43-year-old man with alcoholic dependence syndrome. HE. ×40.

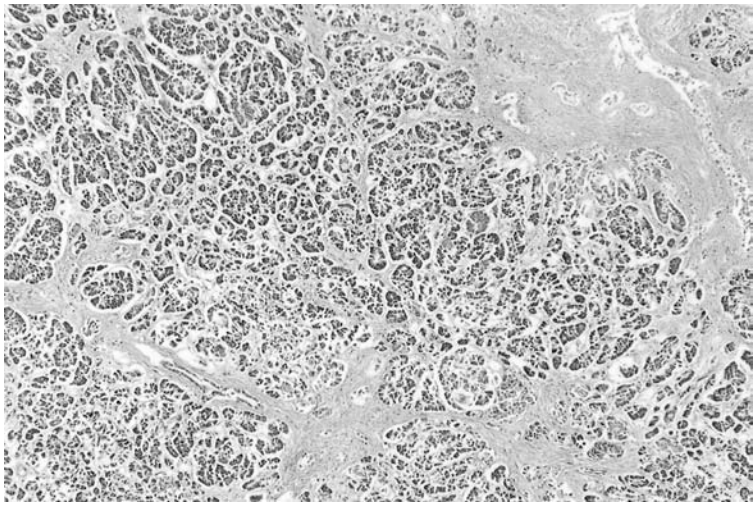

Fig. 3. Mixed intralobular and perilobular sclerosis. Moderately and diffusely distributed mixed intralobular and perilobular or interlobular fibrosis in a 43-year-old man with alcoholic dependence syndrome. HE. ×40.

Table 2. Distribution of types of pancreatic fibrosis in chronic alcohol abuse (94 cases) and clinically diagnosed chronic alcoholic pancreatitis (30 cases)

	Cases	Pancreatic fibrosis	Predominantly intralobular sclerosis	Predominantly perilobular sclerosis	Mixed intralobular and perilobular sclerosis
Alcohol abuse	94	50	16	17	17
Chronic alcoholic pancreatitis*	30	30	0	30	0

*Clinically diagnosed cases.

classification patterns, and chronic alcoholic pancreatitis can be identified by the presence of predominantly perilobular sclerosis, while alcoholic dependence syndrome mainly shows predominantly intralobular and mixed intralobular and perilobular sclerosis, as shown in table 2. Moreover, we think that MS should be included in the IS category, because fibrosis of the MS type is seen mainly in the intralobular or periacinar areas and is uniformly distributed.

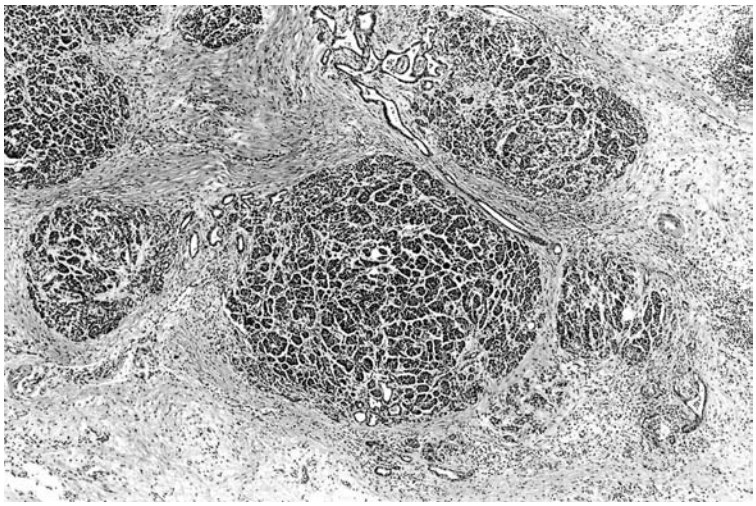

Fig. 4. In a patient with chronic alcoholic pancreatitis, fibrosis was found in the interlobular area, admixing with a 'nodular pancreatitis' pattern. HE. ×25.

Based on the distribution of fibrosis, perilobular, interlobular, or intralobular, and the difference in various components and accompanying diseases such as liver cirrhosis, pancreatic fibrosis can be classified into two distinct pathogenic entities which occur via different mechanisms [11]. Therefore, pancreatic fibrosis can be classified into interlobular and intralobular types; the former is identified with chronic alcoholic pancreatitis, while the latter should be designated as so-called pancreatic fibrosis.

Mechanism of Interlobular Fibrosis of the Pancreas

The most commonly cited theory for the cause of chronic alcoholic pancreatitis is the deposition of protein plugs which later calcify, leading to duct obstruction with subsequent fibrotic replacement of the acinar tissue upstream from the occlusion [12]. However, duct obstruction is considered to be an essential mechanism for fibrosis in chronic obstructive pancreatitis distal to a stricture of the pancreatic duct. Chronic obstructive pancreatitis is observed secondary to slow-growing pancreatic carcinomas, ampullary carcinomas, odditis, and pancreatic duct scars [13]. According to our previous study [2], in chronic alcoholic pancreatitis cases fibrosis is mainly distributed in the interlobular or perilobular areas with a nodular pancreatitis pattern (fig. 4), whereas

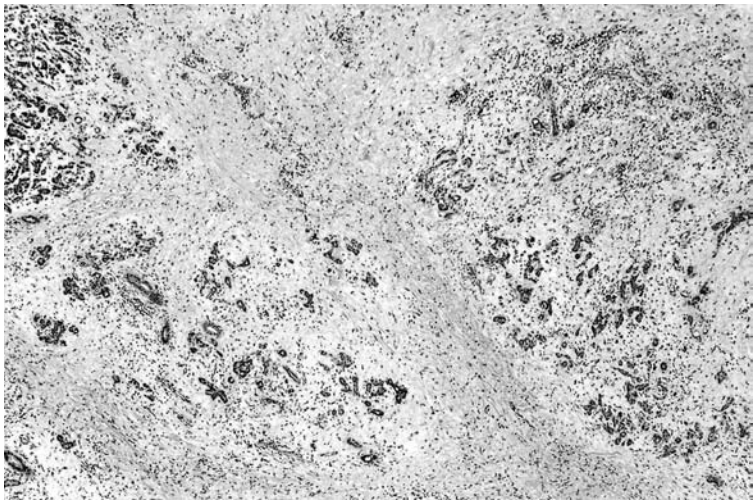

Fig. 5. Chronic obstructive pancreatitis in a patient with pancreas head carcinoma showed inter- and intralobular fibrosis with acinar atrophy. HE. ×50.

in pancreatic head carcinomas, which cause complete obstruction of the main pancreatic duct, fibrosis is found in the inter- and intralobular areas with the lobular/acinar atrophy mentioned above (fig. 5).

Recently, we investigated the mechanism of interlobular fibrosis of the pancreas based on histological changes in the pancreatic tissue in ampullary carcinoma patients with various degrees of stricture in the main pancreatic duct [14]. The results were as follows: incomplete obstruction of the main pancreatic duct caused diffuse mild interlobular fibrosis and an expansive lobular appearance (fig. 6), which may ultimately lead to a nodular lobular pattern. These findings are similar to those of chronic alcoholic pancreatitis, except for excessive fibrosis with patchy distribution. In addition, such fibrosis is accompanied by the appearance of cells with anti-α-smooth muscle actin (α-SMA) immunoreactivity (fig. 7), which is a marker for myofibroblasts [15, 16]. It is well known that myofibroblasts play an important role not only in wound healing, but also in collagenization, which is followed by fibrosis in many organs [17–20]. Moreover, Tanaka et al. [21] demonstrated an experimental canine model of chronic pancreatitis which showed histological changes similar to those of human chronic alcoholic pancreatitis by combining alcohol administration with incomplete pancreatic duct obstruction. Therefore, such incomplete obstruction of the pancreatic duct plays an important role at the onset of interlobular fibrosis, which is categorized as chronic alcoholic pancreatitis [14].

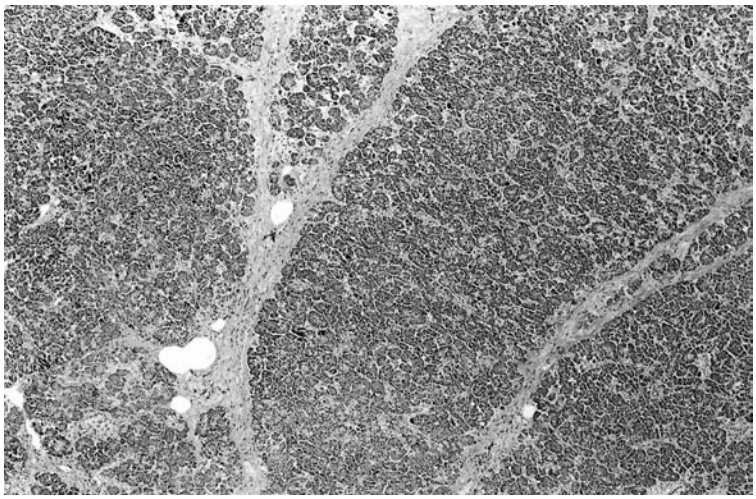

Fig. 6. Mild fibrosis distributed in the interlobular area, with a rather expansive lobular appearance, in a patient with an ampullary carcinoma. HE. ×50. Reproduced with permission [40].

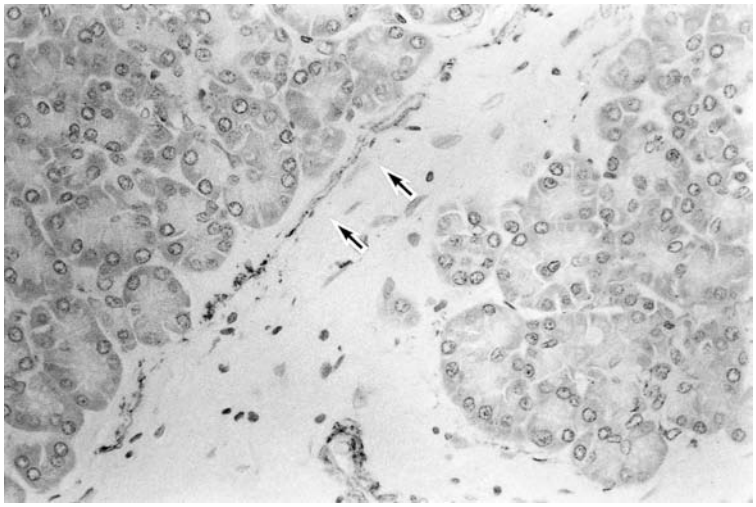

Fig. 7. Anti-α-smooth muscle actin immunoreactivity in the same patient as in figure 6 shows very scant distribution (arrows) in the interlobular area. ×400. Reproduced with permission [40].

Mechanism of Intralobular, or Periacinar, Fibrosis of the Pancreas and Differences to Chronic Alcoholic Pancreatitis

Recently, the identification of pancreatic stellate cells (PSCs) was reported by Bachem et al. [22, 23], and these cells, also known as vitamin A-storing cells, that were isolated from the pancreas were shown to differentiate into myofibroblast-like cells expressing α-SMA and producing collagen type I and III, laminin, and fibronectin. Most of these studies, however, were performed using animal models, such as WBN/Kob rats [24, 25], Otsuka Long Evans Tokushima Fatty (OLETF) rats [26] and Aly mice [27], which are known to develop pancreatitis spontaneously, as well as experimental pancreatitis induced by ethanol feeding [28], arginine injection [29] or trinitrobenzene sulforic acid (TNBS) infusion [30] in rats or mice. These animal model studies and a cell-culture study used activated periacinar, not perilobular, PSCs in fibrogenesis [31–33] that has histological features different from those of chronic pancreatitis in humans [34].

According to Kuroda et al. [35], the myofibroblasts play an important role in pancreatic fibrosis in alcoholics. Based on their diameter and location, the fine filaments (8–15 nm in diameter) presenting between the myofibroblasts and collagen fibrils are considered to probably be collagen filaments in nature (fig. 8). The presence of collagen filaments around the myofibroblasts may indicate that myofibroblasts produce the collagen filaments and fibers. Hence, they suggest that alcohol has an effect on the initial stage of periacinar collagenization in intralobular fibrosis via the activation of myofibroblasts.

Prolyl hydroxylase (PH), located in microsomes, is an enzyme that hydroxylates peptide-bound proline in the process of collagen biosynthesis [36]. Our previous study [37] showed that the immunoreactivity of PH in the pancreas is mainly localized in the acinar cells (fig. 9), and seems to play a role in pancreatic fibrosis. Moreover, Kuroda et al. [35] also notes that protein transport blockage, which is represented by leakage of the contents of zymogen granules, rough endoplasmic reticulum and lysosomes to the cytoplasm and around acinar cells, is preceded by alcohol intake and may contribute to severe damage to acinar cells and the development of periacinar collagenization (fig. 10).

Progression of Pancreatic Fibrosis

There are no precise morphological tools or studies of fibrosis patterns to distinguish between more progressive and steady states in chronic pancreatitis cases. In the end stage, chronic pancreatitis is represented by massive or extensive interlobular fibrosis and total permanent loss of the exocrine pancreatic

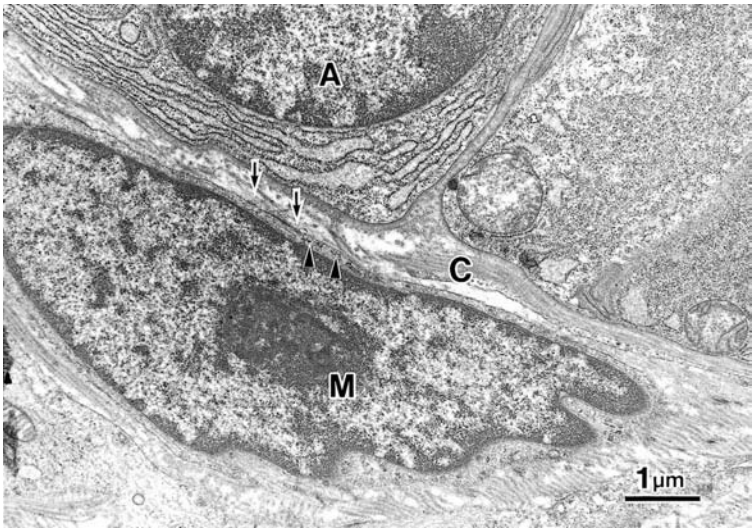

Fig. 8. Collagen fibrils between a myofibroblast and an acinar cell in a 73-year-old male with chronic alcoholism. Note the presence of many fine filaments (arrows), 8–15 nm in diameter, between the myofibroblast (M) and collagen fibrils (C), and many micropinocytic vesicles (arrowheads) in the cytoplasmic surface of the myofibroblast. A = Acinar cell. Uranyl acetate, lead citrate and tannic acid stain.

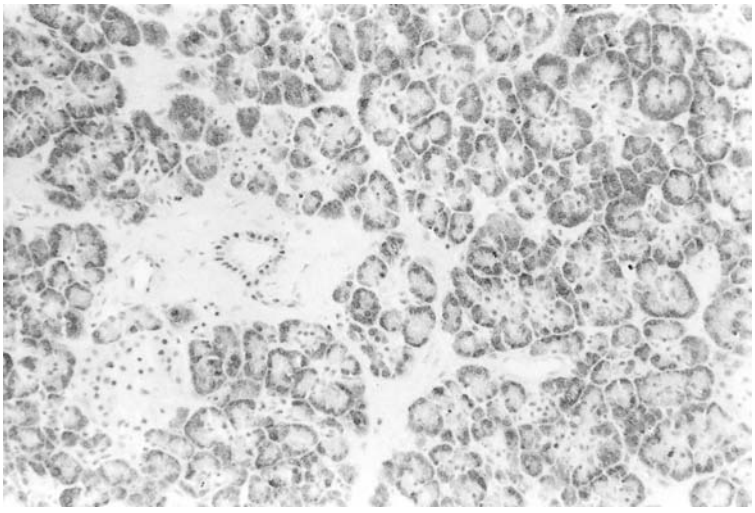

Fig. 9. Immunoreactivity against anti-prolyl hydroxylase was localized mainly in the acinar cells of the pancreas. ×200.

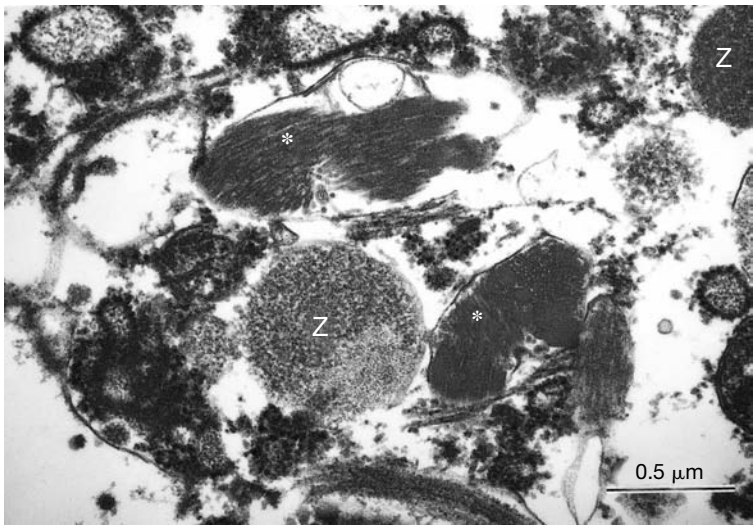

Fig. 10. Leakage of filamentous material (asterisk) via the disrupted limiting membrane of zymogen granules (Z) in a 53-year-old male with chronic alcoholism. Uranyl acetate, lead citrate and tannic acid stain.

tissue, which are frequently found in chronic alcoholic pancreatitis and chronic obstructive pancreatitis. On the other hand, mild cases show both periductal and mild interlobular fibrosis in patients with congenital biliary dilatation, which causes biliary or bile pancreatitis. According to Klöppel et al. [38], biliary pancreatitis is not likely to evolve into chronic pancreatitis. We investigated the difference between progressive pancreatitis and non-progressive, or steady, pancreatitis, and found that the pathologic states and findings common to chronic alcoholic pancreatitis and chronic obstructive pancreatitis are duct obstruction, as mentioned above, and interlobular or perilobular fibrosis, although in chronic obstructive pancreatitis cases fibrosis was also distributed in the intralobular areas. In the latter, interlobular fibrosis was also found in patients with congenital biliary dilatation. In our previous study [9], interlobular fibrosis in patients with congenital biliary dilatation was mostly immunonegative for α-SMA in pancreatoduodenectomized (PD) materials (patients' mean age 35.7 years) (fig. 11). In biopsied cases (patients' mean age 5.0 years), however, α-SMA immunoreactivity was observed in the interlobular fibrotic area in two of three cases, and was also found in the periductal area in all PD materials. Hence, such α-SMA immunoreactivity has occurred at least once in the interlobular area in patients with congenital biliary dilatation and may have subsequently changed in nature. The transient nature of α-SMA

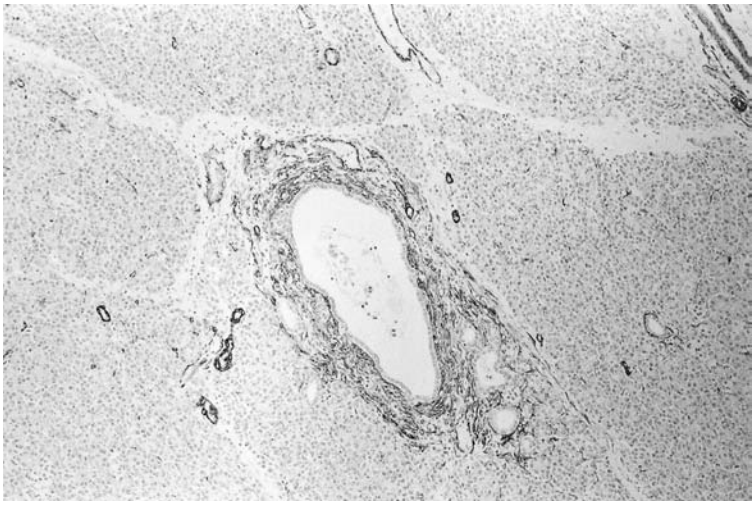

Fig. 11. Interlobular fibrosis was immunonegative for α-SMA, while periductal fibrosis was positive for α-SMA, in a patient with congenital biliary dilatation. ×50.

immunoreactivity may be related not only to the healing of inflammatory injuries, but also to apoptosis [15]. On the other hand, periductal, but not interlobular, α-SMA immunoreactivity which is accompanied by bcl-2 immunoreactivity is considered to be due to reflux of the bile duct contents, resulting in a continuously progressive state of fibrosis. According to Burton et al. [39], the ductal expression of α-SMA decreases in chronic pancreatitis, whereas septal and lobular expression dramatically increases. Hence, α-SMA-negative interlobular fibrosis in patients with congenital biliary dilatation may not show any apparent progression.

Therefore, duct obstruction and interlobular fibrosis admixed with myofibroblast proliferation, as seen in chronic alcoholic pancreatitis and chronic obstructive pancreatitis, are identified as markers or hallmarks of progression in chronic pancreatitis.

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Electron-Microscopic Aspect of Pancreatic Fibrosis: Pancreatic Periacinar Collagenization at the Initial Stage

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Abstract

The exact mechanism of pancreatic fibrosis has not yet been elucidated. We have proposed the following three classifications related to the pathogenesis of pancreatic fibrosis: predominantly perilobular sclerosis in chronic alcoholic pancreatitis, predominantly intralobular sclerosis in alcoholic dependence syndrome, and mixed intralobular and perilobular sclerosis in both obstructive pancreatitis and chronic alcoholism. To examine the development of pancreatic fibrosis, periacinar collagenization at the initial stage of periacinar fibrosis was investigated in patients with chronic alcoholism by electron microscope. Myofibroblasts were found around acini at the initial stage of periacinar fibrosis, and were accompanied by numerous fine filaments 8–15 nm in diameter. In the acinar cells at the initial stage of periacinar fibrosis, various changes in zymogen granules (ZG), lysosomes and lipid droplets were augmented. Mucin-like medium-dense materials were also found in dilated rough endoplasmic reticulum (RER). The contents of ZG and RER occasionally leaked out. The above-mentioned electron-microscopic findings reveal that myofibroblasts play an important role at the initial stage of periacinar collagenization, and that the intracellular transport blockage of protein represented by abnormalities of ZG, ER and lysosomes may contribute to the development of periacinar collagenization. In another of our studies, prolyl hydroxylase was demonstrated in acinar cells immunohistochemically. Furthermore, the intracytoplasmic filaments in close proximity to the degenerative zymogen granules, lysosomes or basal lamina were also been investigated, and were found to be caused by alcohol-enhanced metabolic injury to the acinar cells. It may be confirmed in the near future that the acinar cells themselves participate in periacinar collagenization. Furthermore, our subsequent investigations suggest that the distribution of myofibroblasts in the periacinar and perilobular areas will provide a valuable clue to resolving the mechanism of the three different types of pancreatic fibrosis related to pathogenesis.

Pancreatic fibrosis is one of the representative histopathologic findings in cases of chronic pancreatitis. According to Martin's classification, there are three types: predominantly intralobular sclerosis, predominantly perilobular sclerosis, and mixed intralobular and perilobular sclerosis [1]. We have proposed that this classification is related to the pathogenesis of pancreatic fibrosis. Our previous studies showed that (a) predominantly perilobular sclerosis was found in patients with chronic alcoholic pancreatitis, (b) predominantly intralobular sclerosis mainly in patients with alcoholic dependence syndrome or chronic alcoholism, and (c) mixed intralobular and perilobular sclerosis in both obstructive pancreatitis patients [2] and chronic alcoholism patients [3, 4]. Several other investigators have also discussed the pathogenesis and development of pancreatic fibrosis from a pathological point of view [5–9]. However, the mechanism of human pancreatic fibrosis remains unclear, especially in chronic alcoholism.

To investigate the mechanism of intralobular sclerosis, we focused on the myofibroblasts around the acini as periacinar mesenchymal cells [10, 11]. At that time, the existence of myofibroblasts in the pancreas was not generally acknowledged. The term 'myofibroblast' was introduced by Majno et al. to define the contractile spindle cells in granulation tissue [12], and the electron-microscopic characteristics of both fibroblasts and smooth muscle cells were demonstrated in this cell by Gabbiani et al. [13–15]. Nowadays, it is well known that myofibroblasts play an important role not only in healing, but also in collagenization, which is followed in many organs by fibrosis [16–18]. The role of myofibroblasts in fibrosis has been clarified not only by morphology, but also by biochemistry [19–21]. Many investigators, including Gabbiani and colleagues, have clarified myofibroblast differentiation, the transformation from fibroblasts to proto- or differentiated myofibroblasts with biological changes in variant ED-A fibronectin and TGF-β, the synthesis of α-smooth muscle actin (α-SMA) and stimulation of collagen type I production [22, 23]. Myofibroblasts have been observed in many normal tissues, such as the terminal alveoli of the lungs, the interstitium of the renal cortex and the duodenal villi [18, 24, 25].

In this chapter, electron-microscopic changes in both mesenchymal cells neighboring the acini and zymogen granules at the initial stage of intralobular, or periacinar, fibrosis are briefly reviewed based on previous and current reports by ourselves and others.

Source Materials

To examine the development of pancreatic fibrosis in alcoholics, we investigated by electron microscope the periacinar collagenization at the initial stage of periacinar fibrosis using pancreatic tissues from pancreatoduodenectomized

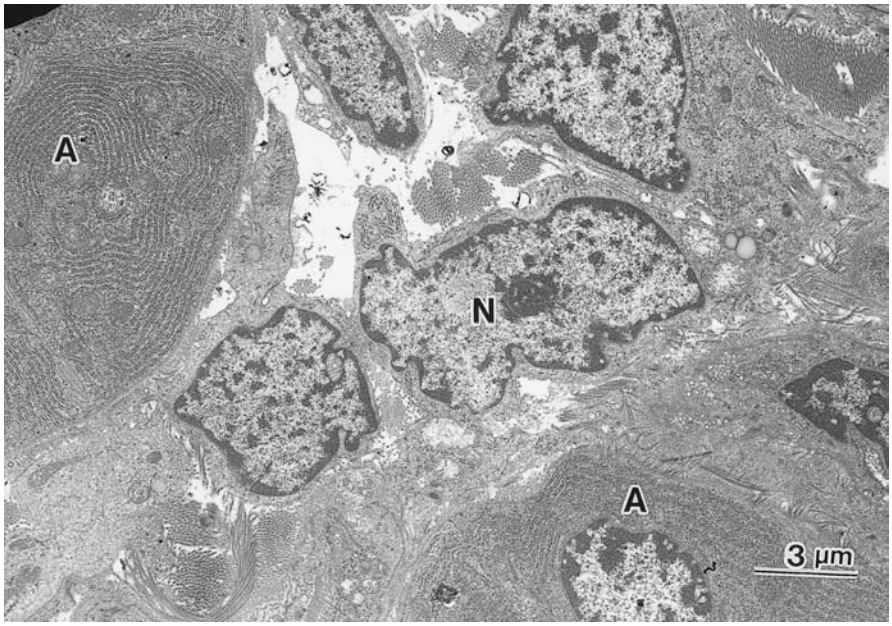


Fig. 1. Periacinar myofibroblasts neighboring the acini (A) at the stage of very slight microscopic peri-acinar collagenization in a 72-year-old male without chronic alcoholism. Note the distinctly folded nuclei (N) of the myofibroblasts. Uranyl acetate, lead citrate and tannic acid stain. From [10] with permission.

patients with common bile duct carcinoma with and without chronic alcoholism, and from heavy drinkers with chronic alcoholism.

Mesenchymal Cells Neighboring the Acini

Myofibroblasts and Fibroblasts

Mesenchymal cells neighboring the acini were morphologically classified into two broad types: myofibroblasts and fibroblasts. Myofibroblasts often had a folded nucleus and many micropinocytic vesicles (fig. 1). Organelles such as the Golgi complex and the endoplasmic reticulum were developed to various degrees. Intracytoplasmic filaments with focal densities and plasmalemmal densities were often found. Occasionally, basal-lamina-like fragments were formed. In contrast, the fibroblasts had few micropinocytic vesicles, no intracytoplasmic filaments, and no basal lamina. At the advanced stage of periacinar

Table 1. The relation between periacinar collagenization and myofibroblasts and fibroblasts

Stage	Periacinar collagenization	Mesenchymal cells	
		Myofibroblasts	Fibroblasts
I	None	−	−
II	Very slight	+	−
III	Slight	+	+

collagenization, the myofibroblasts possessed well-developed endoplasmic reticula and Golgi complexes, and were similar to developed fibroblasts.

The ratio of these two types of mesenchymal cells was different at each stage of the initial periacinar collagenization, which was divided into three stages (table 1). At the stage of no microscopic periacinar collagenization (stage I), no or few myofibroblasts or fibroblasts were found around the acini. Some pericytes and a few collagen fibrils were observed in the interstitium. The morphology of pericytes was often similar to that of myofibroblasts, but they had much more micropinocytic vesicles than myofibroblasts, and had complete basal lamina, like the endothelia of small vessels. When the microscopic periacinar collagenization was very slight (stage II), the myofibroblasts were the only mesenchymal cells neighboring the acini. Numerous fine filaments, 8–15 nm in diameter, were found between the myofibroblasts and collagen fibrils (fig. 2). At the stage of slight microscopic periacinar collagenization (stage III), in which a few collagen fibers were found around the acini, both myofibroblasts and fibroblasts were more spindle-shaped and interlaced with many collagen fibrils close to the basal lamina of the acinar cells. It was clarified that myofibroblasts preceded fibroblasts as mesenchymal cells neighboring the acini, and that the morphological changes of periacinar mesenchymal cells were apparently correlated with the volume of collagen fibers at the initial stage of periacinar collagenization, regardless of alcohol intake.

Many investigators have also morphologically and immunohistochemically demonstrated the characteristics of the mesenchymal cells neighboring the acinar cells using cells isolated and cultured in vitro. The presence of myofibroblasts in the pancreas is now widely accepted [26–28].

Role of Periacinar Myofibroblasts

Our electron-microscope investigation showed the important role of myofibroblasts in pancreatic fibrosis. The fine filaments (8–15 nm in diameter)

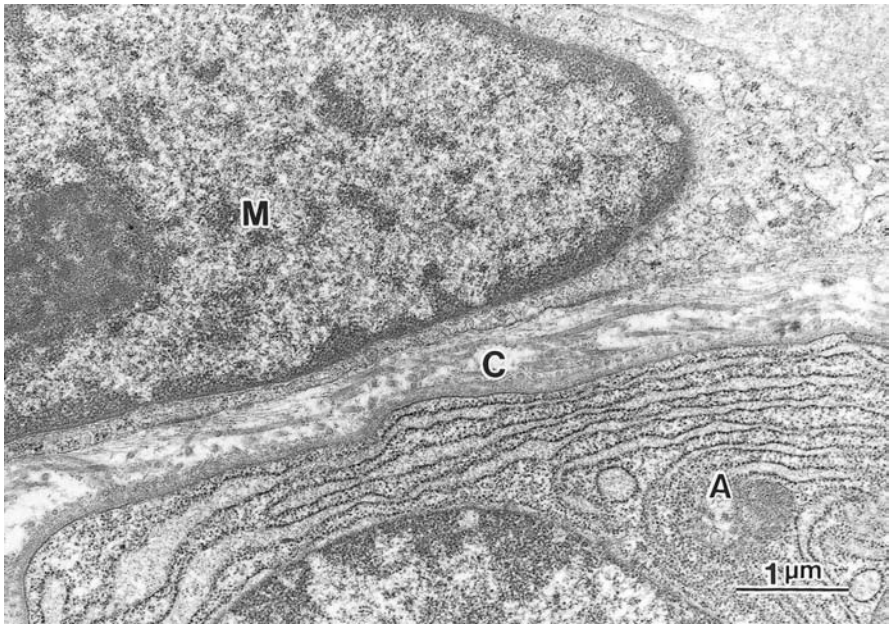


Fig. 2. Collagen fibrils between a myofibroblast (M) and an acinar cell (A) from a 73-year-old male with chronic alcoholism. Note the presence of many fine filaments, which measure 8–15 nm in diameter, between the myofibroblast (M) and collagen fibrils (C), and many micropinocytic vesicles in the cytoplasmic surface of the myofibroblast. Uranyl acetate, lead citrate and tannic acid stain. From [10] with permission.

between myofibroblasts and collagen fibrils are considered to be collagen filaments, based on their diameter and location. The presence of collagen filaments around the myofibroblasts might indicate that myofibroblasts produce collagen filaments and fibers. Our investigation also revealed that myofibroblasts, not fibroblasts, play an important role as periacinar mesenchymal cells, and contribute strongly to the initial stage of periacinar collagenization. At that time, the existence of myofibroblasts in pancreas was not acknowledged by many investigators, but Apte et al. successfully isolated, from rat pancreatic tissue, stellate shaped cells which transformed into myofibroblast-like cells [27]. Based on the results of our investigation, it was suggested that alcohol has an effect on the initial stage of periacinar collagenization in intralobular sclerosis through the activation of myofibroblasts. The myofibroblasts around the acini were sometimes difficult to distinguish morphologically from pericytes or the endothelium in neoformed small vessels. Morphological similarities between myofibroblasts and fibroblasts were also observed at the stage preceding periacinar collagenization. This is inevitable because these cells were considered to be derived from the same stem cells [28].

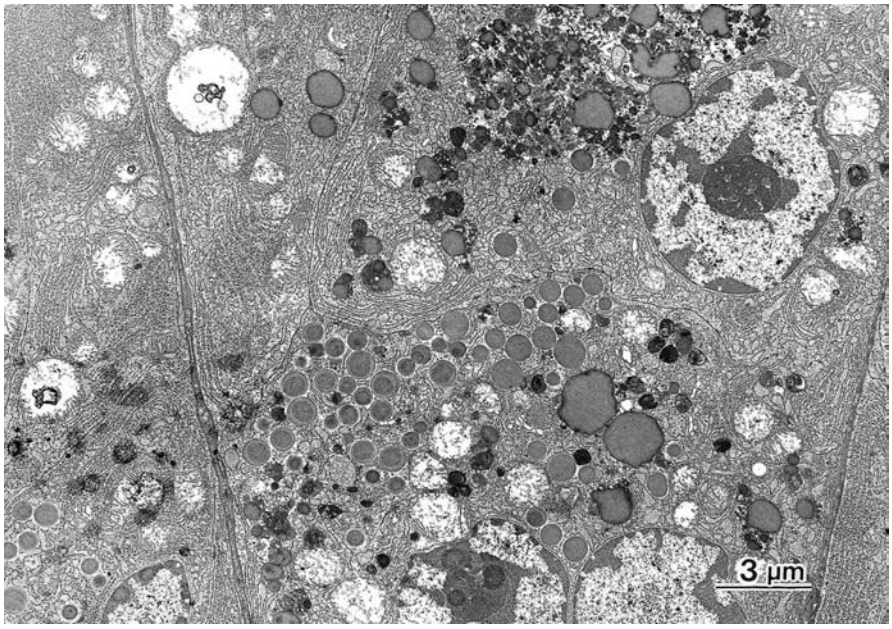


Fig. 3. Degenerative acinar cells from a 59-year-old male with chronic alcoholism. Note the presence of many lysosomes and lipid droplets in the acinar cells. Uranyl acetate, lead citrate and tannic acid stain. From [10] with permission.

It was also reported from a histological investigation using α-SMA immunoreacitivity that the incomplete obstruction of the main pancreatic duct caused the onset of interlobular fibrosis [29]. The distribution of myofibroblasts in both periacinar and perilobular areas might provide a clue to resolve the mechanism of the three different types of pancreatic fibrosis in terms of pathogenesis.

Acinar Cells

Abnormalities Related to Alcohol Intake

At the initial stage of periacinar collagenization, abnormalities related to alcohol intake were found in zymogen granules, lysosomes, lipid droplets and the endoplasmic reticulum. A decreased number of zymogen granules and electron-dense zymogen granules were found in patients with chronic alcoholism. Decreased electron-dense zymogen granules were sometimes seen with peripheral halos and centric or eccentric cores (fig. 3). An increased number of lysosomes was found in patients with chronic alcoholism. Lysosomes were

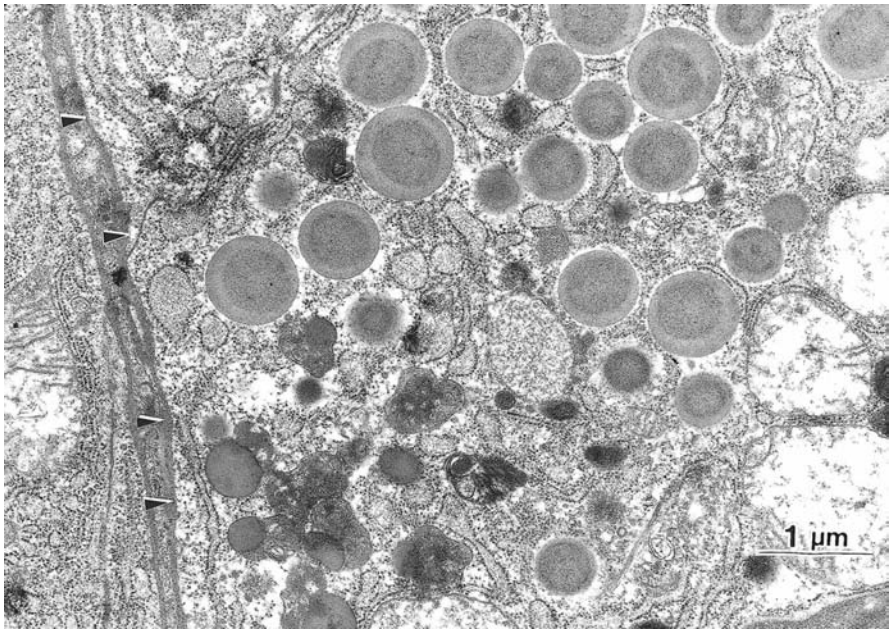


Fig. 4. Higher-powered view of the acinar cell shown in figure 3. Note the presence of many lysosomes and degenerative zymogen granules close to the basal lamina (arrowheads). Some zymogen granules form peripheral halos and reduced the electron-dense contents. Lipid droplets are also found in secondary lysosomes. Uranyl acetate, lead citrate and tannic acid stain. From [10] with permission.

derived both from the Golgi apparatus and from degenerative zymogen granules (fig. 4). Some lysosomes consisted of lipid droplets and highly electron-dense granules, which seemed to be lipofuscin. An increased number of lipid droplets was found in patients with chronic alcoholism. Dilatation of the endoplasmic reticulum was also found in patients with chronic alcoholism. In areas where the degeneration was advanced, dilatation and vesiculation were often observed in the rough endoplasmic reticulum (RER). The vesicles contained medium electron-dense materials similar to mucin, and were sometimes closely packed near the basal lamina (fig. 5). The basal lamina was sometimes obscured by some degenerative acinar cells. Medium electron-dense materials in dilated cisternae of the RER occasionally leaked out via the damaged membranes (fig. 6).

Very thin Filaments in Chronic Alcoholics

In the tissue samples from patients with chronic alcoholism, in addition to the above-mentioned changes, very thin filaments in zymogen granules were found.

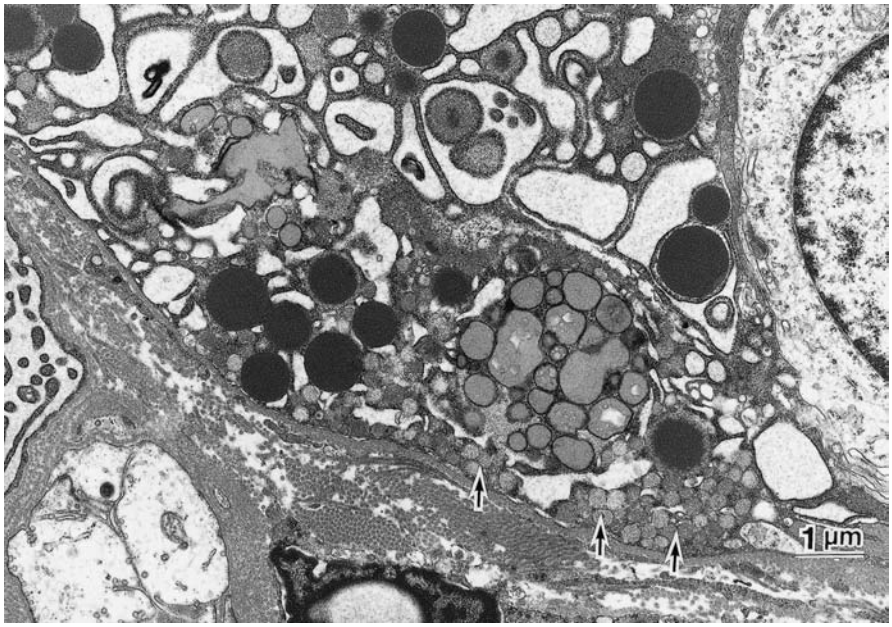
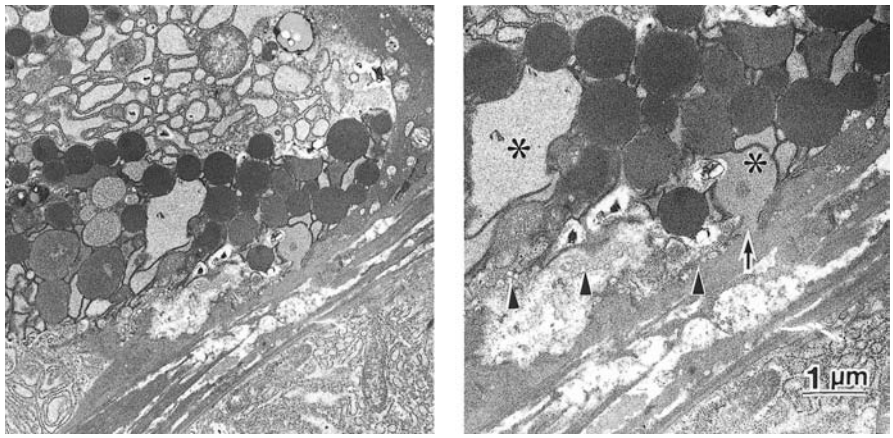


Fig. 5. Degenerative acinar cell from a 58-year-old male with chronic alcoholism. Note the dilation and vesiculation of the RER containing amorphous and medium electron-dense contents similar to mucinous materials. The vesiculated endoplasmic reticulum is sometimes closely packed near the basal lamina (arrows).

These very thin filaments, approximately 3–6 nm in diameter, replaced the osmophilic contents of some zymogen granules. The very thin filaments often spread out to neighboring zymogen granule spaces via a fused limiting membrane (fig. 7). The filamentous structures found near the basal lamina occasionally leaked out from the zymogen granules via the disrupted limiting membrane (fig. 8).

Intracellular Transportation of Materials Related to Periacinar Collagenization

In terms of ultrastructural changes in acinar cells related to alcoholism, various changes were seen in zymogen granules, as well as dilatation and vesiculation of the endoplasmic reticulum containing medium electron-dense material, and an increased number of lysosomes and lipid granules. In normal acinar cells, the secretory proteins are synthesized on ribosomes attached to the membrane of the RER, and the contents of the RER are transported to the Golgi complex where they are condensed and modified. Lysosomes are also transported and



a *b*

Fig. 6. Degenerative acinar cell from the same patient as figure 5. At (***a***), note the group of zymogen granules and dilated RER containing various electron-dense contents. At (***b***) is a higher-powered view of part of the acinar cell shown at (***a***). Note the obscure membranes of some zymogen granules, a markedly dilated RER (asterisks) and the presence of many microvesicles of the endoplasmic reticulum (arrowheads) close to the destroyed basal lamina. The intracisternal contents flowing out via the damaged membrane of the endoplasmic reticulum can be observed (arrow). Uranyl acetate, lead citrate and tannic acid stain. From [10] with permission.

formed in a manner similar to the secretory proteins. Primary lysosomes are thought to produce not only secondary lysosomes, but also zymogen granules. From our studies, various abnormalities in zymogen granules have been reported. Medium electron-dense materials in dilated cisternae of the RER and very thin filaments in the zymogen granules were also found. The abnormalities of zymogen granules, increased lysosomes and lipid droplets were already observed by electron microscope previous to the periacinar fibrosis. Therefore, the Golgi apparatus-endoplasmic reticulum-lysosome (GERL) abnormality and the zymogen granules were considered to disturb the intracellular transportation of various secretory proteins, lipoproteins and lipids and to play an important role in progression towards the advanced stage of periacinar collagenization followed by intralobular fibrosis. The series of changes in GERL and zymogen granules are illustrated in figure 9. The changes in zymogen granules were recognized to have been caused by alcohol-enhanced metabolic injury to acinar cells [5, 30, 31]. Similar changes in zymogen granules, except for the very thin filaments, were previously reported in acute pancreatitis in humans and laboratory animals and in genetic exocrine pancreatic insufficiency syndrome in mice [32, 33]. Medium electron-dense materials in the dilated cisternae of the RER also suggested the blockage of transporting proteins. Normally, the proteina-

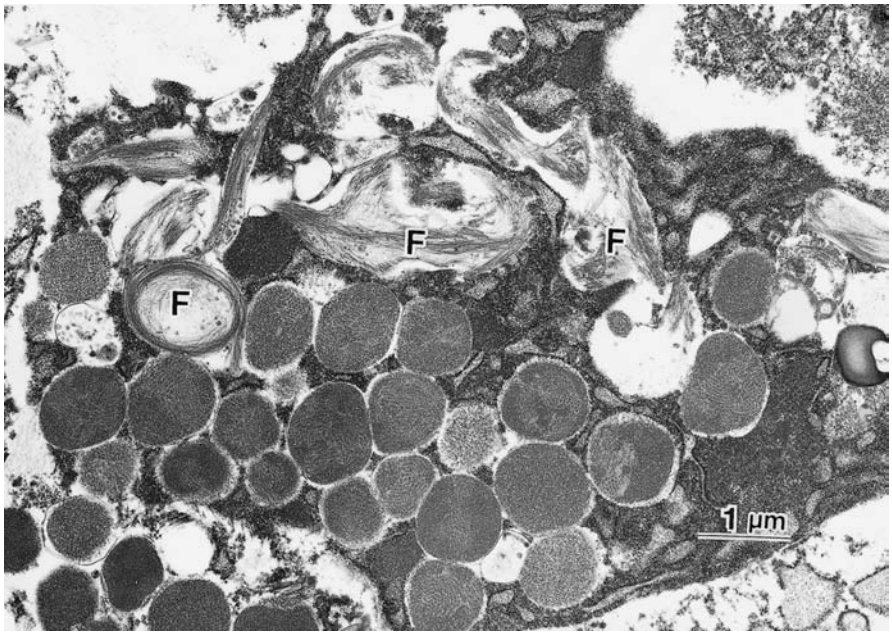


Fig. 7. Degenerative acinar cell from a 52-year-old male with chronic alcoholism. Note the decreased contents of the zymogen granules and the presence of very thin filaments (F), which measure approximately 3–6 nm in diameter, arranged in parallel and sometimes present along the limiting membranes. The thin filaments also occupy the space of the zymogen granules that are without any osmophilic contents. Uranyl acetate, lead citrate and tannic acid stain. From [10] with permission.

ceous substances produced in the RER are transported to the Golgi complex and condensed to form zymogen granules. Zymogen granules contain glycosaminoglycans, mainly heparan sulfate and chondroitin sulfate, which are known to be involved in the mechanism by which collagen fibrils bind these structures together [34, 35]. In addition, heparan sulfate, one of the heparan-related proteoglycans, participates in the differentiation of myofibroblasts [36–39]. In our ongoing study, some filamentous structures of different diameters were identified not only in acinar cells, but also outside them. With reference to the intracytoplasmic process of collagen formation in fibroblasts or the basement membrane formation by epithelial cells, a complex triple helix is formed from an α-chain, a simple amino acid constitution including praline, by hydroxylation of praline with the chains entwining and being transported to the Golgi complex. In the Golgi complex, carbohydrate subunits are synthesized and procollagen molecules are assembled, resulting in tropocollagen formation. Tropocollagen molecules are transported to primary and secondary lisosomes and finally are

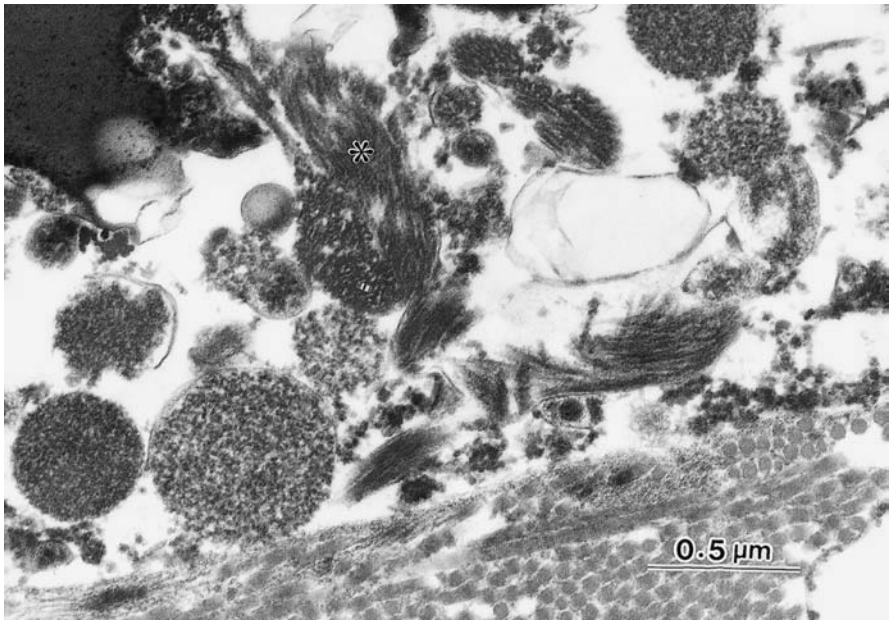


Fig. 8. Leakage of filamentous materials (asterisk) through the disrupted limiting membrane of zymogen granules in a 53-year-old male with chronic alcoholism. Uranyl acetate, lead citrate and tannic acid stain. From [10] with permission.

released from the cell. The morphological changes in a series of filaments in our studies might indicate a similar process to intracytoplasmic collagen formation in fibroblasts or formation of the basement membrane by epithelial cells. Lysosomes are known to play an important role in the turnover of intracellular organelles [39, 40]. Therefore, cell damage, with destruction of the membranes of zymogen granules and GERL, and various filamentous formations, plus the loss of the acinar cell-basal lamina barrier, might enhance periacinar collagenization and lead to intralobular sclerosis. Furthermore, swelling of mitochondria and irregular shaped nuclei with enlarged nucleoli were seen in our studies as abnormalities of organelles in acinar cells. These changes were thought to be related to the severity of the acinar cell damage regardless of alcohol intake. The meaning of these changes is less well understood at present.

The Initial Stage of Periacinar Collagenization

Our electron-microscope investigation revealed that myofibroblasts play an important role at the initial stage of periacinar collagenization [10]. Additionally, the transport blockage of proteins, which was reflected by the leakage of the

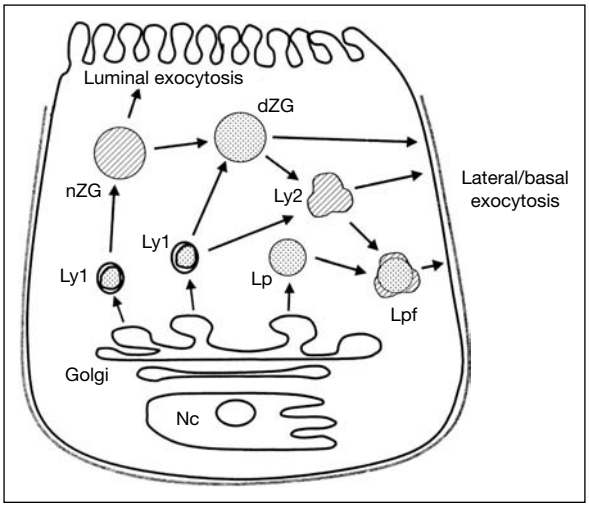


Fig. 9. Transportation of secretory products in the acinar cell. In normal cells, secretory products are condensed and modified in the Golgi complex (Golgi) and transform to small lysosomes and zymogen granules. Normal zymogen granules (nZG) are released by exocytosis into the lumen. In degenerative cells, the electron density of zymogen granules (dZG) is reduced, dilatation and vesiculation of the endoplasmic reticulum containing the amorphous and medium electron dense contents is often observed, and the numbers of lysosomes and lipid droplets (Lp) are increased. Primary lysosomes (Ly1) are fused resulting in secondary lysosomes (Ly2). The lipofuscin (Lpf)-like structure is derived from primary lysosomes and lipid droplets. Degenerative zymogen granules, secondary lysosomes and lipofuscins are extruded from the lateral and basal membranes. Nc = Nucleus. From [10] with permission.

contents of zymogen granules and the rough endoplasmic reticulum to the cytoplasm and around acinar cells, is preceded by alcohol intake, and may contribute to the severe damage to acinar cells and the development of periacinar collagenization. Further studies on the myofibroblasts around the acini and changes in acinar cells, especially the abnormalities in zymogen granules and GERL, will be useful for understanding periacinar collagenization followed by intralobular sclerosis. In another study, we demonstrated prolyl hydroxylase immunohistochemically in acinar cells [41]. Intracytoplasmic filaments up to 7–8 nm in diameter have also been investigated in degenerative acinar cells. The filaments were found in close proximity to the degenerative zymogen granules and lysosomes, and were confirmed to be caused by alcohol-enhanced metabolic injury to the acinar cells [32, 42]. Although it is known that several types of filaments or fibrils have also been found in the acinar cells during the degenerative process in chronic alcoholics, we cannot confirm at present whether or not the intracytoplasmic filaments in the acinar cells are strongly related to periacinar fibrosis. In the future, the relationship between

the intracytoplasmic filaments and each organelle in the acinar cells will be elucidated. The mechanism of periacinar collagenization will be more interesting when it is confirmed that the acinar cells themselves participate in collagenization. The distribution of myofibroblasts in the periacinar and perilobular areas might provide a valuable clue to resolve in the near future the mechanisms of the three different types of pancreatic fibrosis in terms of pathogenesis.

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Paracrine and Autocrine Mechanisms of Pancreatic Fibrosis

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Abstract

Autocrine and paracrine mechanisms play an important role in pancreatic fibrosis. Pancreatic fibrosis is considered to have multiple etiologies, according to the TIGAR-O theory: Toxic-metabolic, Idiopathic, Genetic, Autoimmune, Recurrent and severe acute pancreatitis, and Obstructive pathways. Recently, new concepts have been proposed: the primary duct hypothesis and the sentinel acute pancreatitis event (SAPE) hypothesis. Both are based on new observations of patients and of the fibrogenic mechanism. Stellate cells are located in the periacinar areas and are stimulated to transform into myofibroblasts. Myofibroblasts are characteristic of smooth muscle cells and multifunctional cells. Myofibroblasts express many cytokines, growth factors (especially fibrogenetic) and also their receptors. Thus, myofibroblasts are key players in autocrine mechanisms. In early acute or chronic pancreatitis, acinar cells and ductular cells suffer cellular damage and then express many cytokines, such as interleukin-1, tumor necrosis factor-alpha, interleukin-6, platelet-derived growth factor, and transforming growth factor-beta. These cytokines recruit inflammatory cells such as neutrophils, lymphocytes and macrophages, and also stimulate stellate cells to transform into myofibroblasts. Recruited inflammatory cells express further cytokines to continue inflammation and repair. In the intermediate and late stages, there are inflammatory cells, as well as centroacinar cells, ductular cells, and myofibroblasts in the inflammatory sites of the pancreas. Therefore, the cellular interaction between inflammatory cells, epithelial cells, and myofibroblasts drives the chronic inflammatory process of the pancreas. The histopathology of pancreatic fibrosis is classified as interlobular or intralobular fibrosis. Therefore, the characteristic histopathology of pancreatic fibrosis may be based on complicated cellular interaction caused by multiple etiologies with a final common pathway of fibrogenesis in the pancreas.

Causes of Pancreatic Fibrosis

Pancreatic fibrosis is a disorder associated with loss of pancreatic architecture and progression of fibrosis caused by alcoholism, malnutrition,

metabolic disorders, hereditary diseases such as cystic fibrosis, obstruction of pancreatic ducts, autoimmune diseases, and idiopathic disorders such as retroperitoneal fibrosis. It was recentlyproposed that chronic pancreatitis and fibrosis may be caused by many factors, according to the TIGAR-O theory [1], short for: Toxic and metabolic pathway, Idiopathic pathway, Genetic pathway, Autoimmune pathway, Recurrent and severe acute pancreatitis pathway, and Obstructive pathway. Other hypotheses, such as the primary duct hypothesis and sentinel acute pancreatitis event (SAPE) hypothesis, have also been developed. Many pathways or hypotheses of pancreatic inflammation or fibrogenesis support the concepts of multifaceted regulation of chronic pancreatitis and fibrosis.

TIGAR-O Theory and the New Hypotheses

Toxic and Metabolic Pathway

The most common etiology of chronic pancreatitis involves alcohol. Possible mechanisms of alcohol-related injury include exocrine dysfunction, changes in lipid metabolism, and induction of oxidative stress [2]. However, because pancreatic acinar cells are capable of metabolizing alcohol, it is not proven that alcohol can initiate chronic pancreatitis and/or fibrosis. Oxidative stress may exacerbate chronic inflammation.

Idiopathic Pathway

Some patients with chronic pancreatitis have no clear risk factors. However, patients with the idiopathic type may have unknown genetic alterations.

Genetic Pathway

Hereditary pancreatitis (cationic trypsinogen mutation), cystic fibrosis (CFTR mutations), serine proteinase inhibitor Kazal type 1 (SPINK-1) mutations, and alpha1-antitrypsin deficiency have been found [3].

Autoimmune Pathway

Autoimmune chronic pancreatitis (AIP) is a rare condition. The entity known as non-alcoholic duct-destructive chronic pancreatitis may actually represent AIP. AIP may be associated with other autoimmune diseases, such as Sjögren's syndrome, primary sclerosing cholangitis, and inflammatory bowel disease. Recently, a characteristic high concentration of serum IgG4 was found in patients with sclerosing pancreatitis. Histology reveals lymphoplasmacytic infiltration in periductal non-occlusive fibrosis in the pancreatic tissue, and

immunohistochemistry a predominant infiltration of CD4+ or CD8+ T cells and IgG4+ plasma cells [4, 5].

Recurrent and Severe Acute Pancreatitis Pathway

Severe acute pancreatitis induces severe fibrosis after repair of acute inflammation. Recurrent acute pancreatitis may produce chronic pancreatitis through a necrosis-fibrosis sequence. However, little evidence of acute pancreatitis, such as scarring, has been histologically observed in chronic pancreatitis.

Obstructive Pathway

Obstruction of the main pancreatic duct reproducibly produces changes in chronic pancreatitis within weeks in several animal models. The pathology of obstructive pancreatitis in humans is somewhat distinct from typical alcoholic pancreatitis, such as inter- and intralobular fibrosis and marked destruction of the exocrine parenchyma in the area of obstruction. However, interlobular fibrosis, which is a characteristic of chronic alcoholic pancreatitis, is also observed in the early stage of obstructive pancreatitis [6].

Primary Duct Hypothesis

In the primary duct hypothesis, the primary pathogenic factor is an attack of the pancreatic duct epithelium leading to inflammation and destruction of the ductal architecture, such as primary sclerotic cholangitis [7]. The target epithelial cells may express specific acquired or genetic antigens attacked by antibodies, such as carbonic anhydrase I and II [8].

Sentinel Acute Pancreatitis Event (SAPE) Hypothesis

The cellular and molecular mechanism of chronic pancreatitis and/or fibrosis is well understood. This hypothesis could explain why many etiologies lead to a final common pathway of chronic inflammation and/or fibrosis in the pancreas. During early acute inflammation in the pancreas, acinar cells and other cells may be induced by necrosis-fibrosis or toxic-metabolic, or oxidative stress. These cells express inflammatory cytokines to recruit inflammatory cells, such as neutrophils, lymphocytes, and macrophages. Then, if the inflammatory stimuli are removed, this inflammatory reaction disappears and the pancreas heals to a normal state. However, if the inflammatory stimuli continue to attack the pancreatic tissue, pro-fibrotic cells such as stellate cells are reactivated and transformed into myofibroblasts, which then synthesize extracellular matrix. Therefore, pancreatic fibrosis may occur through interaction between the cells producing stimulating factors and those synthesizing extracellular matrix, such as collagen type I and III. Recently, stellate cells were found in the

pancreas. Also, cytokines may play an important role in fibrogenesis of the pancreas. Both stellate cells and fibrogenesis-related cytokines are briefly reviewed below.

Stellate Cells

Stellate cells were first described in the mechanism of liver cirrhosis. In the liver, Ito cells are present as storage cells, which contain lipids such as vitamin A. Ito cells are stellate cells in the liver and can metamorphose into myofibroblasts under various conditions. Myofibroblasts play many roles in inflammatory processes. In the liver, viral infections, such as hepatitis B or C, induce Ito cells and stellate cells into becoming myofibroblasts to proliferate around Glisson's sheath. Stimulated myofibroblasts produce collagen fibers to form fibrosis in the liver. In the skin, lung, prostate gland and many other organs, the fibrotic process, i.e. wound healing, is associated with myofibroblasts. In the pancreas, stellate cells are present around the lobules and/or pancreatic ducts. They can transform into activated myofibroblasts. Myofibroblasts are smooth-muscle-like fibroblasts. Immunohistochemically, myofibroblasts are characterized by immunopositivities for α-smooth muscle actin (α-SMA), vimentin, desmin, and CD34 [9]. Stellate cells metamorphose into myofibroblasts in the pancreas as same as in the liver by being induced by various stimulators, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, macrophage inflammatory protein (MIP)-1, and IL-8, all of which are inflammatory cytokines or chemokines, as well as transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF), all of which are associated with increasing cell mobility, proliferation, and differentiation. Myofibroblasts are capable of synthesizing collagen types I, III and fibronectin [10–15].

Cytokines and Fibrogenesis

Proinflammatory cytokines, such as IL-1, TNF-α, IL-6, MIP-1, PDGF, and transforming growth factor-beta 1 (TGF-β1), are expressed by inflammatory cells such as neutrophils, lymphocytes, and macrophages, and also by centroacinar duct cells, endothelial cells, and fibroblasts [16]. Pancreatic stellate cells are stimulated by those cytokines and then transform into myofibroblasts, crucial players in pancreatic fibrosis. Myofibroblasts also express many cytokines, growth factors and chemokines and their receptors [17]. TGF-β may

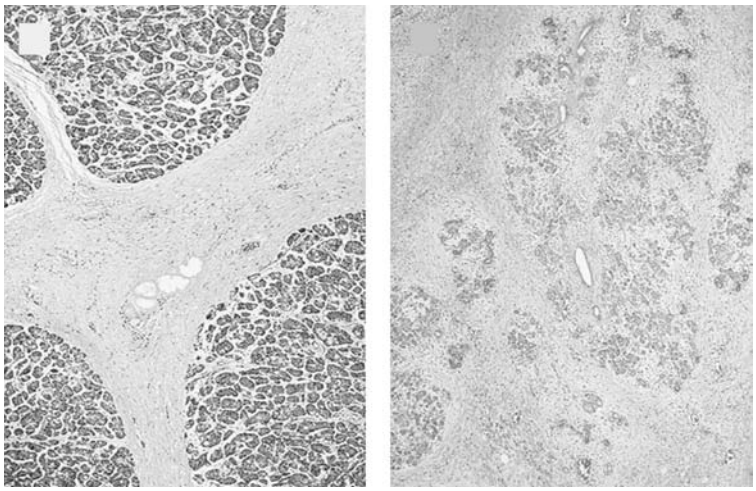


Fig. 1. Typical histology of interlobular fibrosis and intralobular fibrosis. Chronic alcoholic pancreatitis shows fibrosis between nodular pancreatic lobules with wide deposition of collagenous matrix (***a***), while chronic obstructive pancreatitis shows fibrosis in and around the destructive pancreatic lobules with inflammatory cells (***b***).

play an important role in pancreatic fibrogenesis [18, 19]. The source of TGF-β in damaged tissue may be from neutrophils, epithelial cells, parenchymal cells or myofibroblasts [11]. This indicates the important role of autocrine and paracrine mechanisms in chronic pancreatitis and fibrosis.

Histopathology of Pancreatic Fibrosis

The pancreas consists of lobules composed of the exocrine acinar and centroacinar cells and the endocrine islets of Langerhans, and a tree-like ductular system, which drains pancreatic juice excreted by acinar cells into the duodenum. Accordingly, the pancreatic lobules are the smallest structural unit of the pancreas. Pancreatic fibrosis is histopathologically subdivided into (1) interlobular fibrosis, (2) intralobular fibrosis, and (3) mixed fibrosis (fig. 1). However, intralobular fibrosis sometimes has interlobular elements and, when interlobular fibrosis progresses, it includes intralobular elements. In typical interlobular fibrosis, nodular pancreatic lobules remain with wide fibrotic areas in between. In contrast, intralobular fibrosis shows irregular or stellate-shaped thin or broad fibrotic areas within the lobules.

Pancreatic Inflammation and Fibrosis

In early pancreatitis, interlobular thin fibrosis and mild intralobular fibrosis are simultaneously observed. Inflammatory cells appear to various degrees in and around the pancreatic lobules. In the intermediate stage, intralobular fibrosis is observed to a much greater extent. It appears particularly as periductular fibrosis and dilatation of the ductules in the centers of the lobules together with inflammatory cells such as neutrophils or lymphocytes, but fewer macrophages. In end-stage pancreatitis, pancreatic parenchymal tissue decreases and fibrosis increases in and around the lobules, or replaces the original tissue, resulting in loss of function.

In the early stage of obstructive pancreatitis, fibrosis is located in the periductal and intralobular areas. In the intermediate to late stages, fibrosis extends into the interlobules and the intralobules resulting in diffuse fibrosis, i.e. severe pancreatitis. In this stage, many myofibroblasts between collagen fibers can be identified by immunohistochemistry, especially underneath the pancreatic ductal epithelial cells, with erosion.

Are myofibroblasts to be found in these stages of pancreatic inflammation? In general, a few myofibroblasts can be found in the periductal areas of pancreatic tissue without inflammation. Normally, pancreatic stellate cells are located in the periacinar areas. Pancreatic acini are connected to the central pancreatic duct system via centroacinar cells and also ductules. Immunohistochemistry for α-SMA indicates that myofibroblasts are located not only in the periductal areas, but also in the periacinar or perilobular areas where normal myofibroblasts are not usually present. In intermediate obstructive pancreatitis, a few myofibroblasts proliferate between the lobules, showing as thin fibrosis. Myofibroblasts surround the peripheral acini, but are not present in the central acini of the lobules. This suggests that the influence of inflammation is different between the peripheral areas and the central areas in obstructive pancreatitis.

Autocrine and Paracrine Mechanisms in Chronic Obstructive Pancreatitis

In the case of chronic obstructive pancreatitis, pressure or enzymatic effects of pancreatic juice may cause damage to the pancreatic acinar cells and ductal epithelial cells. The pancreatic acini show a declining number of acinar cells and transformation of acinar cells into ductular cells, resembling the proliferation of cholangioles in chronic cholestasis of the liver. In the intermediate or late stage of obstructive pancreatitis, the ductules transformed from centroacinar cells proliferate at the centers of the pancreatic lobules. These ductules are surrounded by

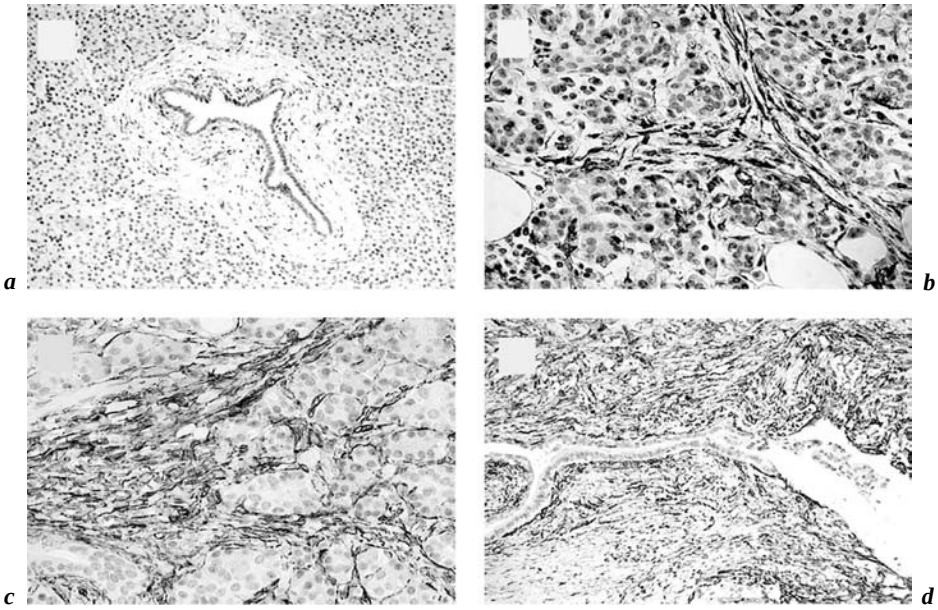
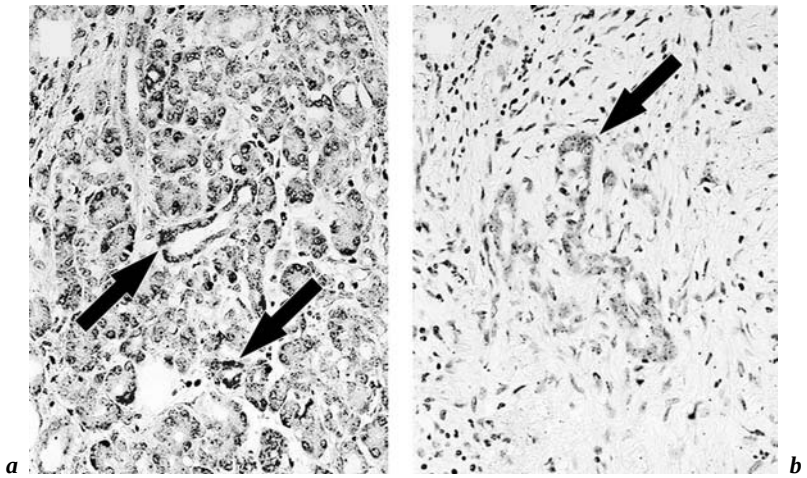


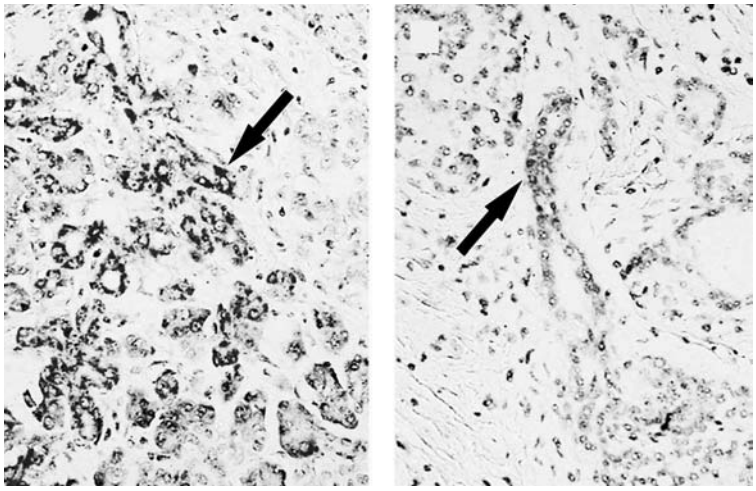
Fig. 2. A few myofibroblasts are observed around the pancreatic ducts, but not in the pancreatic lobules (***a***). Activated myofibroblasts are observed in the periacinar areas in the early stage of chronic obstructive pancreatitis (***b***) and also in the perilobular areas in the mid stage (***c***). In the late stage, diffuse proliferation of activated myofibroblasts is observed in the fibrosis, especially around the pancreatic ducts (***d***). Immunostaining for α-SMA.

myxomatous stroma and collagen fibers. In the late stage, the pancreatic parenchyma, except for the ducts, disappears and the collagenous matrix increases. Immunohistochemistry reveals that myofibroblasts proliferate around the lobules in the early stage of pancreatic obstruction, similar to interlobular fibrosis, which is frequently observed in chronic alcoholic pancreatitis. In the intermediate stage, myofibroblasts are present in and around the lobules, similar to mixed fibrosis. In the final stage, fibrosis is extended into all pancreatic parenchyma except for the pancreatic duct. A number of myofibroblasts are observed in the fibrotic parenchyma, especially around the pancreatic ducts, at this stage. This indicates that myofibroblasts are induced by diverse stimuli at each stage during progression of fibrosis in chronic obstructive pancreatitis (fig. 2). We focused on expression of TGF-β1, 2 and 3, PDGF and IGF-1 in the paracrine mechanism of chronic obstructive pancreatitis. Interestingly, TGF-β1 expresses on neutrophils, and TGF-β2 and 3 on ductal or ductular cells. All subtypes of TGF-β are expressed on myofibroblasts [20]. PDGF and IGF-1 are



a ***b***

Fig. 3. In situ hybridization of TGF-β mRNA in chronic obstructive pancreatitis. Centroacinar cells or pancreatic ductular cells express TGF-β mRNA in chronic obstructive pancreatitis. ***a*** Positive centroacinar cells in the early stage. ***b*** Positive ductular cells in the late stage.



a ***b***

Fig. 4. In situ hybridization of PDGF mRNA in chronic obstructive pancreatitis. ***a*** Many centroacinar cells express platelet-derived growth factor mRNA in the mid stage. ***b*** A few ductular cells express it in the late stage.

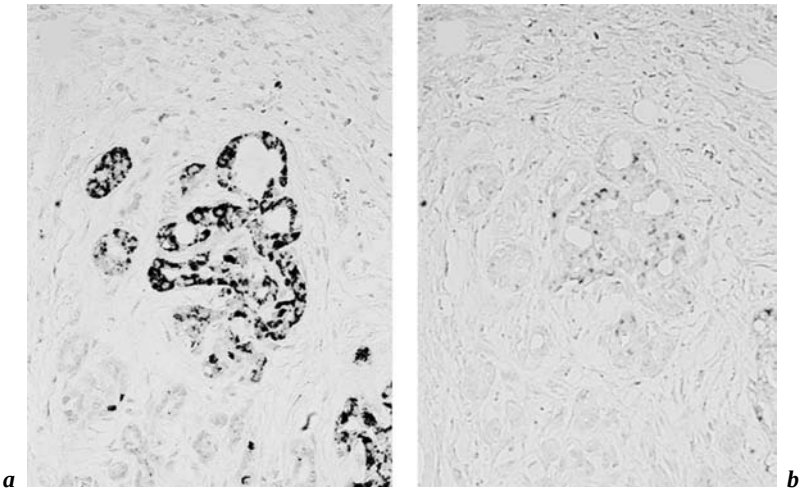


Fig. 5. In situ hybridization of IGF-1 mRNA in chronic obstructive pancreatitis. Centroacinar cells strongly express IGF-1 mRNA in the early stage. ***a*** Anti-sense probe. ***b*** Sense probe.

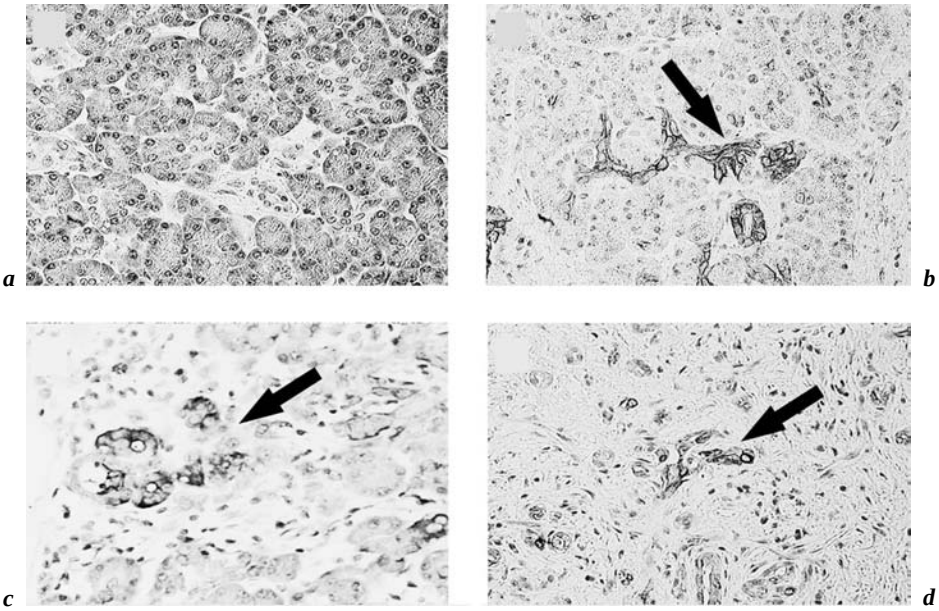


Fig. 6. Immunohistochemistry for IGF-1 and its receptor in chronic obstructive pancreatitis. Normal pancreatic tissue is negative for immunostaining of IGF-1 (***a***), while centroacinar cells in the normal pancreatic tissue are positive for immunostaining of its receptor (***b***, immunopositive for IGF-1 receptor indicated by arrow). In chronic obstructive pancreatitis, centroacinar cells are immunopositive for both IGF-1 (***c***, immunopositive for IGF-1 indicated by arrow) and its receptor (***d,*** immunopositive for IGF-1 receptor indicated by arrow).

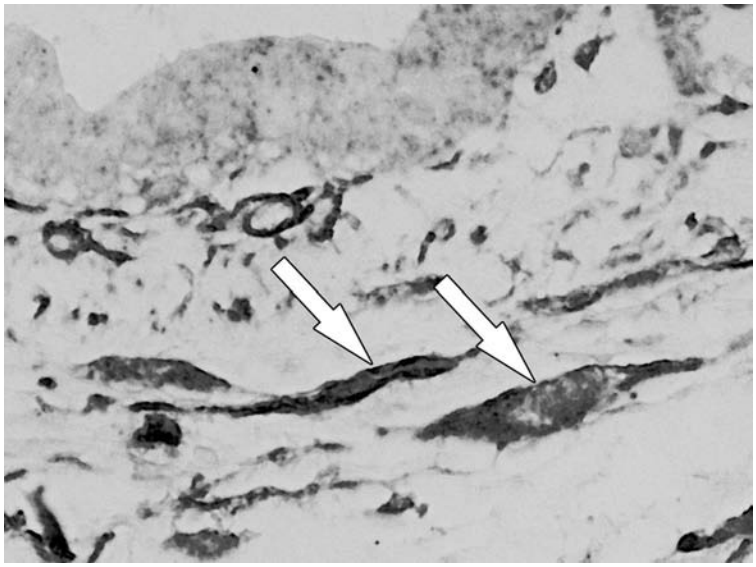


Fig. 7. Double immunostaining of α-SMA and TGF-β in chronic obstructive pancreatitis. A few myofibroblasts, which are immunopositive for α-SMA, express TGF-β around the pancreatic duct (white arrows).

expressed on centroacinar cells, but not inflammatory cells (figs. 3–6). Myofibroblasts around the pancreatic ductules express TGF-β (fig. 7), suggesting that chronic obstructive pancreatitis has an autocrine mechanism.

In conclusion, autocrine and paracrine mechanisms play a crucial role in pancreatic fibrosis. However, many cells express different growth factors or growth factor subtypes in chronic pancreatitis and fibrosis, indicating that these may lead to different morphologies, such as interlobular and intralobular fibrosis.

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Experimental Animal Models of Pancreatic Fibrosis

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Abstract

Animals that spontaneously show phenotypic characteristics similar to those seen in human disorders, or animals subjected to various procedures that empirically induce symptoms resembling human clinical symptoms, can be used as models for human disorders. Such animal models are mainly used to clarify the mechanisms of diseases or to evaluate possible treatments. An understanding of the mechanisms involved in animal models should help researchers to uncover the mechanisms underlying the corresponding human disorders. Moreover, successful treatment of the symptoms in animal models suggests that similar treatment might be effective in humans. In any event, it is important to consider fully the anatomical and physiological differences between humans and animals. Also of importance is the choice of a suitable model(s) – it is often the case that more than one model is needed for clarification of a human disorder. As regards pancreatitis, the mechanisms involved are very complex in humans, and animal models can reflect only a part of them. Nevertheless, animal models may provide useful information, especially when they are carefully selected in accordance with the experimental purpose or design. Therefore, researchers need to have a sufficient knowledge of the characteristics of the various models. Pancreatic fibrosis was spontaneously observed in WBN/Kob rats, ALY mice and OLETF rats. Experimentally induced pancreatic fibrosis models include caerulein- or dibutyltin dichloride-treated rats and a pancreatic duct-ligation model. We describe the pancreatic histology observed in our experiments with various animal models.

Numerous animal models of pancreatitis in humans have been established, but the complexity of the human disease, especially chronic pancreatitis, means that it is difficult to extrapolate the findings in such models to the human situation [1]. The differences between these animal models and humans with pancreatitis are probably largely due to anatomical and physiological species

differences. On the other hand, there seems little doubt that animal models are useful, since studies using them often help to clarify the mechanisms at work in human disorders, or to define medical guidelines for treatments [2–10]. For the proper utilization of animal models in studies on human pancreatitis, it is first necessary to know the characteristics of acute and chronic pancreatitis.

In general, acute pancreatitis is classified into alcoholic, biliary, idiopathic, and other types, in accordance with the major causes [11–14]. The alcoholic and biliary types are closely associated with alcohol abuse and biliary abnormalities, respectively [15–17]. On the other hand, idiopathic pancreatitis, which occurs at high incidence, may be caused by anatomical abnormalities, circulatory disorders, metabolic disturbances etc. [18, 19]. Ductular, lymphogenous, hematogenous, nervous, metabolic and other factors are considered to be associated with the acute disease, and to interact with each other [20–23]. Several theories to explain the formation or progression of acute pancreatitis have been proposed, including the common channel theory [24–27] and the obstruction-hypersecretion theory [28–30]. In the common channel theory, the underlying condition is thought to be the existence of an anatomical channel between the common bile duct and the main pancreatic duct. This channel permits the reflux of bile into the pancreatic duct, resulting in activation of pancreatic enzymes [29, 31–34], and self-digestion of pancreatic tissues ensues. Disturbance of the outflow through aberrant pancreatic secretion also induces acute pancreatitis [35, 36]. On the other hand, the obstruction-hypersecretion theory is better suited to explain the mechanism of alcoholic pancreatitis [28, 30]. This theory has been further subdivided into the big duct theory [29, 31] and the small duct theory [34, 37]. According to the former, edema or spasmus of the duodenal papilla occurs after alcohol ingestion, constricting the orifice of the pancreatic duct, while according to the latter, alcohol-induced protein plugs occlude the small pancreatic ducts. As suggested above, more than one mechanism may exist, with interaction between them. In general, acute pancreatitis is reversible, except for severe cases, which may have a fatal progression [38–40].

Unlike most acute pancreatitis, chronic pancreatitis is irreversible and the pathological condition gradually progresses [41–45]. The causal factors for chronic pancreatitis are thought to be the same as those for acute pancreatitis, i.e. alcoholic, biliary, idiopathic, and so on [28, 34, 40, 46–48]. Moreover, some cases evolve from acute pancreatitis [38, 39]. It is noteworthy that irreversible fibrosis is a major characteristic of chronic pancreatitis [43, 44, 49, 50]. Fibrous elements expand into the periductular, interlobular and intralobular areas to various extents. The characteristics of fibrous spreading, the kinds of inflammatory cells, and the existence of cysts, protein plaques or calculus are used for classification [28, 51–53]. Autoimmune pancreatitis, a concept proposed in recent years, is also a type of chronic pancreatitis with the involvement of immune mechanisms [54]. In

such cases, nodular lesions consisting of accumulated lymphocytes are often observed around the pancreatic ducts [51, 55–58]. If the mechanisms of the various forms of pancreatitis, including the autoimmune type, are to be established in greater detail, animal models must be further categorized with respect to their detailed characteristics, so that they can be utilized more effectively. It should also be noted that chronic pancreatitis is often reactivated [40, 59, 60]. The necrosis-fibrosis sequence theory has been proposed to explain the mechanism of reactivation. This theory claims that pancreatitis-induced fatty necrosis triggers stenosis or occlusion of the pancreatic ducts, resulting in elevation of the intraductal pressure and leakage of pancreatic fluid into the peripheral tissue, leading to further necrosis of the adipose tissues [28, 59, 61].

In summary, numerous animal models have been used in studies of pancreatitis, and each of them has particular pathological features, which cannot necessarily be extrapolated to pancreatitis in humans. Therefore, it is critically important to utilize appropriate models depending on the particular research aim in each case.

Animal Models of Pancreatitis

As mentioned above, the mechanisms responsible for pancreatitis in humans are complex. Animal models can reflect only part of them. However, they may provide useful information, especially when the models are carefully selected in accordance with the experimental purpose or design. Therefore, it is necessary that researchers have a sufficient knowledge of the characteristics of the various models.

In the subsections below we describe the pancreatic histology observed in our experiments using various animal models. First, we focus on the characteristics of pancreatic fibrosis. Later, the results of animal models which unfortunately failed to form fibrosis under our experimental conditions are also mentioned, since the models have been most often used in the studies on pancreatitis.

Pancreatitis in Male WBN/Kob Rats

Male WBN/Kob rats show hemorrhage and edema in intralobular and interlobular areas at the age of about 2 or 3 months, as well as widespread acute pancreatitis with slight fibrosis (fig. 1a). At 4 months of age, the fibrosis becomes marked (fig. 1b). Because of these features, this rat strain is often used as a chronic pancreatitis model [62–65]. In some reports on acute pancreatitis in this strain, initial bleeding was thought to arise around the islets of Langerhans [65]; however, we could not identify the site of bleeding in our studies [66]. Female WBN/Kob rats show no changes in their pancreas; therefore, sex hormones

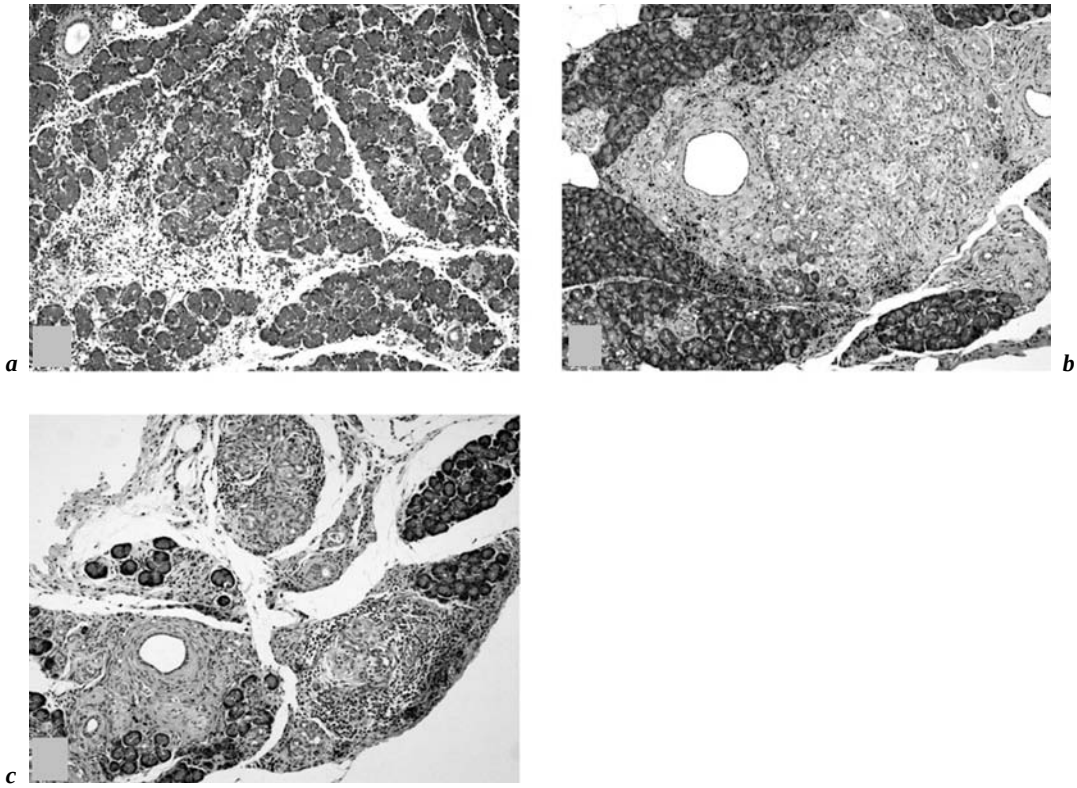

Fig. 1. Characteristics of spontaneously occurring pancreatitis in WBN/Kob rats. ***a*** Pancreas from male WBN/Kob rat at 2 months of age. Interlobular hemorrhage and edema are seen. ***b*** Pancreas from male WBN/Kob rat at 4 months of age. Inter- and intralobular fibrotic changes are seen. Destruction of pancreatic islets has occurred as the result of the widespread fibrosis. ***c*** Pancreas from male WBN/Kob rat at 8 months of age. Inflammatory changes have continued, while the fibrosis shows a tendency toward resolution.

must be involved in the occurrence of pancreatitis. In the males, fibrosis was prominent in the periductal or interlobular areas, extended into the intralobular area, and disrupted the islet tissues. This fibrotic change is thought to be why diabetes occurs in male WBN/Kob rats. Unfortunately, fibrosis in this strain is also reversible; in other words, the fibrosis reaches a peak at 4 months of age and gradually decreases thereafter (fig. 1c). This model can thus not be considered a suitable model for human chronic pancreatitis. In contrast, we investigated WBN/Kob rats, taking their fibrous characteristics into account, and found some factors related to the formation and resolution of the fibrosis (described later).

In addition to other minor changes, we also observed signs of apoptosis [67, 68] in acinar cells and inflammatory cells in the fibrotic and inflamed area. Protein plugs were not observed during the acute phase (about 2 months of age) but were recognized in inflamed areas from 4 months of age onwards.

Caerulein-Induced Pancreatitis

Caerulein, which structurally resembles cholecystokinin (CCK) and gastrin, is known to exert pharmacological effects similar to those of CCK [69–73]. Therefore, this model has often been used to clarify the pathogenetic mechanism of pancreatitis in relation to metabolism [72, 74, 75]. Various administration routes and doses have been selected in many investigations and on the whole the results indicate that caerulein can induce acute pancreatitis.

In our experiments, caerulein-treated mice were used to investigate the relationship between the histopathologic severity of pancreatitis, presence or absence of calculi, and dosage frequency. Ten-week old C57BL6 mice were intraperitoneally injected with 100 μg/5 ml/kg of caerulein 4, 7, 10 or 13 times at 1-hour intervals, and sacrificed at 7, 10, 13, or 24 h or 2, 3 or 8 days after the last injection. In the mice dosed 4 times, inflammatory changes were comparatively slight and there were almost no advanced changes in the pancreas (fig. 2b). On the other hand, the histopathology of mice treated with 7 doses showed massive and severe edema, necrosis of acinar cells, and hemorrhage, and resembled that of mice treated with 10 (fig. 2c) or 13 doses (fig. 2d), when the animals in these groups were examined 13 h after the final dosing. However, at 8 days after the final dosing, there were great differences in the changes between the mice treated with 7 or 10 doses and those dosed 13 times. At that point, the mice treated 7 or 10 (fig. 2e) times showed slight changes, i.e. slight necrosis of acinar cells with low-grade inflammation and ductal proliferation. In contrast, mice treated 13 times showed more severe necrosis of acinar cells, inflammatory cell infiltration, ductal hyperplasia, and fibrosis (fig. 2f). Interestingly, many calculi were observed in the pancreatic ducts from the mice treated with 13 doses. It is more than probable that calculi formation, which was not clear at 13 h after the final dosing, occurred later, and induced severe pancreatic changes in the animals treated with 13 doses. This hypothesis may indicate that the cause of caerulein-induced pancreatitis is related to acceleration of pancreatic secretion and autolysis. Furthermore, the formation of calculi may be a significant step toward the development of pathological features including fibrosis [76–78].

Pancreatitis in the DBTC Model

In recent years, there have appeared many reports on models of pancreatitis induced by dibutyltin dichloride (DBTC) [79–82]. Rats or mice treated

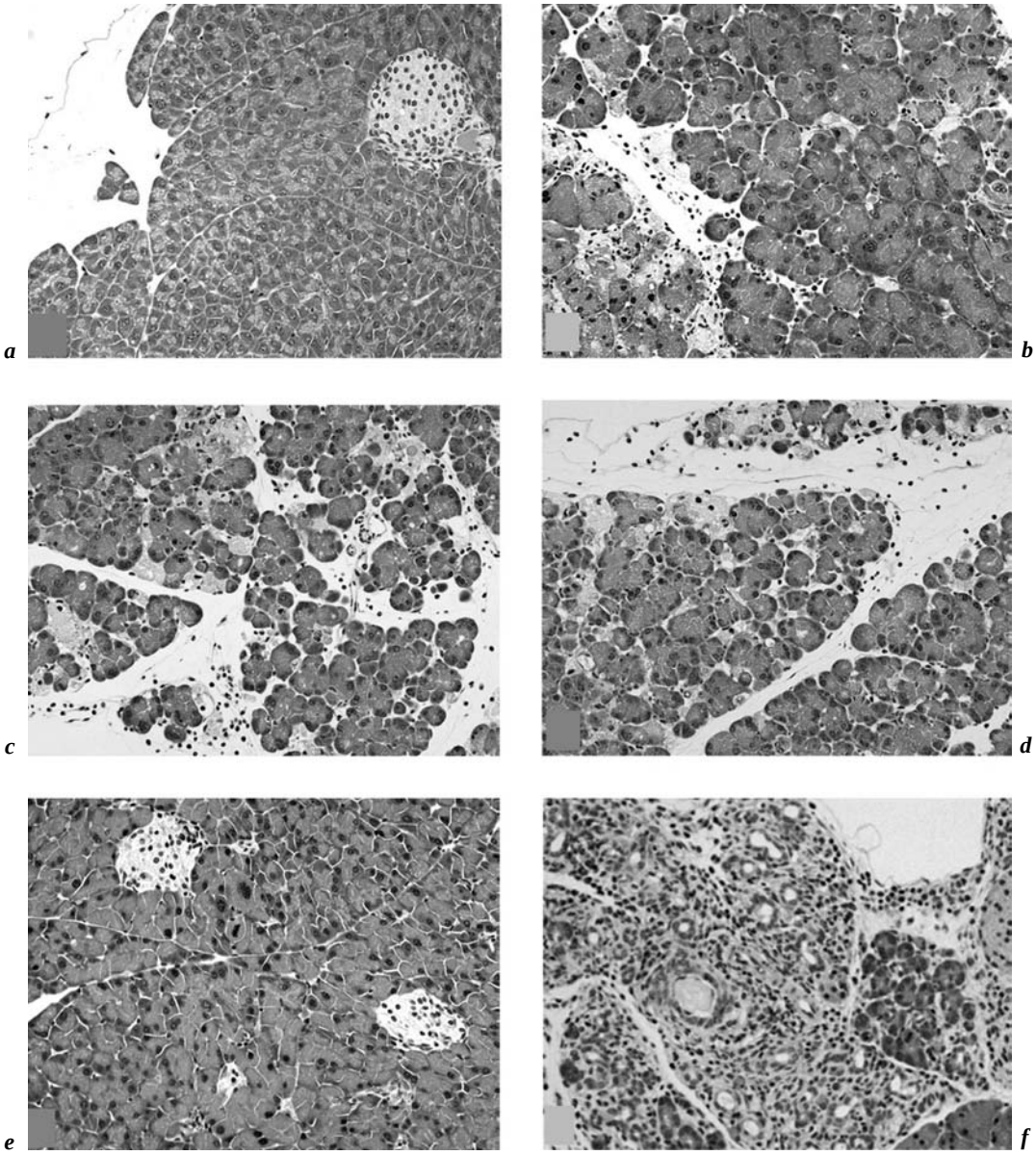

Fig. 2. Characteristics of cerulein-induced pancreatitis. ***a*** Pancreas from control mice. No abnormalities are seen. ***b*** Pancreas at 13 h after 4 treatments with 100 μg/kg. Slight infiltration by inflammatory cells is noted. Edema and necrosis are seen. ***c*** Pancreas at 13 h after 10 treatments with 100 μg/kg. Severe edema has occurred. Necrosis and inflammatory cell infiltration are also observed. ***d*** Pancreas at 13 h after 13 treatments with 100 μg/kg. Edema, inflammation, and necrosis are at the same levels as in (***c***). ***e*** Pancreas at 7 days after 10 treatments with

intravenously with DBTC were reported to show epithelial lesions in the extrahepatic bile duct, leading to chronic inflammation and cystic dilation of the bile duct. Also, tri- and dibutyltin or diethyltin diiodide compounds, which are alkylstannane compounds like DBTC, induce similar lesions in rats or mice, but not in cats, guinea pigs or rabbits [83, 84]. These facts may suggest that the involved cause for the lesion may be partly due to the anatomical character of the pancreaticobiliary duct, since the pancreatic duct and bile duct normally become confluent in rats and mice [85].

In our study, Lewis rats were injected intravenously at a single dose of 8 mg/kg of DBTC, and histopathologically examined. Also in our case, cystic dilation of the bile duct was marked several days after treatment. Necrosis and desquamation of bile duct epithelial cells, severe edema and inflammatory cell infiltration around the bile duct, and obstruction of the pancreaticobiliary duct by debris preceded the cystic changes. In the pancreas from mice with a cystic bile duct, scattered colliquative necrosis of the ductal epithelium and peripheral tissue, as well as fatty necrosis, was observed at 1 day after treatment (fig. 3a). Unlike those in ethionine- or arginine-induced pancreatitis (accumulation of secretion components in acinar cells or self-digestion, as described later), the lesions in the DBTC model were localized around the pancreatic ducts, interlobular spaces, and also inconspicuously periductule sites in the intralobular areas (fig. 3b). It was thus probable that the starting point for pancreatitis in the DBTC model was leakage of pancreatic juice or bile into the periductal space, which occurs as a sequela of the destruction of the ductal wall. In the chronic situation, acinar cells became atrophied, and ductal proliferation and fibrosis appeared (fig. 3c, d). In the liver (fig. 3e), it was not surprising that proliferation of bile ducts occurred.

Interestingly, fibrosis in the DBTC model persists for a comparatively long time around the pancreatic ducts and interlobular spaces (fig. 3f). It is just conceivable that the persistent fibrosis may be attributed to fibers composed chiefly of type I collagen, which is known to be much more stable than type III collagen (described later). For instance, fibrosis in male WBN/Kob rats is due to type III collagen, and is known to disappear spontaneously.

100 μg/kg. Inflammatory changes are mild or nonexistent. Evidence of sporadic regenerated acinar cells is present. ***f*** Pancreas at 7 days after 13 treatments with 100 μg/kg. Unlike in the case of 10 treatments (***e***), inflammatory changes have progressed. Decrease in acinar cells with severe proliferation of pancreatic ducts is noteworthy. Protein plugs are seen in the pancreatic ducts, which may indicate that such plugs or calculi are factors that exacerbate or reactivate the pancreatitis. Areas of fibrosis have formed in the spaces between the proliferative ducts.

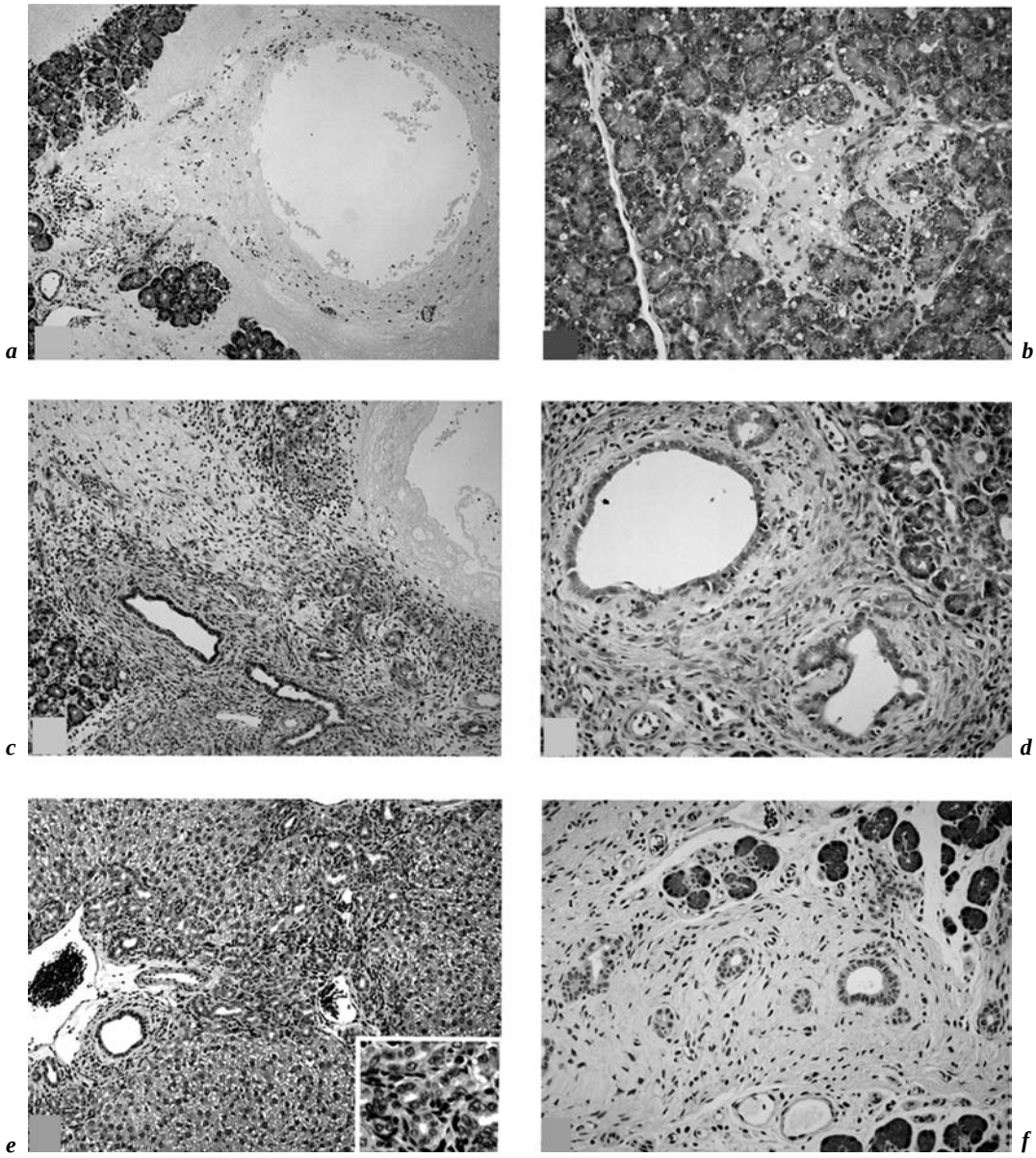

Fig. 3. Characteristics of DBTC-induced pancreatitis. ***a*** Pancreas at 1 day after treatment with 8 mg/kg. Necrotic epithelium of the pancreatic duct is seen. Acinar cells and adipose tissue around the duct have also become necrotic. Hemorrhage and inflammatory cells are observed. ***b*** Pancreas from the same animal as (***a***). Focal necrosis is also seen in the intralobular areas.These areas are probably located around the intralobular pancreatic ducts. ***c*** Pancreas

It must be noted that in this model there are individual differences between rats. In our experiments, only approximately half of the treated rats developed pancreatitis. So, especially in therapeutic experiments, preconsideration of progress levels of pancreatic lesions in individual animals is necessary before any procedures are started. Finally, we also believe this to be suitable model for congenital cystic dilatation of the common bile duct in humans.

Pancreatitis in the Duct-ligation Model

Pancreatic ducts in the rat are composed of several large ducts and numerous narrow ones, and they open directly into the common bile duct without forming main or accessory pancreatic ducts. Also, the rat pancreas is segmented by the pathways of its ducts and blood vessels into splenic, duodenal, gastric, and parabiliary segments [85]. Various methods for ligation of the pancreatic duct were reported years ago [86–91]. For study purposes, complete or partial ligation was performed at various segments of the pancreas, and duct obstruction was concluded to be the cause of pancreatitis [88, 91, 92].

As described, the pancreatic duct and bile duct of the rat are interfluent outside the duodenum. So, it was thus expected that ligation closer to the duodenum would induce influx of bile into the pancreatic duct. In a study using the rat ligation model, we investigated the relationship between human anomalous arrangement of the pancreaticobiliary duct and pancreatitis; therefore, ligation was done at a position closer to the duodenum.

As a result, dilation of the pancreatic ducts was remarkable in both inter- and intralobular areas. Severe inflammation was observed in the intralobular and periductal spaces. Fatty necrosis was also scattered (fig. 4a, b). At 7 days after ligation, atrophy of acinar cells, proliferation of intralobular pancreatic ducts, and periductal fibrosis were seen (fig. 4c).

Moreover, the cystic dilation of the bile duct was remarkable . It was clear that pancreatic juice flowed into the bile duct, since the contents of the cyst showed amylase activity. On the other hand, bile was not morphologically detected in pancreas specimens in our experiments, and hence there was no evidence of influx of bile into the pancreas. The main causes of occurrence and progression of pancreatitis in the ligation model, as well as in the DBTC model, may therefore be elevation of ductal pressure, and not bile influx into the pancreatic duct.

at 3 days after treatment. Necrosis, infiltration by fibroblastic cells and proliferation of pancreatic ducts have progressed. ***d*** Pancreas at 2 weeks after treatment. Fibrosis has become firm by this time. Regenerative epithelial cells are arranged at the luminal surface of the pancreatic ducts. ***e*** Liver at week 2 after treatment. Evidence of proliferation of the bile duct with slight infiltration of inflammatory cells is seen. ***f*** Pancreas at 24 weeks after treatment. Extensive fibrosis is seen around the ducts. Fibrosis has expanded into the intralobular spaces.

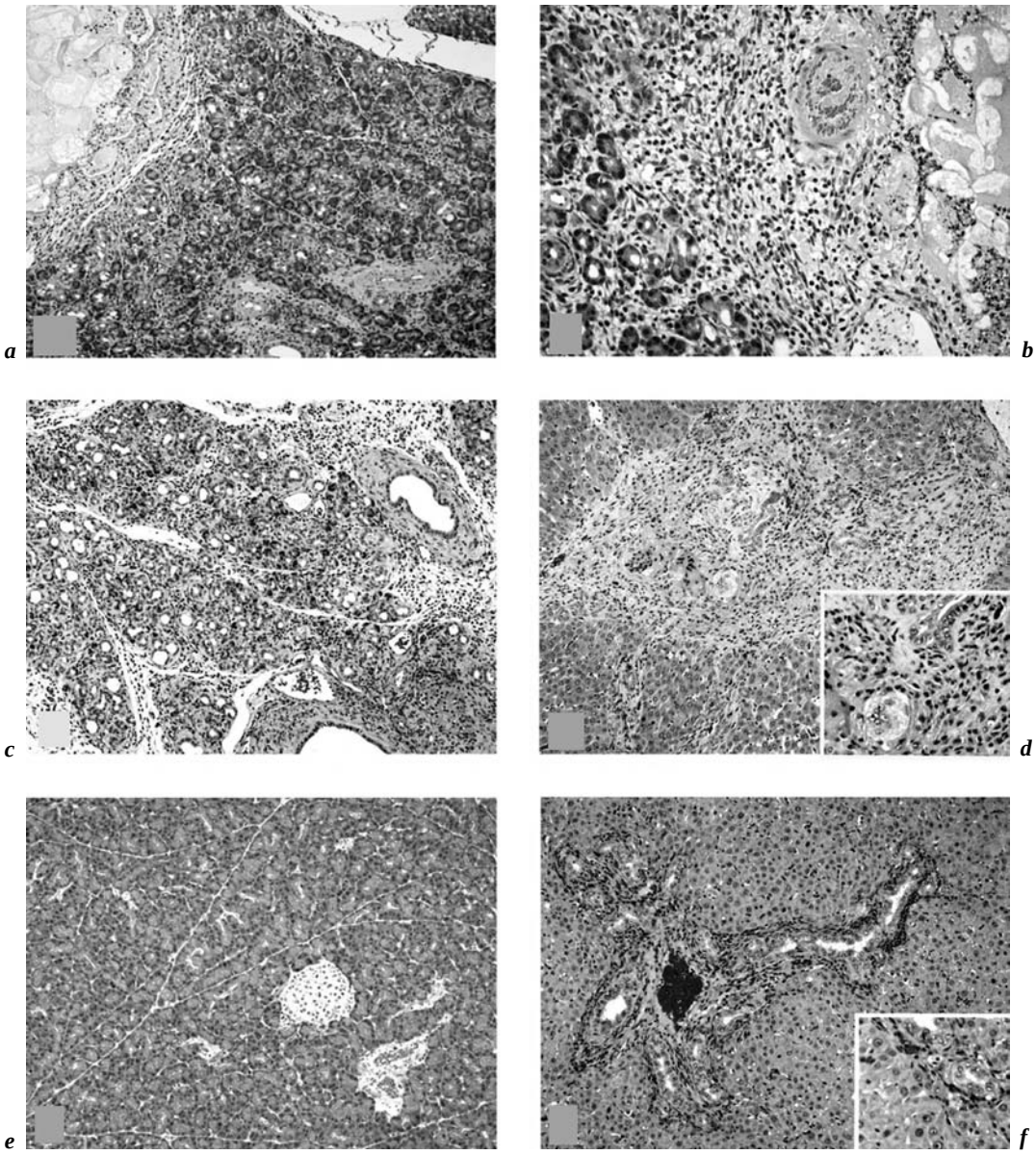

Fig. 4. Characteristics of pancreaticobiliary duct-ligation model. ***a*** Pancreas at 3 days after ligation near the duodenum. Drastic changes, such as necrosis of the epithelium and acinar cells, inflammatory cell infiltration, and proliferation of pancreatic ducts, are notable. ***b*** Higher magnification of pancreas from the same animal as in (***a***). Small ducts also show epithelial necrosis. Ducts obstructed by protein plugs or debris are seen. Peripheral tissues have undergone colliquative necrosis. ***c*** Pancreas at 7 days after ligation near the duodenum. Acinar cell atrophy and

In the liver, destruction and proliferation of the bile duct, along with inflammation, were noticeable (fig. 4d). These changes were more severe in the rats with ligation closer to the duodenum than in those with ligation at the porta hepatica (fig. 4f). It was therefore considered that influx of pancreatic juice into the bile duct might affect the progression of hepatic lesions.

Pancreatitis in ALY mice

Autoimmune pancreatitis is defined as pancreatitis involving autoimmune responses [54, 55]. This type of pancreatitis is characteristically accompanied by increased levels of autoantibody and γ-globulin in the serum, and by fibrosis with lymphocytic infiltration; but clinically there is a lack of acute symptoms [51, 56, 58, 93, 94]. As an experimental model for autoimmune pancreatitis, thymectomized neonatal BALB/c mice immunized with carbonic anhydrase II or lactoferrin have been used, since these mice show T-lymphocyte-dominated infiltration into exocrine glands such as salivary glands and pancreas [55, 57, 95]. In contrast, as a model of spontaneously-occurring autoimmune pancreatitis, the alymphoplasia (ALY) mouse is well recognized [96–98]. The ALY mouse is immunodeficient, as it lacks generalized lymph nodes and Peyer's patches and, moreover, shows structural abnormalities in its spleen and thymus. It was mentioned that histopathological characteristics were chronic inflammatory changes in exocrine organs such as the salivary gland, lacrimal glands and pancreas in homozygotes (aly/aly), but not in heterozygotes (aly/+) [97, 98]. In our examinations, inflammation was located in periductal areas in the early stage (fig. 5a), fibrosis gradually appeared in an age-related manner, and there was marked proliferation of the pancreatic ducts (fig. 5b, c). Inflammatory changes were severer in the pancreas than in the salivary glands (fig. 5d) or lacrimal glands (fig. 5e). In 30-week-old aly mice, the acinar cells had become atrophic and the ductal proliferation increasingly dominant. On the other hand, the fibrosis around the pancreatic ducts did not undergo involution during the experimental period. We attribute the irreversibility of the pancreatic fibrosis in ALY mice to persistent inflammation around the pancreatic ducts.

ductal hyperplasia are widespread. Ductal proliferation is accompanied by periductal fibrosis. Inflammatory cell infiltration also has continued. ***d*** Liver at 7 days after ligation near the duodenum. Unlike in the case of ligation at the porta hepatica (***f***), various changes are observed around Glisson's sheath. Epithelial necrosis of the bile ducts is similar to that of the pancreatic ducts. Necrosis of hepatocytes, inflammatory cell infiltration, and fibrosis can be observed in this model. This hepatic necrosis is thought to be a major difference between the DBTC and ligation models in our experiments.The latter possibly involves the influx of pancreatic juice into the bile duct. ***e*** Pancreas at 3 days after ligation at the porta hepatica. In the case of common bile duct ligation (***a***), no abnormalities are seen in the pancreas. ***f*** Liver at 7 days after ligation at the porta hepatica. Proliferation of bile ducts with infiltration of inflammatory cells is seen.

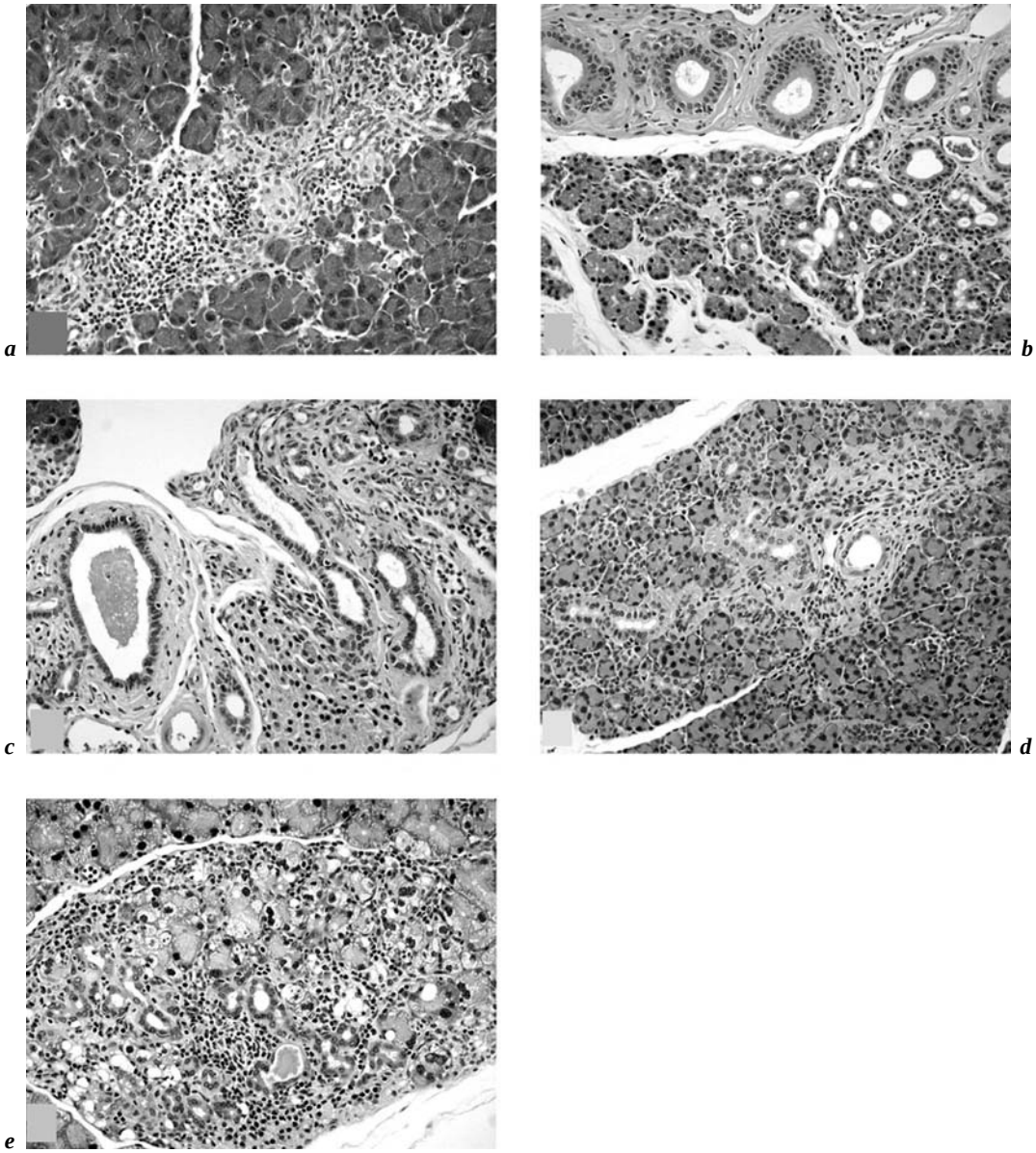

Fig. 5. Characteristics of spontaneously occurring autoimmune pancreatitis in ALY mice. ***a*** Pancreas from an ALY mouse at 8 weeks of age. Inflammatory cells accumulate around the pancreatic duct. Slight hemorrhage is seen. ***b*** Pancreas from an ALY mouse at 20 weeks of age. Fibrosis is formed around the pancreatic ducts. Inflammatory cell infiltration into the periductal area and proliferation of ducts are seen. ***c*** Pancreas from an ALY mouse at 30 weeks of age. Atrophy of acinar cells is notable. Inflammatory cells continue to

Pancreatitis in OLETF Rats and STZ Model

Otsuka Long Evans Tokushima Fatty (OLETF) rats are known as a strain which lacks the CCK-1 receptor and spontaneously develops diabetes, and they have been used as a model of human type II diabetes [99–101]. It was reported that infiltration of connective tissue into the islets of Langerhans occurred in OLETF rats at 20 weeks of age or later, and that thereafter the fibrosis in the islets progressed, the islets became enlarged, and fibrosis subsequently spread to the peripheral tissue [102, 103]. The islets were eventually divided into many pieces and functional disorder ensued. Also in our experience, fairly extensive fibrosis around the islets occurred with atrophy of acinar cells. In the enlarged islets or the surrounding fibrotic area, hemosiderin or inflammatory cell infiltration was often observed, and edema was noted in exocrine tissue (fig. 6a–d). However, fibrosis in this strain is also considered to be reversible, and, moreover, adipose tissue gradually replaces the areas of fibrosis.

On the other hand, streptozotocin (STZ) treatment is known to induce dysfunction of islets, and also has been used to prepare a model of type II diabetes. In our experience, the time of occurrence or degree of diabetes after treatment can vary a great deal among STZ-treated rats (intravenously injected with 60 mg/kg). Also, in this model, we found that fibrosis occurred, dividing the islets, and partly extending around the islet tissue (fig. 6e, f). However, under our experimental conditions, fibrosis and atrophy of acinar cells in the STZ-model rats were weaker than in the OLETF rats.

Pancreatitis in DahlS Rats

DahlS rats were reported to be a salt-sensitive model of hypertension [104]. There have been many reports about the nephrotoxicity [105, 106] in these animals but little information about pancreatic changes [107]. Although this strain is not a major model for studying pancreatic fibrosis, fibrosis can be sporadically seen in these animals. In our experience, slight hemorrhage or degeneration and necrosis of acinar cells could be occasionally seen in lobules of DahlS rats at 10 weeks of age or older (fig. 7a, b). However, the fibrosis, which became marked as the animals aged further, was limited to periarterial areas (fig. 7c). We believe that the periarterial fibrosis in this strain may not be associated with intralobular changes, and that the characteristic fibrosis is an after-effect of hypertension.

infiltrate and fibrosis is widespread. ***d*** Parotid gland from an ALY mouse at 30 weeks of age. Inflammatory cell infiltration into the periductal area is seen. Periductal fibrosis is not as pronounced as in the pancreas. ***e*** Lacrimal gland from an ALY mouse at 30 weeks of age. Changes are at the same level as those in the parotid gland (***c***).

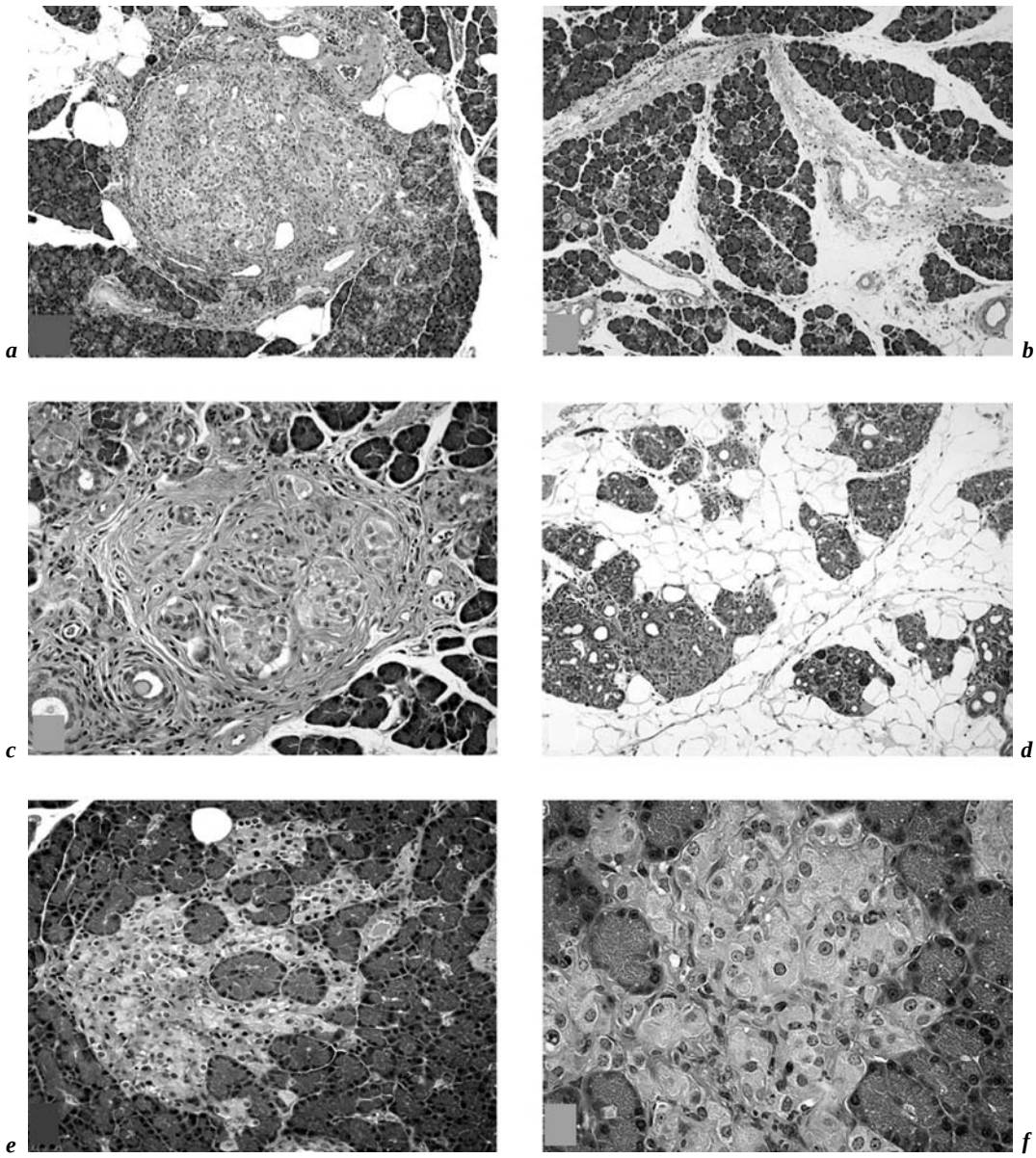

Fig. 6. Characteristics of islet fibrosis in OLETF rats and STZ-treated rats. ***a*** Pancreatic islets from an OLETF rat at 4 months of age. The islets are enlarged and divided by fibrous elements. ***b*** Pancreas from the same animal as (***a***). Acute pancreatitis is seen, i.e. widespread edema is noted. There is slight hemorrhage and infiltration by inflammatory cells. ***c*** Pancreatic islets from an OLETF rat at 8 months of age. Fibrosis has

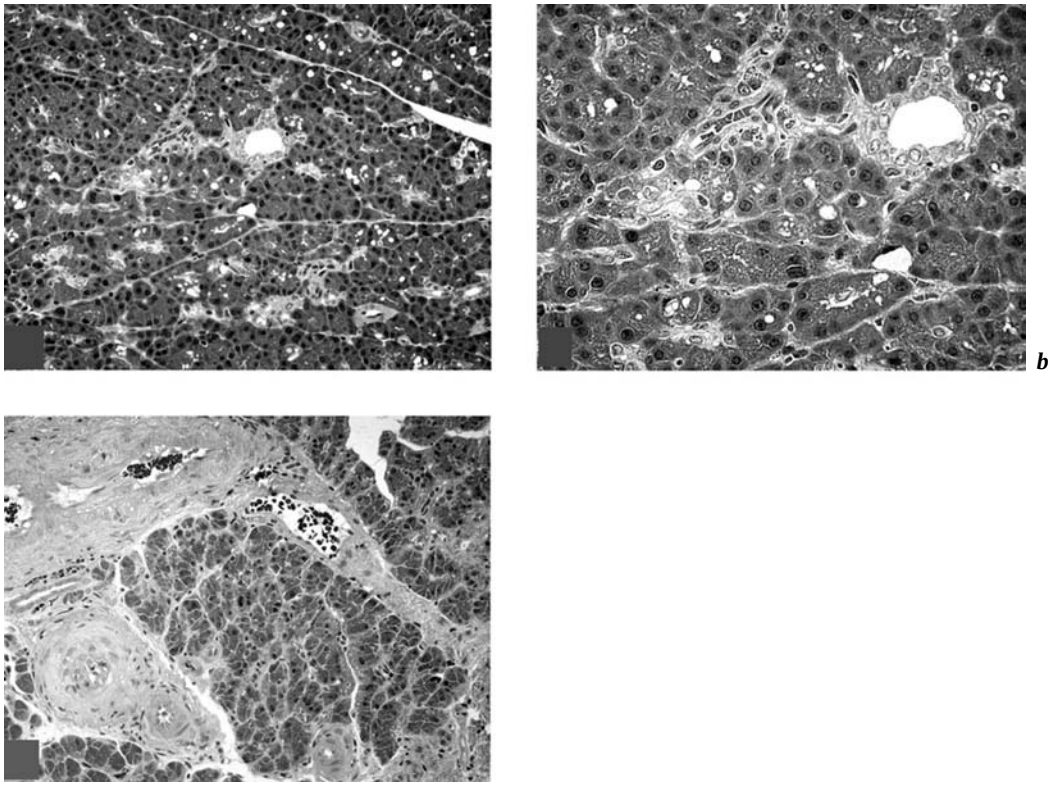

Fig. 7. Characteristics of pancreatic fibrosis in DahlS rats; a spontaneously occurring hypertensive model. ***a*** Pancreas from a DahlS rat at 10 weeks of age. Vacuoles are observed in acinar cells. Atrophic acinar cells are scattered. ***b*** Higher magnification of pancreas from the same animal as in (***a***). Hemosiderin deposited by the side of the small arteries. ***c*** Pancreas from a DahlS rat at 20 weeks of age. Apart from arterial changes such as medial necrosis, periarterial fibrosis can be seen. Also a small amount of fibrous material can be seen in the lobules.

expanded around the islets. Evidence of atrophy of acinar cells and proliferation of pancreatic ducts is present. ***d*** Pancreas from the same animal as (***c***). Atrophy of acinar cells is notably widespread, and these cells have been displaced by adipose tissues. Fibrosis in and around islets still remains. ***e*** Pancreatic islets from a rat at 4 days after treatment with 60 mg/kg of STZ. Fibrous elements can be observed in the islets but there are fewer than in OLETF rats. Also, hemosiderin deposits are seen. ***f*** Pancreatic islets from a rat at 2 weeks after treatment with STZ. Progression of fibrosis and destruction of islets are not as advanced as in OLETF rats.

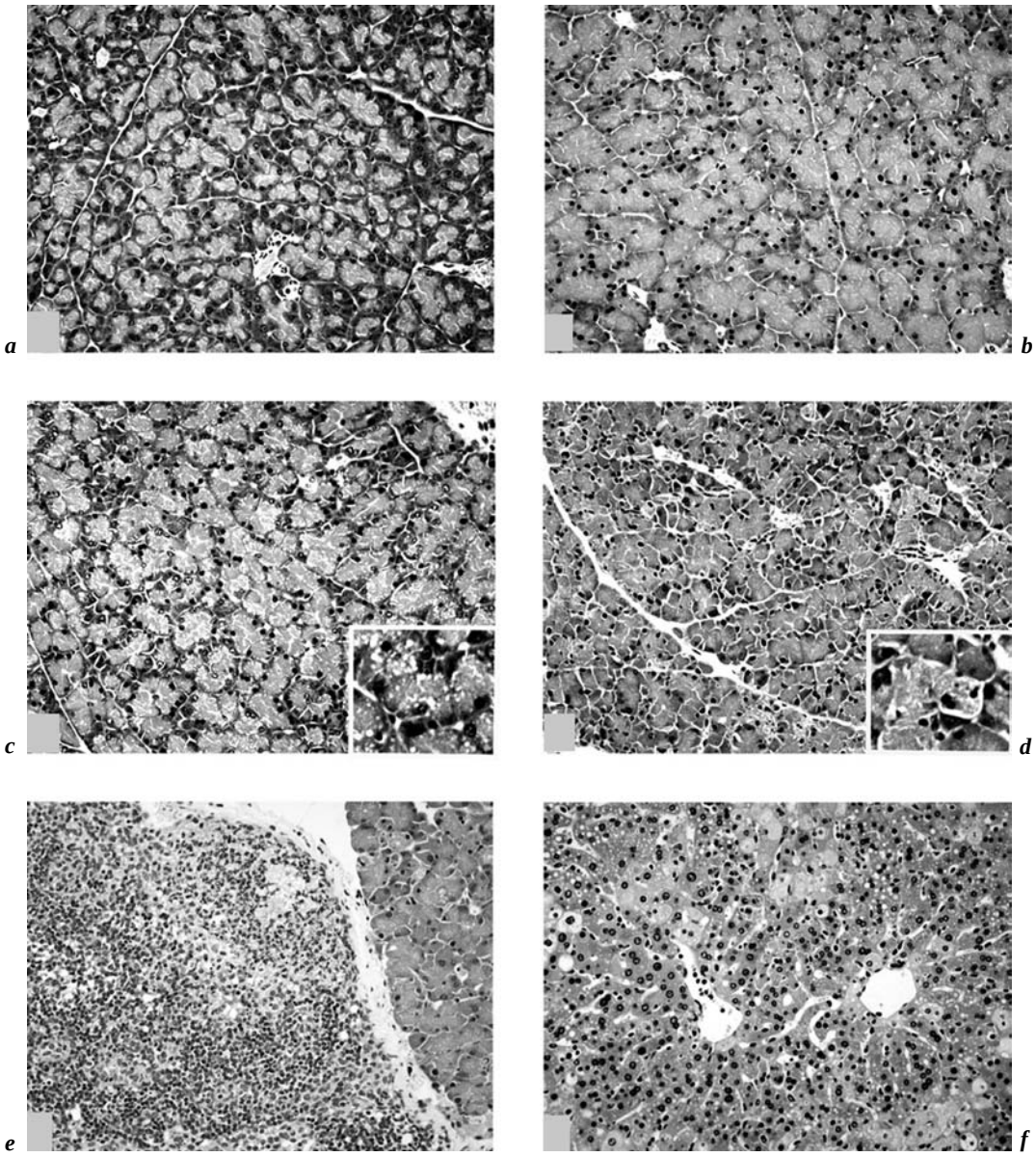

Fig. 8. Characteristics of ethionine-induced pancreatitis. ***a*** Pancreas from a control mouse. No abnormalities are seen. ***b*** Pancreas at 1 day after feeding a choline-deficient plus 0.5% ethionine diet. Swelling of acinar cells is noticeable. ***c*** Pancreas at 2 days after treatment. Vacuoles are seen in acinar cells. A scattering of necrotic acinar cells is evident. ***d*** Pancreas at 5 days after treatment. Necrosis of acinar cells has become prominent. Inter- and intralobular edema is observed. ***e*** Juxtapancreatic lymph node at 5 days after treatment.

Ethionine-induced Pancreatitis

Ethionine, a metabolic analogue of methionine, is an inhibitor of protein synthesis and has been used for investigations on metabolic disturbances in pancreatitis [108–112]. There are some reports indicating that ethionine alone can induce pancreatitis in animals, although ethionine treatment of animals given a choline-free diet has an indisputably greater inductive effect [113, 114]. The causes of this experimental pancreatitis evoked by ethionine in the context of choline deficiency are commonly believed to be deficient secretion and intracellular activation of zymogen granules, since a marked increase in the number of zymogen granules in acinar cells in the initial stage and a decrease in pancreatic enzyme concentrations in the blood have been found in model animals [113, 115].

We performed the following experiment treating choline-deficient mice with ethionine: Five-week-old mice were given a choline-deficient diet with or without 0.5% ethionine and sacrificed at 1, 2, 3, 4 or 5 days after initiation of the treatment.Their pancreata were removed and histologically examined. One day after initiation of the treatment, the acinar cells swelled and the number of their zymogen granules increased (fig. 8b). After 2 days, fine vacuoles were noted in the acinar cells (fig. 8c). Necrosis of these cells became prominent at 5 days (fig. 8d). Although apoptotic figures were seen, self-digestion was considered as the main cause of cell death. However, fibrosis did not progress in pancreas during the experiment period. Therefore, it might be necessary to use animals following withdrawal after a certain period of ethionine treatment for investigations on chronic pancreatitis or pancreatic fibrosis.

In addition, in the juxtapancreatic lymph nodes, infiltrates of white blood cells and their necrosis were often conspicuous. These changes in lymph nodes seemed to be associated with the pancreatitis (fig. 8e). Furthermore, hepatic injury and pancreatic failure remarkably occurs in this model [116–118]. In our experiments, fatty changes and necrosis of hepatocytes were obvious even just 1 day after the start of treatment (fig. 8f). It is therefore necessary to be aware that severe hepatic failure occurs in this model even more than pancreatitis, and to consider the degree of involvement of other factors, especially those from hepatic failure, in the study of therapeutic effects on acute pancreatitis.

Edema, neutrophilic infiltration and necrotic debris are observed. ***f*** Liver at 1 day after treatment. Widespread necrosis of hepatocytes is seen. This may indicate that the treatment induced not only pancreatic failure but also undeniable hepatic injury.

Arginine-induced Pancreatitis

Arginine-induced pancreatitis is also classified as a metabolic disorder and is used in many studies to clarify the mechanism of pancreatits [119–125]. Amino acid imbalance and alteration of protein metabolism initially occur, followed by cessation of synthesis of zymogen granules and necrosis of acinar cells [121–123]. Moreover, there are reports indicating that excess arginine reduces pyrimidine biosynthesis and thus inhibits nucleic acid and protein synthesis [124]. Swelling and rounding of mitochondria or dilation of the endoplasmic reticulum are also reported to be predominant changes in pancreatic acini [125].

We did an experiment on arginine-induced pancreatitis in mice. Mice were intraperitoneally injected with L-arginine-HCl at doses of 3, 6 or 9 g/kg twice, with a 1-hour interval between injections, and were sacrificed at 3, 6, 9, 12 or 24 h, or 2, 3, 7 or 14 days after the treatment. At 24 h after treatment, vacuoles of various sizes and necrosis were seen in the acinar cells (fig. 9b, c). Hypertrophy of acinar cells, which was observed in the pancreatitis induced by the ethionine plus choline-deficient diet, was not observed in the case of the ariginine-induced disease. Necrosis and inflammatory changes continued for 3 days afterward (fig. 9d). At 7 days after treatment, there was a tendency toward recovery. In any case, there was no fibrosis or ductal proliferation under our experimental conditions.

Fas Ligand or LPS-induced Pancreatitis

There are many reports that indicate a relationship between apoptosis and pancreatitis [126–133]. It is prudent to distinguish apoptosis from necrosis and to consider the involvement of fas/APO-1 ligands [134–136]. In our investigation on the appearance of apoptotic cells, detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method [137, 138], TUNEL-positive cells were observed in the inflamed area in the pancreas from animal models and humans. It is therefore possible that apoptosis arises in association with pancreatitis. However, we were interested in the order of events in pancreatitis, i.e. whether apoptosis was induced after inflammation or whether apoptosis induced inflammation. We therefore treated animals with Jo2, an antibody with the capability to mediate apoptosis. Hundred micrograms of Jo2/animal was intravenously injected into ICR, C3H/HeN and DBA/1J mice [139, 140]. It had been earlier confirmed that this dosage was sufficient to induce apoptosis in immune-system organs. As a result, at the stage when abundant apoptotic figures were seen in thymus, lymph nodes and spleen, apoptotic acinar cells in the pancreas were very few. Inflammatory changes were not induced in the pancreas (fig. 10a, b). These facts suggest that pancreatic acinar cells are less likely to express fas/APO-1 on their surface in comparison with immune cells and that the cells rarely go into apoptosis in the presence of

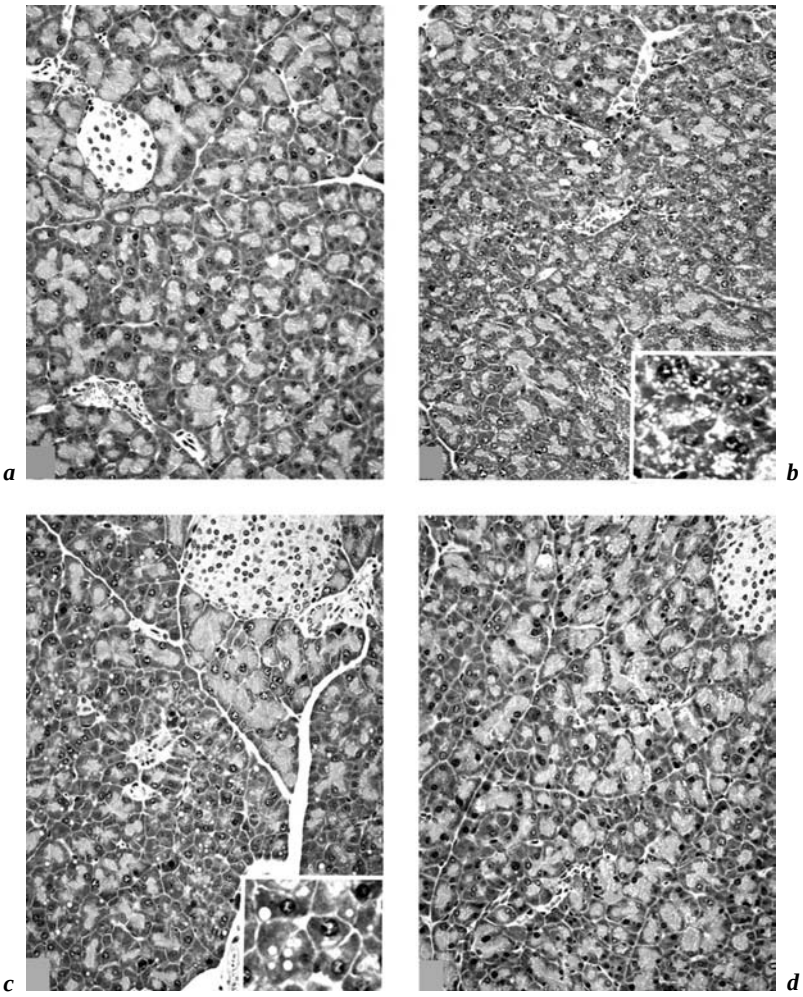

Fig. 9. Characteristics of arginine-induced pancreatitis. ***a*** Pancreas from a control mouse. No abnormalities are seen. ***b*** Pancreas at 6 h after the last treatment with 9 g/kg. Small vacuoles are seen in acinar cells. ***c*** Pancreas at 12 h after the last treatment with the same dose as (***b***). Comparatively large vacuoles have appeared. Scattered necrosis of acinar cells is evident. ***d*** Pancreas at 3 days after the last treatment with the same dose as (***b***). There is scattered colliquative necrosis. Inflammatory changes are very slight and not much different from (***c***).

sufficient quantities of fas/APO-1 ligands in serum. Moreover, it might also be possible to conclude that apoptosis is not a main cause of pancreatitis. At any rate, animals treated with Jo2 died at an early date but did not show any signs of fibrosis at the time of their death.

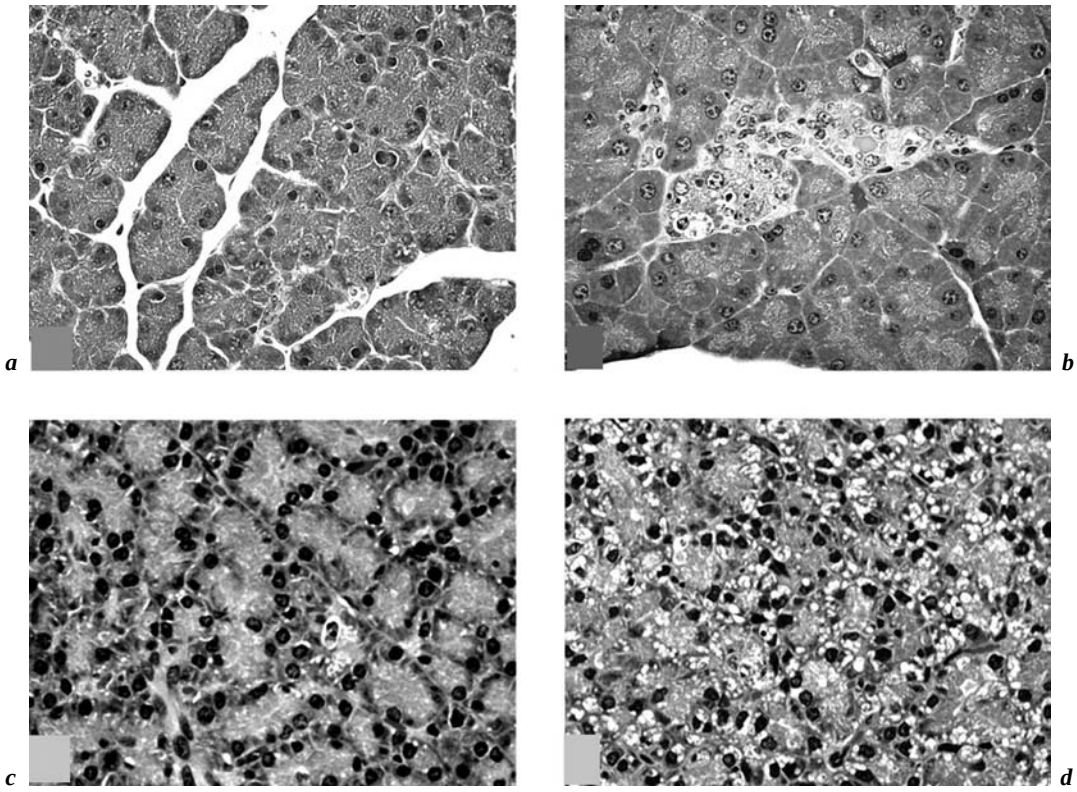

Fig. 10. Characteristics of pancreas from animals treated with Jo2 (anti-Fas antibody) or LPS. ***a*** Pancreas from a C3H/HeN mouse at 1 h after treatment with Jo2. Only a few apoptotic cells are seen. ***b*** Pancreas from a C3H/HeN mouse at 4 h after treatment with 100 μg of Jo2 per animal. A few apoptotic cells are seen. At the same time, many more cells showing apoptosis are seen in the liver, spleen, lymph nodes, bone marrow, intestinal epithelium, and other organs. The pancreas is therefore possibly not so sensitive to Fas-ligand. After withdrawal of the treatment, fibrosis is not observed. ***c*** Pancreas from a New Zealand White (NZW) rabbit at 4 h after the last treatment with LPS. Apoptotic cells are scarce. ***d*** Pancreas from a NZW rabbit at 6 h after the last treatment with LPS. Apoptotic cells have increased in number, and vacuolar degeneration is marked.

On the other hand, approaches to induce pancreatitis by treatment with LPS have been attempted for a long time. There are many reports on models prepared by intraductal injection of endotoxin for the purpose of elucidating the mechanisms of infection or immunization [141–144]. We also have had experience in studying LPS-induced pancreatitis in rabbits. In brief, New Zealand White rabbits received LPS (5 μg/kg, purified from *Salmonella minnesota*)

[145, 146] at 0, 5 and 24 h were histopathologically investigated. At 24 h after the last treatment with LPS, the rabbits showed signs of apoptosis in acinar cells (fig. 10c, d), however, the signs were more frequent in inflammatory cells than in pancreatic acinar cells. When the pancreata from the LPS-injected rabbits showed changes indicative of apoptosis, hemorrhage, thrombus formation and necrosis had already appeared in the livers, kidneys, spleens or hearts from the same rabbits. It goes without saying that these pancreatic changes would depend on the factors leading to multiple organ failure, which is commonly noted in septicemic diseases, rather than on the direct effect from LPS.

Mechanisms of Fibrous Regression in Animal Models

We have had some experience in investigating the mechanisms involved in the process of fibrous regression after the peak of fibrous formation in animal models of chronic pancreatitis [66]. In our studies, it became apparent that fibrous regression depends on a change in the balance between factors promoting fibrosis and those causing its dissolution. That is to say, the WBN/Kob rats used in the study, showed accelerated collagenase activity in the pancreas after the fibrosis had occurred. On the other hand, the activity leading to fibrosis seemed to remain, because the amount of prolyl hydroxylase, which plays a role in collagen synthesis, was still maintained at a high level. We hence concluded that the reversibility of fibrosis in the WBN/Kob rats was partly due to the ability of the pancreas to elevate its collagen-degrading activity above its collagen-forming activity. In recent years, there have appeared a growing number of reports on studies that have analyzed the mechanism underlying the resolution of pancreatic fibrosis in relation to both formation and degradation [147–150].

Also, we immunohistochemically investigated pancreatic fibrosis in animal models of pancreatic fibrosis. We compared fibrosis in WBN/Kob rats with that in the DBTC model. Fibrosis in WBN/Kob rats, which is reversible, was due to fibers composed of type III collagen (fig. 11a, b). In contrast, in the DBTC model, the fibrosis around the pancreatic ducts is irreversible, and was due to fibers composed of type I collagen (fig. 11c). However, the interlobular or intralobular fibrosis in the DBTC model, which resolved with time, was immunohistochemically positive for type III collagen (fig. 11d). As type I collagen is known to be more resistant to enzymic degradation than type III [151, 152], fibrosis involving type III collagen would be more easily disrupted or resolved. This indicates that the irreversible fibrosis seen in human chronic pancreatitis may be due to the fact that the fibers involved are mostly type I collagen [150, 153] and that approaches to limit synthesis of type I collagen may be very significant for establishing treatment guidelines. If it is possible that

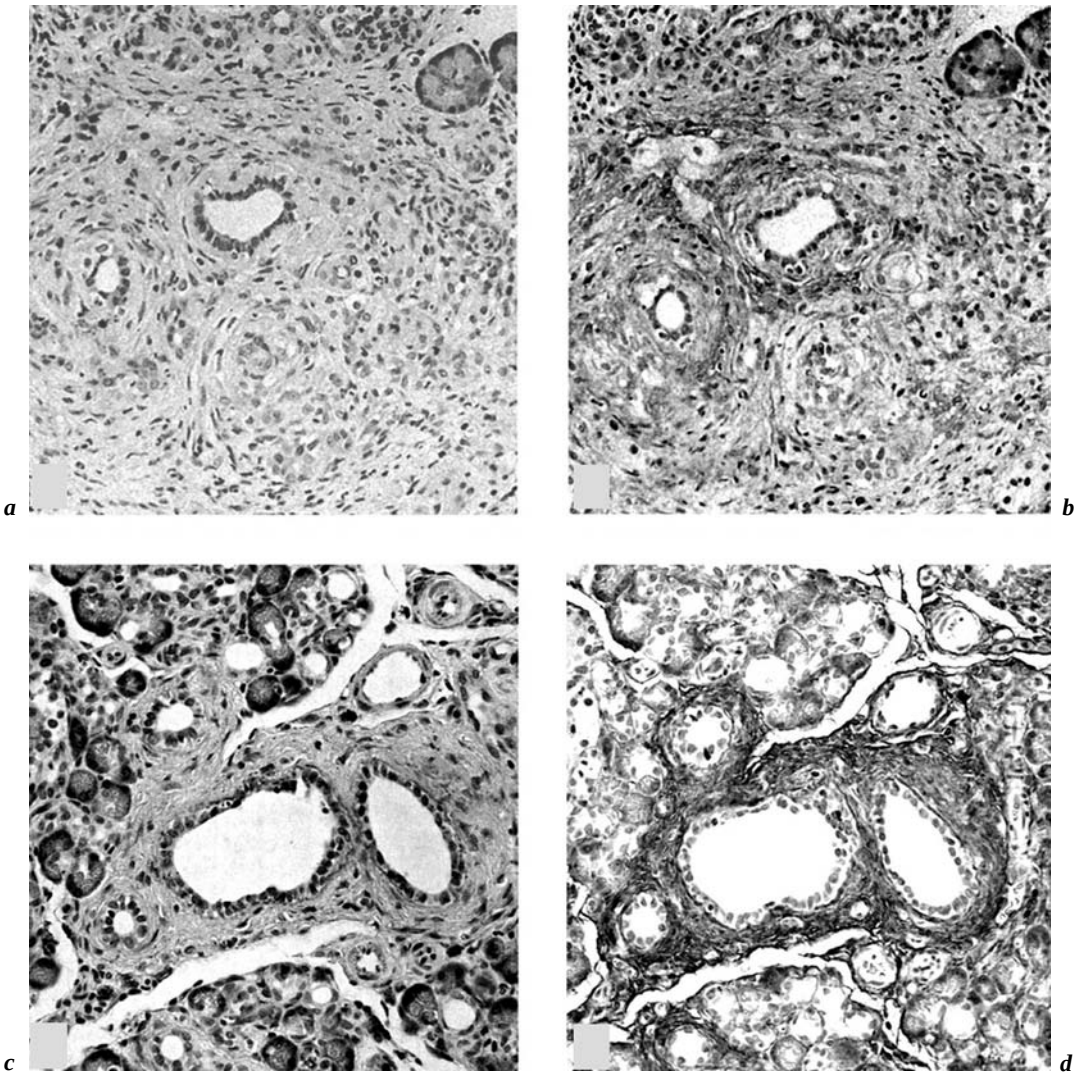

Fig. 11. Immunohistochemical characteristics of pancreatic fibrosis from male WBN/Kob rats and DBTC model rats. ***a*** Type I collagen immunohistochemistry of pancreas from a WBN/Kob rat at 4 months of age. Scarcely any positive immunoreaction is seen in the area of fibrosis. ***b*** Type III collagen immunohistochemistry of the pancreas from the same animal as in (***a***). It is evident that the fibrosis in WBN/Kob rat is mostly due to fibers composed of type- III collagen. ***c*** Type I collagen immunohistochemistry of pancreas from a DBTC model rat at 2 weeks after the treatment. Positive staining is seen in fibrous elements around the pancreatic ducts, indicating a great difference between the fibrosis in WBN/Kob rats and that in DBTC model rats. ***d*** Type III collagen immunohistochemistry of pancreas from the same animal as in (***c***). As in the case of the WBN/Kob rats, type III collagen is

fibrosis in human pancreatitis could be controlled by promoting the formation of type III collagen while inhibiting that of type I collagen, it might be possible to prevent acute pancreatitis from progressing to pancreatic fibrosis or to treat recrudescence of chronic pancreatitis.

Additional Statements

As mentioned above, animal models of pancreatitis show various features. Obviously, it is not possible to extrapolate these characteristics to pancreatic fibrosis in humans. However, use of these models for study purposes might be effective and may provide medical guidelines for treatment of the disease. It is therefore important that researchers understand well the features of each model, estimate the reactions of these models to treatment with accuracy and, moreover, clearly focus on those features that can be applied to the human case.

In the future, more and more pancreatic fibrosis-related proteins and genes will undoubtedly be discovered [147–149, 154–165]. Particularly the identification of the regulatory genes that control the genes involved in the synthesis of type I collagen may result in better therapeutic strategies [150, 157, 166, 167]. At present, genetic research on the pancreas is still difficult, as the pancreas is too rich in digestive enzymes to allow collection of its DNA or RNA. When the techniques in genomic analysis, which have markedly developed in recent years, become more practicable, the pancreatitis-related genes will be found and the cause of irreversible fibrosis may be revealed in detail, hopefully leading to more effective therapies for pancreatitis.

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present in the intralobular and interlobular areas of fibrosis, which areas undergo resolution. The appearance of type I collagen around the ducts is thought to be the reason why ductal fibrosis in DBTC model rats is hard to resolve.

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Experimental Pancreatitis in Animal Models

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Abstract

Chronic pancreatitis pathogenesis, toxic-metabolic agents, oxidative stress, obstruction, recurrent and severe acute pancreatitis (necrosis-fibrosis), autoimmune and genetic factors, and mechanisms of chronic pancreatitis remain under investigation. Chronic pancreatitis is histologically characterized by progressive, dense irregular fibrosis with destruction and loss of the exocrine parenchyma. Fibrosis is a potentially reversible condition in early stages. In recent years, the identification and characterization of pancreatic stellate cells (PSCs) indicate a key role for activated PSCs in the early stages of fibrogenesis. The role of PSCs has been examined in vivo (using pancreatic tissue from animal models of experimental pancreatitis) and in vitro (using PSCs in culture). The reversibility of fibrosis in ethionine-induced pancreatitis in dogs has been observed in the early stages. Activated PSCs, α-SMA-positive cells morphologically produce and secret extracellular matrix proteins, colagen and fibronectin. It is likely that dense and irreversible fibrosis in chronic pancreatitis was formed after recurrent and severe pancreatic injury.

Chronic pancreatitis is histologically characterized by dense irregular fibrosis with destruction and loss of the exocrine parenchyma. It is not known whether the progression of fibrosis occurs from acute to chronic pancreatitis. Neither is it known whether destruction and loss of the exocrine parenchyma induce pancreatic fibrosis. Prominent theories of chronic pancreatitis pathogenesis, the toxic-metabolic theory, the oxidative stress hypothesis, the stone-and-duct-obstruction theory and the necrosis-fibrosis hypothesis are known. Experimental duct ligation and ethanol feeding induce collagen synthesis in the rat pancreas [1, 2]. Ethanol feeding induces metabolic disturbance in pancreatic

exocrine parenchyma. However, experimental fibrosis in the pancreas of small animals such as rats probably decreases after such injuries. After injury, the rat pancreas regenerates with a decrease and resolution of fibrous deposition. It is difficult to experimentally induce dense fibrosis in the rat pancreas. Recently, activated pancreatic stellate cells (PSCs) have been implicated in the pathogenesis of pancreatic fibrosis and inflammation [3–5]. PSCs are located in interlobular areas and periacinar spaces of the rat pancreas. PSCs (also known as vitamin A-storing cells) were first described in the pancreas of mice given vitamin A by Watari et al. in 1982 [6]. These cells were identified by electron microscopy in normal rat and human pancreatic tissues [7], and are morphologically similar to the hepatic stellate cells (HSCs) that play a central role in the inflammation and fibrogenesis of the liver. HSCs, also called vitamin A-storing cells, Ito cells, or fat-storing cells, are predominantly located in the space of Disse [8]. During liver fibrogenesis, HSCs are activated and transformed into myofibroblasts. There is increasing evidence demonstrating a central role for PSCs in pancreatic fibrogenesis. PSCs are identified using antibodies to desmin and glial acidic protein (GFAP) [4]. During pancreatic injury PSCs are activated and transformed into myofibroblasts in a manner similar to HSCs. Activated PSCs expressing smooth muscle actin (SMA) produce and secrete the extracellular matrix proteins, collagen, fibronectin and laminin [9]. Cytokines such as transforming growth factor-β and platelet derived growth factor mediate activation of PSCs. These cytokines are produced and secreted by parenchymal cells, inflammatory cells, and PSCs. PSC activation occurs in fibrotic areas of pancreatic tissue from patients with chronic pancreatitis and from experimental animal models [10].

Materials and Methods

To investigate the mechanism of fibrosis progression in chronic pancreatitis, we studied the process of pancreatic fibrosis in male dogs (approximately 12 kg body weight) after oral administration of DL-ethionine (ethionine) as methionine analogue.

Group 1: Ethionine at a dose of 130 mg/kg/day was given orally for 7 days. Dogs were sacrificed 1, 2, 3, 7 and 30 days after the last feeding.

Group 2: Ethionine at doses of 10, 50 and 100 mg/kg was given once or twice a week for 10 or 20 weeks. Dogs were sacrificed 7 days after the last feeding.

Group 3: Ethionine at a dose of 75 mg/kg was given once a week for 15 weeks. Dogs were sacrificed 2, 3, 4 and 26 weeks after the last feeding.

Pancreas tissues were fixed in glutaraldehyde (2%) phosphate buffer (0.1 m, pH 7.4) at 4°C for 2 h and subjected to electron microscopic analysis. Other pancreas tissues were frozen for immunohistochemistry and fixed in formaldehyde (10%) for histochemistry and immunohistochemistry.

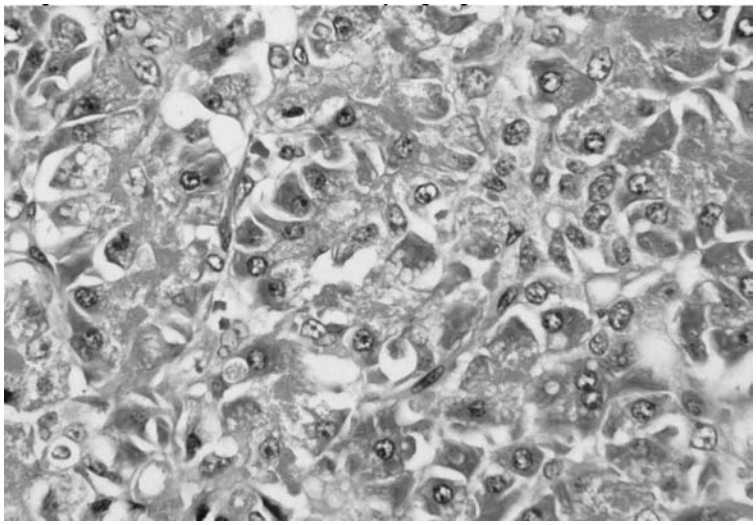

Fig. 1. Group 1. Day 3. Mild proliferation of spindle shaped cells in the periacinar area. Degeneration with decreased number of zymogen granules in acinar cells.

Results

Only few spindle-shaped cells were found in the periacinar and interlobular areas in the normal pancreas. Fibrous elements were found in the ductal wall, periductal area and interlobular area.

Group 1: On day 3 after the last feeding of ethionine, mild proliferation of spindle cells staining for α-smooth muscle actin (α-SMA) was found in the periacinar and periductal areas (fig. 1). Under the electron microscope, zymogen granules, decreased in number and size, were found in acinar cells. On day 7, there was considerable fibrosis with proliferation of α-SMA positive cells (myofibroblasts) (fig. 2). On day 30, fibrous deposition had decreased, but mild fibrosis was focally found in the periductal and regenerated periacinar areas (fig. 3).

Group 2: Marked pancreatic atrophy and mild to moderate fibrosis in the periacinar, interlobular and peri-ductal areas was found.

Group 3: Marked pancreatic atrophy was found 2, 3 and 4 weeks after the last feeding (fig. 4a). The pancreas, showing normal macroscopic appearance and weight, was regenerated after 26 weeks (fig. 4b). Formation of fibrous connective tissues was most manifest 2 weeks after the last feeding. Fibrosis was found in the periductal and inter- and intra-lobular areas with destruction and

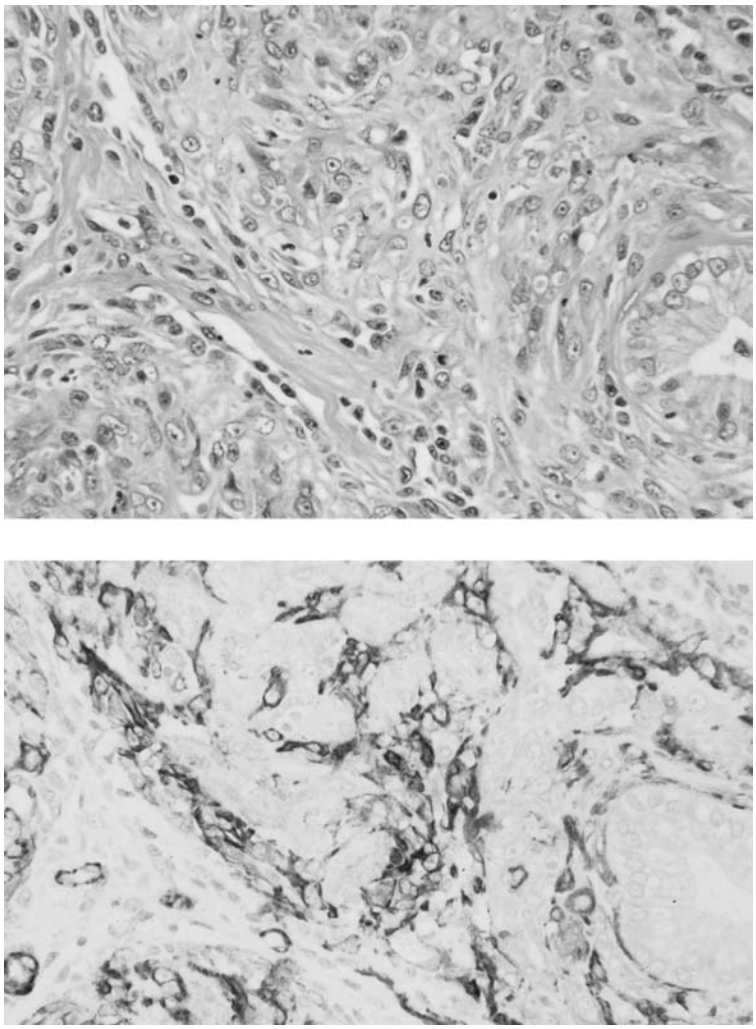

Fig. 2. Group 1. Day 7. ***a*** Proliferation of spindle shaped cells and collagen fibers in the periductal and intralobular areas. ***b*** Spindle shaped cells and capillary staining for α-SMA.

loss of the exocrine parenchyma (fig. 5a). An increased staining of type III collagen and a lesser staining of type I collagen were found in all zones of fibrosis. Fibronectin was also found between the collagen bundles. Electron microscope findings showed fine collagen bundles in this area. Such fibrosis decreased thereafter and mild fibrosis was found in the periductal, perivascular and interlobular areas after 3 weeks (fig. 5b). Fibrosis had largely disappeared

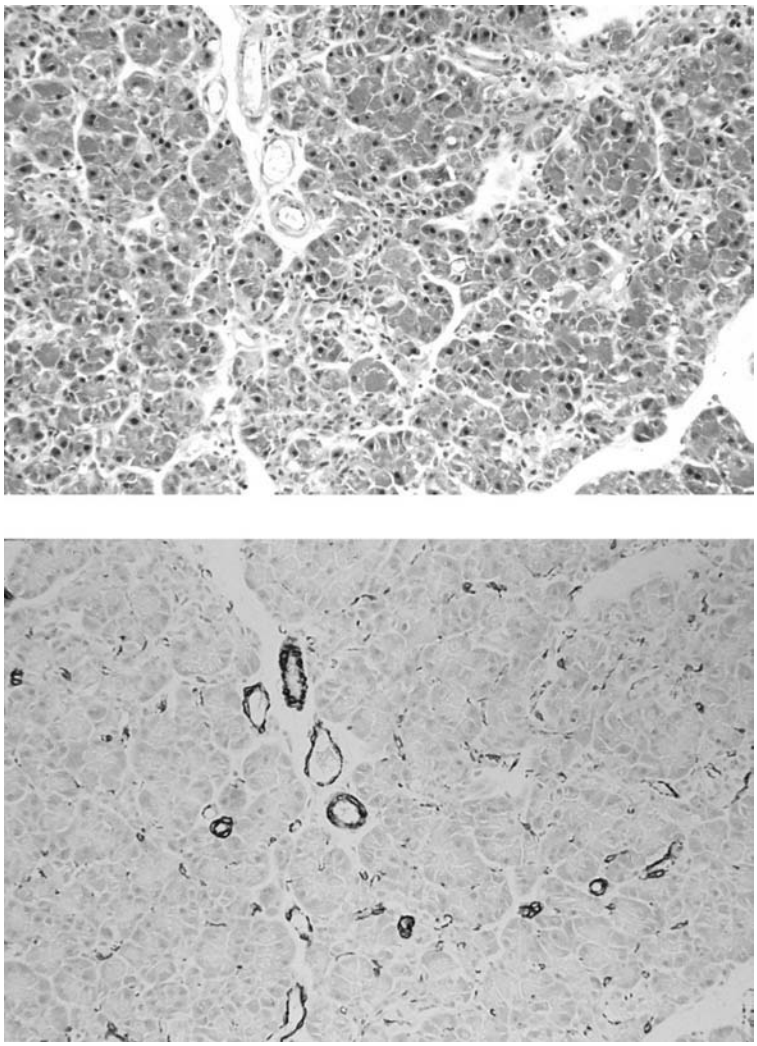

Fig. 3. Group 1. Day 30. ***a*** Slight proliferation of spindle shaped cells in the periacinar and interlobular areas of the regenerated pancreas. ***b*** Slight proliferation of α-SMA positive spindle cells.

after 26 weeks. Pancreas lobules were almost regenerated, but focally remodelled with aggregated pancreatic ductules. Mild fibrosis was found only in these periductules and focally in interlobular areas after 26 weeks (fig. 6a, b). Slight proliferation of α-SMA positive cells (myofibroblasts) was found in these areas (fig. 7a, b) [11].

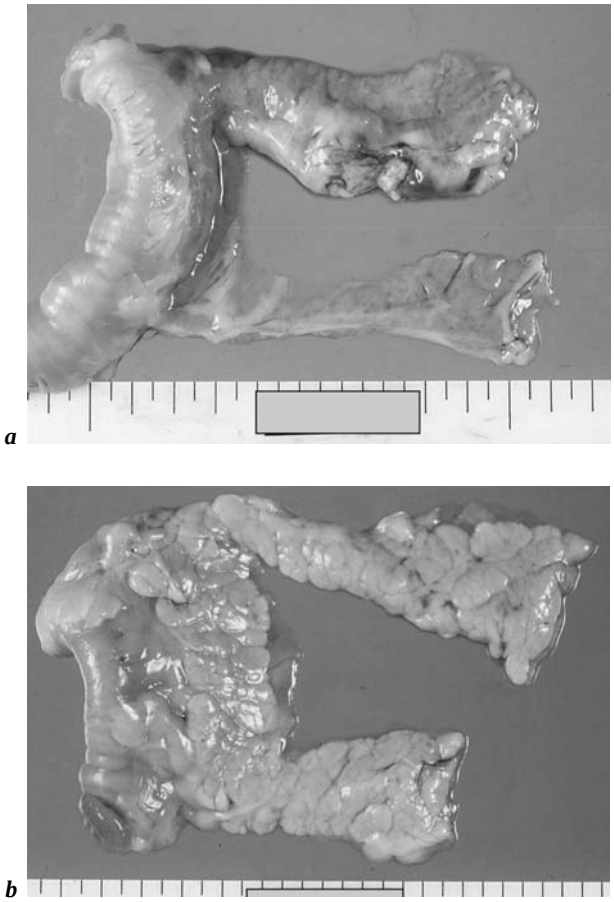

Fig. 4. Group 3. ***a*** Week 4. Marked atrophy of the pancreas. ***b*** Week 26. Regenerated pancreas showing almost normal macroscopic appearance.

Discussion

This study demonstrated the reversibility of fibrosis in ethionine-induced pancreatitis in dogs in the early stages. Destruction of the exocrine parenchyma and decreased number and size of zymogen granules in acinar cells were mainly found in early stages of injury. Infiltration of lymphocytes was slight. Activated PSCs and α-SMA positive cells (myofibroblasts) play a central role in pancreatic fibrogenesis. Morphologically these cells produce and secrete the extracellular matrix proteins collagen and fibronectin. It is therefore likely that the

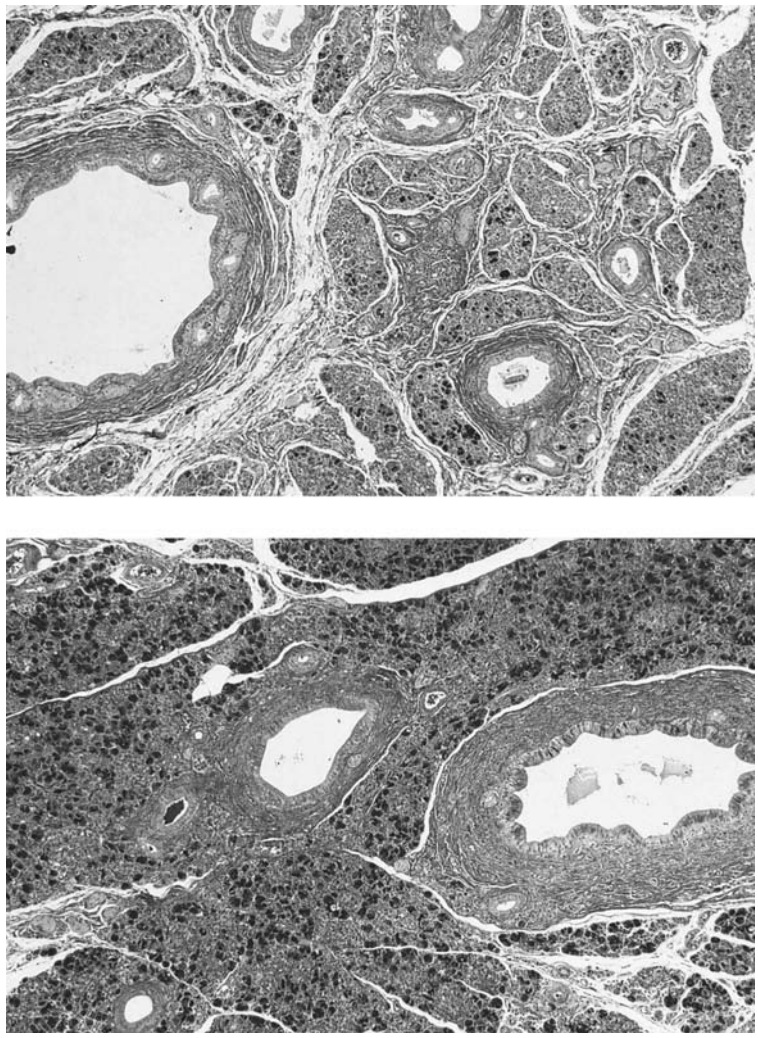

Fig. 5. Group 3. ***a*** Week 2. Fibrosis in the periductal and intra- and inter-lobular areas with atrophic exocrine parenchyma. Azan. ***b*** Week 3 Mild fibrosis in the periductal and inter-lobular areas. Azan.

formation of collagen bundles and resolution of fibrous depositions occur simultaneously between 2 and 4 weeks after the last feeding of ethionine, and that only the resolution of fibrous depositions occurs thereafter. These findings support the view that fibrosis in acute pancreatitis does not generally progress

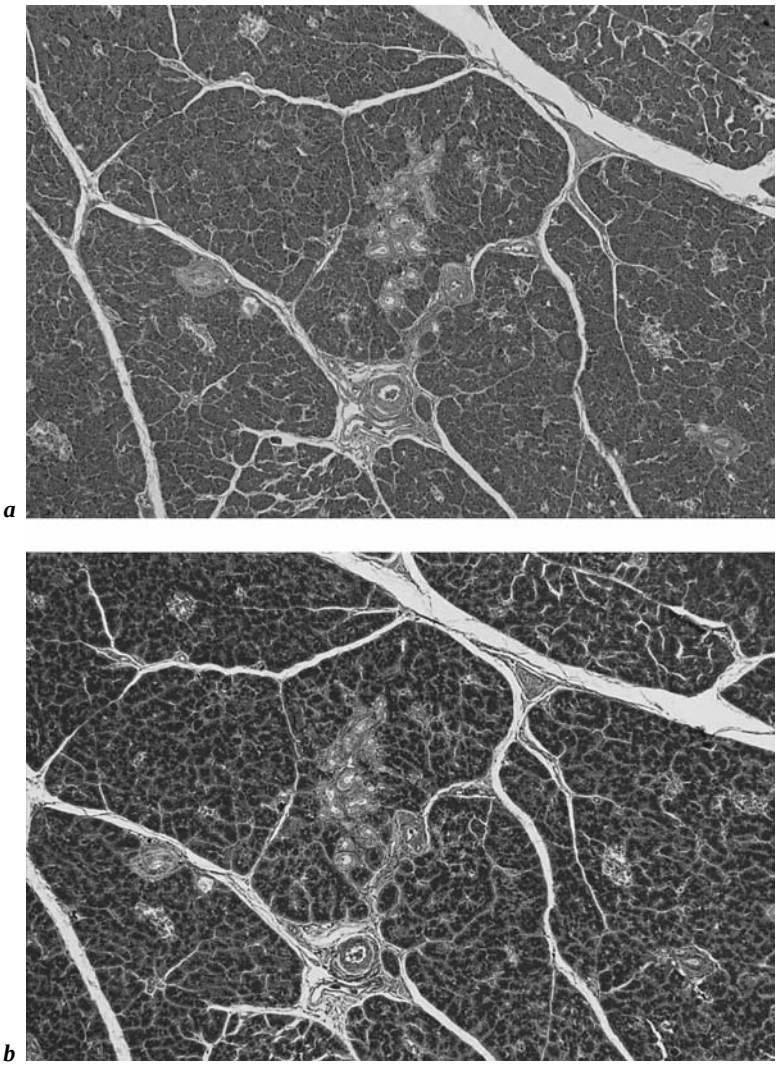

Fig. 6. Group 3. Week 26. ***a*** Focal mild fibrosis in the periductal, perivascular and interlobular areas. HE. ***b*** Mild proliferation of collagen bundle. Azan.

to that in chronic pancreatitis and that fibrosis in chronic pancreatitis probably occurs due to continuous pancreatic injury, which causes the release of pancreatic enzymes. Mild fibrosis remained only in the periductule, interlobular and perivascular areas. It is likely that collagen bundles bound to existing fibrous

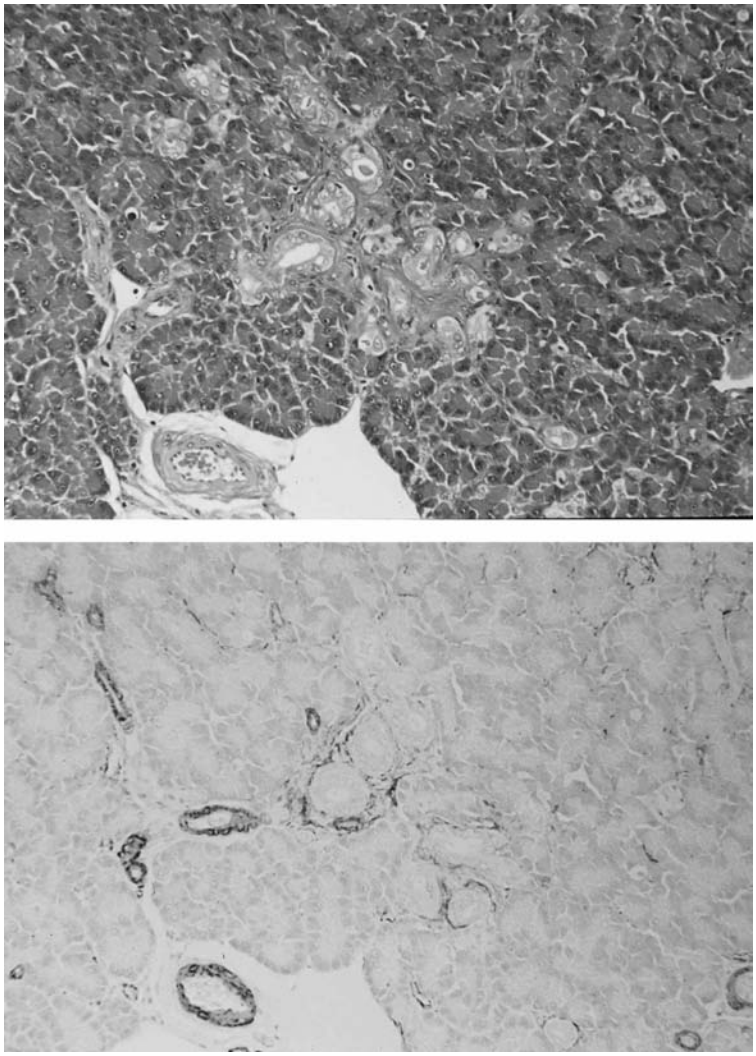

Fig. 7. Group 3. Week 26. ***a*** Mild fibrosis in the periductal area. Aggregation of ductules in the regenerated pancreas. ***b*** Mild proliferation of α-SMA positive spindle cells in the periductal and periacinar areas.

tissues such as pancreatic duct wall, vascular wall and interlobular stroma were slowly resolved.

In activated PSCs, a cell-cycle inhibitory protein $p21^{Cip1/WAF1}$ was present in the nucleus. With conversion of PSCs to fibroblasts, $p21^{Cip1/WAF1}$ translocated

to the cytoplasm [12]. It is proposed that the fibroblastic phenotype of the stellate cell is resistant to apoptosis and a terminally differentiated state, due to similarities in the pattern of changes in $p21^{Cip1/WAF1}$ associated with differentiation in monocytic cells and neuronal cells [13]. Fibroblastic cells not expressing α-SMA also play a role in pancreatic fibrosis in experimental models of pancreatitis. This phenotype is resistant to apoptosis. In this study fibroblastic cells were also found in fibrotic foci. However, α-SMA-positive stellate cells and also fibroblastic cells were decreased in number after removal of the injury.

In addition, PSCs were activated on exposure to ethanol in an in vitro model [14]. PSCs also have a capacity to metabolize ethanol to acetaldehyde and to synthesize collagen.

Pancreatic fibrosis in the animal model depends on the dose and duration of exposure to the metabolic agent. Activated PSCs play a central role in fibrogenesis, especially in early stages of the pancreas injury. PSCs were activated on exposure to cytokines, such as transforming growth factor-β and platelet derived growth factor, and also ethanol. Activation of PSCs occurs early in long-term pancreatic injury.

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Histological Characteristics of Chronic Pancreatitis, Based upon Etiology

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Abstract

Histological characteristics of alcoholic, bile, autoimmune, and obstructive pancreatitis are reviewed and summarized. Chronic alcoholic pancreatitis has characteristics such as interlobular fibrosis, nodular appearance of remaining lobules, and formation of protein plugs or stones in the pancreatic ducts. The difference in the distribution of fibrosis between alcoholic pancreatitis and chronic alcoholism is noted briefly. The histopathological changes of bile pancreatitis are degeneration/disappearance of the pancreatic ductal epithelium, and less progressive interlobular/periductal fibrosis. In autoimmune pancreatitis, narrowing of pancreatic ducts due to dense lymphoplasmacytic infiltration is the main remarkable feature. A significant number of these inflammatory cells are IgG4-positive plasma cells. Characteristics of obstructive pancreatitis are both inter- and intralobular fibrosis with duct dilatation especially in the progressive stage. Recognizing these characteristics helps not only to elucidate causes of pancreatic damage whose etiologies remain clinically unknown, but also to investigate the pathogenic mechanisms of chronic pancreatitis.

Chronic pancreatitis is an irreversible and progressive disease, and its pathological hallmarks are inflammation, glandular atrophy, ductal changes, and fibrosis [1]. Although excess alcohol consumption is the most common cause of chronic pancreatitis in developed countries, several other etiologies are known and there are many cases whose etiologies remain unclarified. Also, the exact pathogenic mechanism of chronic pancreatitis has so far remained elusive, in spite of a great deal of cellular, genetic and molecular research.

Over the past twenty years, we have observed and reported that there are several specific and characteristic pathological changes connected to some etiologies. To recognize these histological characteristics is not only helpful in

Table 1. Etiology of chronic pancreatitis

	Male	Female	Sex uncertain	Total
Alcoholic	525 (78.6%)	43 (29.7%)	14	582 (69.7%)
Idiopathic	98 (14.7%)	73 (50.3%)	8	179 (21.4%)
Bile	19 (2.8%)	10 (6.9%)		29 (3.5%)
Pancreas divism	6 (0.9%)	8 (5.5%)		14 (1.7%)
Hyperlipidemia	4 (0.6%)	4 (2.8%)		8 (1.0%)
Obstructive	5 (0.7%)	2 (1.4%)		7 (0.8%)
Chronic renal failure	5 (0.7%)	0 (0.0%)		5 (0.6%)
Hereditary	4 (0.6%)	1 (0.7%)		5 (0.6%)
Abnormal pancreatic choledochoductal junctions	1 (0.1%)	1 (0.7%)		2 (0.2%)
Stenosis of papilla	0 (0.0%)	2 (1.4%)		2 (0.2%)
Injury	1 (0.1%)	0 (0.0%)		1 (0.1%)
Drug	0 (0.0%)	1 (0.7%)		1 (0.1%)
Total	668 (80.0%)	17.4%		835

trying to elucidate the causes of pancreatic damage in cases whose etiologies remain clinically unknown, but also necessary to investigate the pathogenic mechanism of chronic pancreatitis.

The etiologies of chronic pancreatitis in Japanese patients are categorized in table 1 [2]. Autoimmune pancreatitis, whose disease concept has been recently established, is excluded from this table. Among these etiologies, this chapter focuses on histological characteristics of alcoholic, bile, and autoimmune pancreatitis. The histopathology of obstructive pancreatitis, whose mechanism of tissue damage has been well elucidated, is also described here.

Chronic Alcoholic Pancreatitis

In chronic alcoholic pancreatitis (CAP), pancreatic damage is observed in a patchy pattern throughout the pancreas. Parenchymal loss and fibrosis occur mainly interlobularly, leaving remaining lobules with a nodular appearance [3, 4].

Histopathological View of CAP and Progression

CAP is a progressive disease and the pathological characteristics differ depending on the stage of the disease and the area being investigated [5, 6].

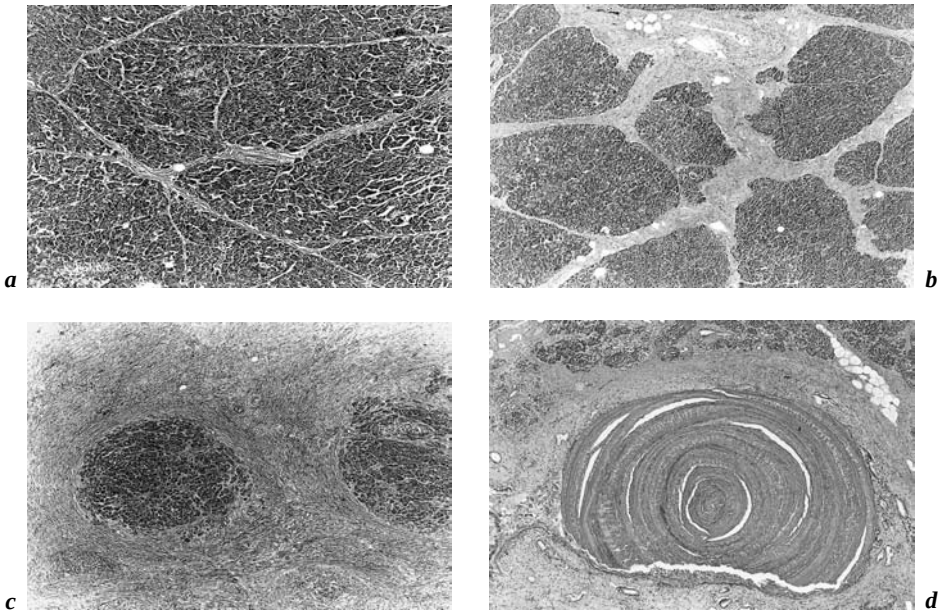

Fig. 1. Chronic alcoholic pancreatitis (CAP) by disease stages. ***a*** Mild fibrosis is seen at interlobular spaces in the early stage of CAP. HE. ×10. ***b*** Even when lobules become more atrophic and fibrosis has progressed in advanced stages of CAP, fibrosis is mainly localized interlobularly. HE. ×20. ***c*** Note the island-like or nodular appearance of the remaining lobules. HE. ×20. ***d*** In CAP, lamellar arranged collagen and mucoprotein, a protein plug, is often observed in pancreatic ducts. HE. ×20.

In the early stage of the disease, mild interlobular fibrosis is seen; the original interlobular stroma becomes lengthened or widened with fibrosis (fig. 1a). When this interlobular fibrosis progresses, and protein plugs or stones are formed in the pancreatic duct, atrophy of the lobules commences; atrophy and loss of acinar cells are seen [3]. So called 'nodular pancreatitis' is observed in some cases, in which severe inflammatory and repair reactions leave some lobules island-like, or nodular [7, 8]. In the advanced stage, most parenchyma is lost, leaving ducts and Langerhans islets isolated. Even in such severe cases, the shapes of the lobules usually retain their nodular, or so-called cirrhosis-like, appearance (fig. 1b, c).

There is a tendency that parenchymal loss and fibrosis is more severe at peripheral/pericapsular sites of the pancreas. Hence, progressed fibrosis sometimes results in stenosis of the common bile duct, obstruction of the splenic vein, or duodenal obstruction. So-called 'groove pancreatitis' is the state in which inflammation and fibrosis occur at the 'groove' surrounded by the

common bile duct, duodenal C-loop and pancreas head, resulting in duodenal stenosis or obstruction [9]. Also, in CAP localized portal hypertension sometimes occurs as a consequence of stenosis or obstruction of the splenic vein [10].

It is worth noting that, in chronic alcoholism or heavy drinkers, fibrosis is distributed intralobularly, in particular, periacinarly [5, 11]. Hence, the pathology of chronic alcoholism is quite distinct from CAP. Clinically, patients with chronic alcoholism also tend to develop liver cirrhosis rather than pancreatitis [12].

Protein Plugs and Stones

In CAP, protein plugs or protein stones are often observed in the pancreatic ducts [13] (fig. 1d). Protein plugs consist of protein derived from zymogen granules, erythrocytes, duct epithelium cells, and mucoprotein secreted by the pancreatic duct [14]. With time, a lamellar arrangement of collagen and mucoprotein can be seen. Pancreatic stones are formed in the protein plugs by the deposition of calcium ions derived from pancreatic juice.

Changes in Islets

In CAP, islets appear in a variety of sizes and irregular shapes, particularly in fibrotic lesions. In fibrotic lesions, a depletion of B cells and a relative increase in A, D and PP cells are observed. Clinically, the risk of complication of diabetes mellitus is higher in CAP, and even higher in CAP cases with pancreatic stones.

Other Histopathological Changes

Several metaplastic and hyperplastic changes are often observed in pancreatic duct epithelia, such as pyloric-gland-like mucinous metaplasia/hyperplasia and squamous metaplasia. Cystic changes of branch ducts are also often seen.

These changes, however, also occur as senile changes, and are not specific to CAP.

Bile Pancreatitis

Bile pancreatitis is observed in cases of cholelithiasis and abnormal pancreatic choledochoductal junctions. The degeneration/disappearance of pancreatic ductal epithelium is often seen in this type of pancreatitis [15]. Intraluminal aggregation of bacilli may be noted in some pancreatic ducts. Fibrosis progresses periductally and interlobularly (fig. 2), and in this type of pancreatitis, is generally mild and less progressive [16].

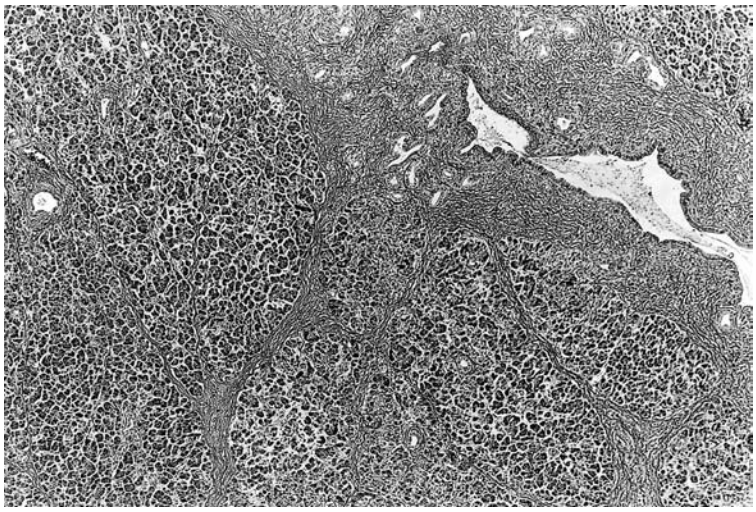

Fig. 2. Bile pancreatitis. Fibrosis is distributed periductally and extends to the interlobular spaces. Most of the pancreatic ductal epithelium has been lost due to the damage (choledochal cyst in a 52-year-old female). HE. ×20.

Autoimmune Pancreatitis

It has recently been elucidated that there is a group of patients with duct-narrowing pancreatitis in whom steroid therapy is very effective [17, 18]. Since this group of patients often has other autoimmune diseases such as Sjögren syndrome, and their serum γ-globulin, IgG, or particularly, IgG4, often shows a high value, the autoimmune mechanism has been suggested as the cause of this particular form of chronic pancreatitis, and it was named autoimmune pancreatitis [19–21]. In autoimmune pancreatitis, narrowing of the pancreatic ducts due to dense lymphoplasmacytic infiltration is seen [22, 23] (fig. 3a). These inflammatory cells are distributed especially within and around the pancreatic ducts, and often include a significant number of IgG4-positive plasma cells (fig. 3b). This contrasts with other types of chronic pancreatitis in which only mild infiltrations of inflammatory cells are usually seen, and IgG4-positive plasma cells are, if any, usually not so many. Inter- and intralobular fibrosis admixed with acinar atrophy are characteristics observed in autoimmune pancreatitis (fig. 3c), contrasting with the fibrosis pattern of CAP. Also, inflammatory processes sometimes cause obliterative phlebitis in autoimmune pancreatitis, contrasting with CAP, whereby thrombosis of the splenic vein is the usual finding.

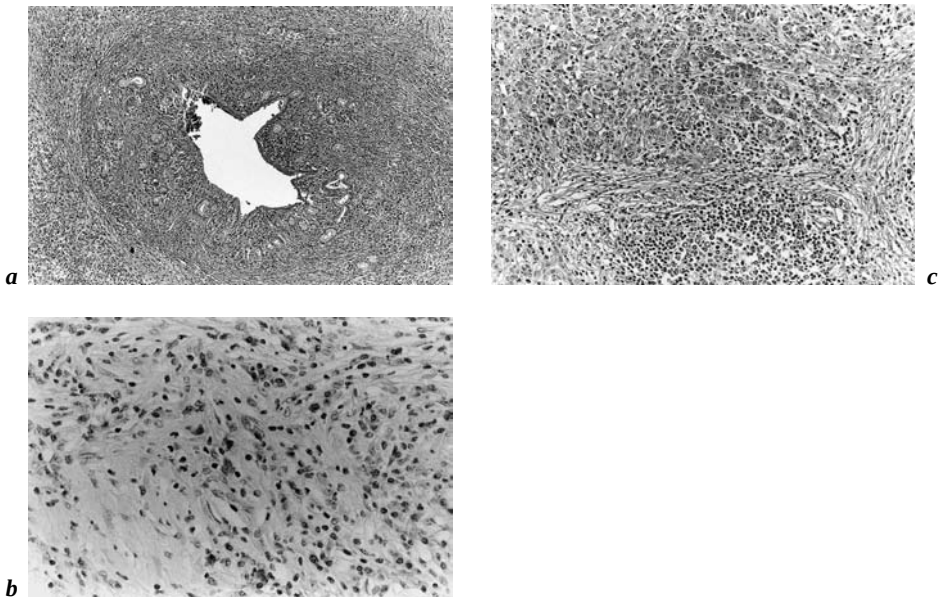

Fig. 3. Autoimmune pancreatitis. ***a*** Periductal lymphoplasmacytic infiltration and narrowing of the pancreatic ducts are evident. HE. ×20. ***b*** Some of the narrowing is caused by IgG4-positive plasma cells. Immunostain for IgG4. ×60. ***c*** Diffuse inter- and intralobular fibrosis as well as acinar atrophy is marked in this disease. HE. ×40.

Obstructive Pancreatitis

Obstructive pancreatitis is a form seen in pancreatic tissue at the distal side of duct-obstructive changes. This type is noted in cases of carcinoma of the ampulla of Vater, pancreas head, or pancreas body, and in some cases of pancreatic divism, where pancreatic damage is caused due to the impairment of pancreatic juice outflow [16].

Fibrosis is seen interlobularly in the early stage of obstructive pancreatitis, as in the early stage of CAP (fig. 4a). However, in contrast to CAP, intralobular fibrosis with marked acinar atrophy is also seen in progressed stages of obstructive pancreatitis (fig. 4b). Infiltration of a usually small, but sometimes moderate number of inflammatory cells is seen in fibrotic regions in all stages of obstructive pancreatitis, i.e. inflammatory cells such as neutrophils and macrophages infiltrate the interlobular spaces in the early stages, while they infiltrate both the inter- and intralobular spaces in progressed stages [24]. Dilatation of large pancreatic ducts is also observed in this type of pancreatitis.

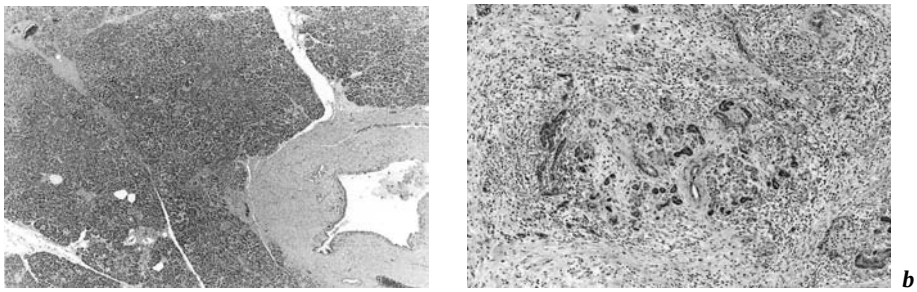

Fig. 4. Obstructive pancreatitis by disease stages. ***a*** In the early stage, fibrosis is seen mainly interlobularly. HE. ×10. ***b*** Both inter- and intralobular fibrosis occurs in progressed stages. Also note the moderate amount of inflammatory cells seen in both fibrotic regions. HE. ×20.

Comments

Recently, the TIGAR-O classification of chronic pancreatitis was devised to categorize the various known risk factors [25]. This classification is based on the idea that multiple genetic and environmental cofactors interact even in a case of chronic pancreatitis with an identified etiology. Hence, the histological characteristics based on etiology, as mentioned above, may be further subdivided or differentiated according to such cofactors interacting in the progression of pancreatitis.

As mentioned above, observation of the pathological courses and changes in each type of pancreatitis gives important information to clarify the pathogenesis. For example, histological differences of CAP from obstructive pancreatitis, such as interlobular-oriented fibrosis and frequent protein plug formation in ducts, may suggest that duct obstruction caused by protein plugs is not the main factor in the genesis of alcoholic pancreatitis [26].

This gives us abundant and valuable messages to continue careful observation and to identify the histopathological differences according to the interacting etiologies and cofactors. It helps not only in the clarification of pathogenesis, but also for pre-recognition of the clinical course, and deciding on the therapeutic approach.

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Groove Pancreatitis

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Abstract

Groove pancreatitis (GP) is often diagnosed in middle-aged alcoholic men presenting nausea, vomiting, upper abdominal pain and jaundice due to common bile duct stricture and duodenal stricture. Due to the absence of ultrasonographic and radiographic evidence of cancer and no definitive criteria for pre-operative diagnosis, diagnostic imaging is helpful to distinguish GP and pancreatic cancer. An intraoperative histologic examination using frozen sections may provide useful information. Etiology is not yet elucidated. Currently, two types of research approach have reported on the etiopathogenetic possibilities of this type of pancreatitis: inflammatory process of the duodenal wall, and around Santorini's duct. By histologic examination using paraffin sections of the pancreas, common bile duct and duodenum, GP should be confirmed after surgery. ERCP shows narrowing of the intrapancreatic common bile duct. Ultrasonography, CT and MRI demonstrates a mass lesion in the pancreas head. Preoperative diagnostic criteria may help to make a correct diagnosis of GP.

The anatomic space between the head of the pancreas, the right lateral and lower borders, and the duodenum is called a groove, where the common bile duct descends behind and where the anastomosing superior and inferior pancreaticoduodenal arteries run. Groove pancreatitis (GP) is defined as a special form of pancreatitis, arising in the groove. Scarring is mainly found in GP.

In 1973, Becker et al. [1] reported 117 cases of GP, first reported in German as 'Rinnenpankreas', from 600 resected pancreata. In terms of pathomorphological characteristics, they distinguished between (1) pure GP, (2) segmental pancreatitis of the head with groove involvement and (3) chronic homogeneous pancreatitis with groove involvement. In 1983, Stolte et al. [2] reported 30 cases of GP in 123 cases of chronic pancreatitis. They distinguished between pure and segmental GP. In pure GP, no fibrosis of the pancreatic parenchyma is seen, and in segmental GP, both pancreas and groove are affected.

Pathogenesis

With regard to the etiopathogenetic possibilities, Stolte et al. summarized four clinical characteristics of GP: (1) previous diseases of the biliary system, peptic ulcer and gastric resection; (2) mild dilation of the pancreatic duct; (3) higher frequency of narrowing of the duodenal lumen, and (4) cyst formation in the duodenal wall. The etiology of GP, however, so far remains controversial. Currently, two types of research approach have reported on etiopathogenetic possibilities.

Firstly, the disturbance of pancreatic juice outflow in Santorini's duct is highlighted, because stricture of the duct may be caused by inflammation around the duct. This can affect the intrapancreatic common bile duct and lead to fibrosis, inflammatory infiltrates and scarring. Other organic changes such as duodenal wall cysts, pancreatic head cysts, pancreatitis in duodenal pancreatic heterotopia and Brunner's gland hyperplasia may lead to develop stricture and disturbance of secretion of the orifice of the minor papilla. Chronic consumption of alcohol seems to be an important pathogenic factor.

Adsay and Zamboni [3] have proposed a new clinicopathologic entity named paraduodenal pancreatitis, unifying (1) cystic dystrophy of heterotopic pancreas, (2) pancreatic hamartoma of the duodenum, (3) paraduodenal wall cyst, (4) myoadenomatosis and (5) groove pancreatitis, because these lesions have common characteristics. They considered that fibrosis of the duodenum may affect the groove area and produce GP. They provided a new view on the pathogenesis of GP.

Case Presentation

Case 1

A 56-year-old man, an alcohol user, presented with a 1-month history of mild acute pancreatitis and obstructive jaundice, followed by increasing general fatigue and severe epigastric pain. CT showed a mass in the head of the pancreas (fig. 1). ERCP demonstrated a smooth stricture of the intra-pancreatic main bile duct and an irregular, incomplete, stricture in the main pancreatic duct (fig. 2). There were no definitive criteria for pre-operative diagnosis. Intraoperative histopathogical examination using a frozen section of the pancreas was performed to rule out a carcinoma. The diagnosis was chronic pancreatitis and no features of cancer were seen (fig. 3). The patient underwent a biliary stent placement in the common bile duct via Vater's papilla, which procedure provided a good outcome with recovery of bile duct drainage and complete pain relief. The histologic diagnosis of the paraffin sections was chronic pancreatitis. The patient had an uneventful postoperative course until his death in a traffic accident 2 years later.

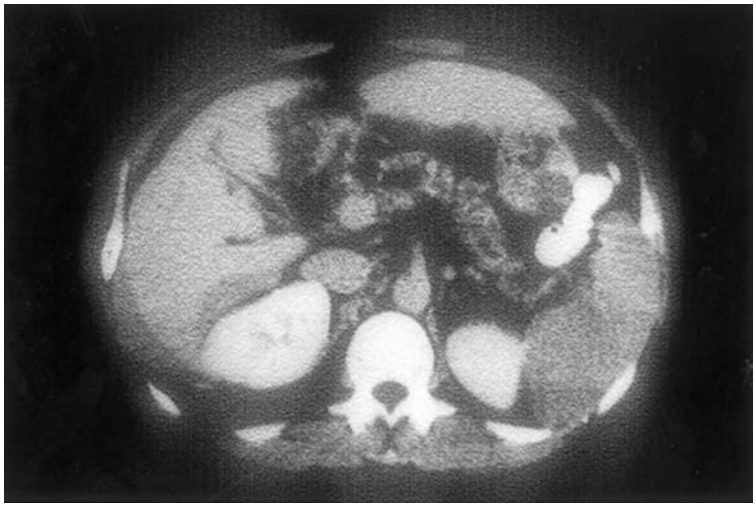

Fig. 1. Iso-hypodense mass (CT).

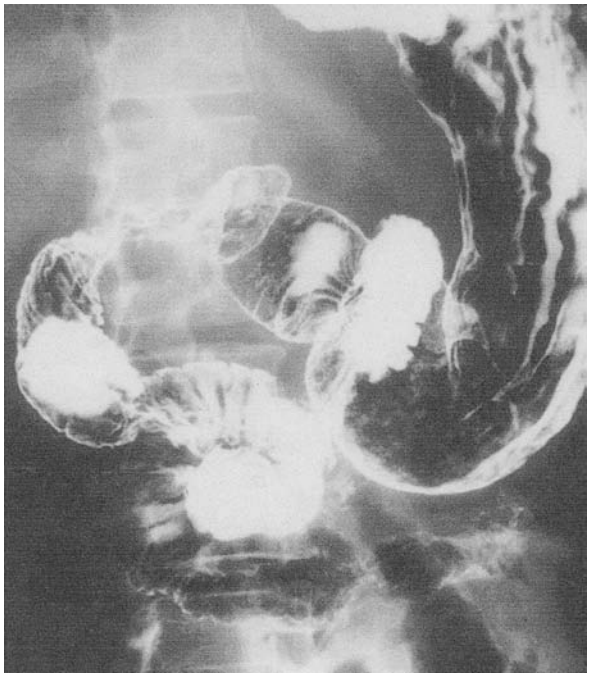

Fig. 2. Segmental stricture of duodenal C-loop (hypodonic duodenography).

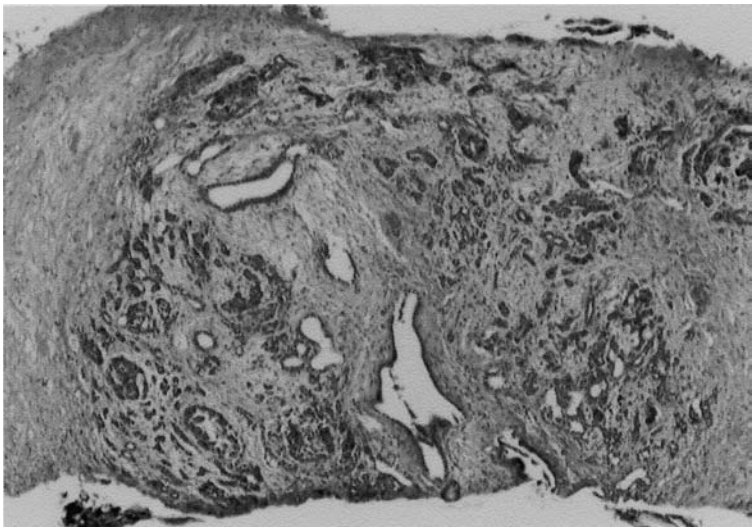

Fig. 3. Chronic pancreatitis (frozen section; HE).

Case 2

A 44-year-old man, with a history of alcohol abuse, presented with a 3-day history of nausea, vomiting and general fatigue after heavy drinking. In the hospital, anemia was noted. An endoscopic examination disclosed a marked thickening of the duodenum (fig. 4). ERCP showed narrowing of the intrapancreatic common bile duct (fig. 5). Ultrasonography and CT demonstrated a mass lesion in the pancreatic head. A duodenal cancer could not be excluded, and he underwent a pancreatoduodenectomy. Intraoperative histopathogical examination using a frozen section of pancreas was not performed. The histologic diagnosis of the resected specimen was chronic pancreatitis and Brunner's gland hyperplasia (fig. 6). The lumen of the common bile duct was narrowed by marked fibrosis. The Santorini duct was dilated and contained protein plugs. The postoperative course was uneventful.

Pathophysiology

Patients with common chronic pancreatitis suffer from repeated vomiting, severe epigastric pain and weight loss. GP presents various clinical symptoms due to stricture of the common bile duct and the duodenum. Inflammatory infiltration and fibrosis induce scarring in the groove, and result in obstructive jaundice by narrowing the common bile duct. Stricture of the duodenum by marked thickening of the duodenal wall causes abdominal fullness, nausea and vomiting.

The function of Brunner's glands is not well known. The cytoplasma of the Brunner's gland epithelial cells contains neutral mucin that is PAS-positive and

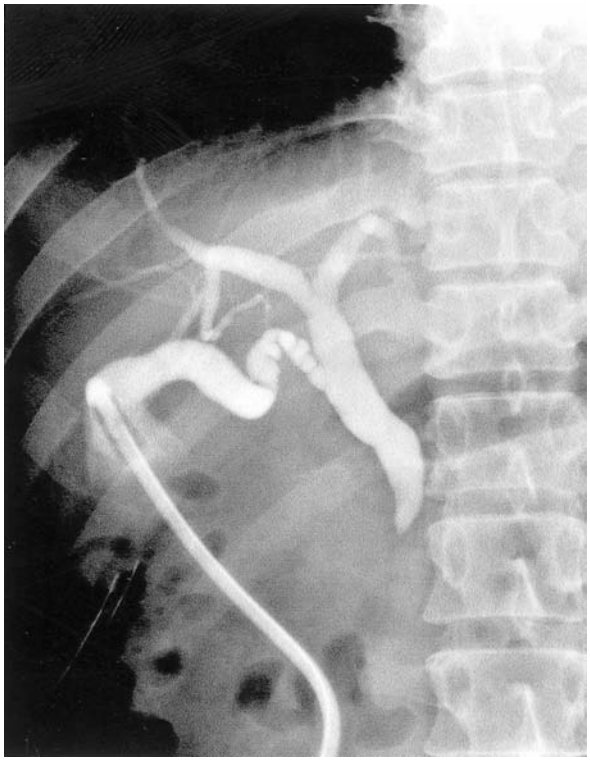

Fig. 4. Stricture of the common bile duct (ERCP).

diastase resistant. The mucin might play a role in neutralizing the duodenum content of elevated gastric acid. Hyperplasia of Brunner's glands is probably an adaptive reaction to the exocrine insufficiency of the pancreas or the changes in gastric function (hyperacidity, accelerated emptying of the stomach) caused by chronic pancreatitis.

Pathomorphological Features and Diagnostic Imaging

A clear understanding of the characteristic features of GP, including documentation of the size and location of the mass, may be necessary when diagnostic imaging tests are employed. Imaging of the pancreas, common bile duct and duodenum by extra-abdominal ultrasound (US), endoscopic ultrasound (EUS), computed tomography (CT) and magnetic resonance imaging (MRI) of

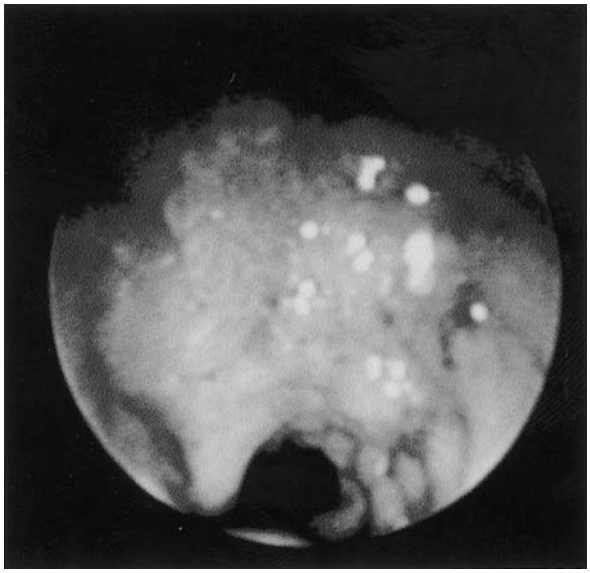

Fig. 5. Marked thickening of the duodenal wall (endoscopy).

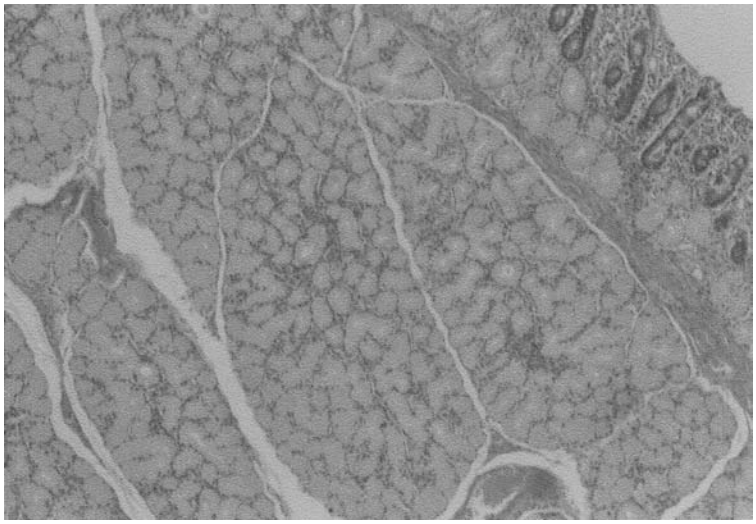

Fig. 6. Diffuse Brunner's gland hyperplasia.

the abdomen may help to distinguish between GP scarring and neoplastic proliferation. The benign narrowing of the common bile duct in GP may be confirmed by endoscopic retrograde cholangiopancreatography (ERCP).

Pancreas

The mass located on the upper surface of the pancreas is whitish yellow with an elastic hard surface. Morphologically, various features such as fibrous scarring of the groove area and pericholedochal fibrosisis with inflammatory infiltrates are commonly seen. The Santorini duct is dilated and contains protein plugs.

The fibrous lesion is clearly imaged by US as a hypoechoic mass [4]. CT [5] reveals swelling of the pancreatic head and a heterogeneously enhanced low-density lesion in the groove. A sheet-like or plate-like mass is hypointense relative to the pancreatic parenchyma on T1-weighted images and iso- to slightly hyperintense on T2-weighted images [6]. The main pancreatic duct is not narrowed in pure GP, but narrowed in segmental GP. MR cholangiopancreatography (MRCP) and endoscopic retrograde pancreatography (ERP) can also provide useful information.

Common Bile Duct

The biliary stricture is produced by fibrous scarring and chronic inflammation around the distal common bile duct. Because of scarring of the groove and thickening of the duodenum, the common bile duct bends to the left. An ultrasound endoscopy shows a dilated duct in the pancreatic head. ERCP shows that a stricture of the intra-pancreatic common bile duct is smooth, symmetrical and tapering, and that a stricture of the main pancreatic duct is occasionally irregular and incomplete. MRCP, like ERCP, shows stricture of the intrapancreatic common bile duct.

Duodenum

The duodenum shows marked thickening of the wall due to fibrous scarring and hyperplasia of Brunner's gland. Microscopic foci of heterotopic pancreas with mild fibrosis are rarely seen in the wall of the minor papilla. Endoscopy demonstrates inflamed mucosa with a humped shape and a narrowed lumen of the duodenum. Hypotonic duodenography demonstrates the segmental narrowing of the supra-ampullary area and/or the upper portion of the C loop of the duodenum. T1-weighted images on dynamic study show the medial wall thickening of the descending duodenum, several small cysts in the groove and thickened duodenal wall.

Table 1. Distinct morphologic features of pancreatic carcinoma and groove pancreatitis (proposed by Mohl et al., 2001)

	Pancreatic carcinoma	Groove pancreatitis
Transabdominal ultrasound and endoscopic ultrasound; abdominal CT	Enlarged mass, mostly in the pancreatic head, sometimes infiltration of the duodenum and/or vessels; often dilatation of the major pancreatic duct and/or the common bile duct	'Groove' shown as hypoechoic zone between pancreatic head and duodenum, thickening of the duodenal wall; at most minimal dilatation of the common bile duct
MRI	As in CT, frequently encasement of vessels detectable. Cholangiopancreaticography studies: as in ERCP (see below)	'Sheet-like mass' in the groove, cysts, duodenal wall thickening. No encasement of vessels. Cholangiopancreaticography studies: long and smooth common bile duct stenosis or medial shift of the duct
Endoscopy	Sometimes compression of the duodenum or tumorous infiltration of the duodenal wall	Often duodenal stenosis caused by a thickened wall, histopathologic any hyperplasia of Brunner's glands
ERCP	lrregular stenosis of the major pancreatic duct, frequently accompanied by distal common bile duct stenosis ('double duct sign')	Often tubular common bile duct stenosis, usually intact pancreatic duct, sometimes stenosis and/or dislocation of the pancreatic duct

Differential Diagnosis

Most reported patients underwent a Whipple procedure because preoperative differentiation between groove pancreatitis and pancreatic cancer was difficult [7]. A mass lesion should be verified by intraoperative pathological examination to rule out a malignant lesion and to avoid unnecessary radical surgery. In addition, a duodenal mucosal biopsy of a markedly thickened duodenum is required, when endoscopy reveals luminal narrowing of the duodenum. When Brunner's gland hyperplasia is confirmed histopathologically, GP is favored, in patients with no history of gastrectomy, peptic ulcer or disease of the biliary tree.

Taya et al. [8] reported a case of minute cancer of Santorini's duct, and preoperative diagnosis was GP. If a biopsy fails to demostrate the malignant lesion of minor papilla and/or Santorini's duct, a pancreatoduodenectomy may be avoided and the patient may undergo a biliary stent placement. For this kind of unrecognized cancer, further and adequate examinations are recommended.

This type of pancreatitis is still unknown to most clinicians and pathologists, possibly because major textbooks of medicine and surgery do not describe it. Summarizing distinct morphologic features of pancreatic cancer and GP, in 2001 Mohl et al. [9] proposed diagnostic criteria (table 1) on differential diagnosis, in order to lead to a more reliable preoperative diagnosis and to avoid unnecessary radical surgery.

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Complications of Chronic Inflammation

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Abstract

In this chapter, chronic inflammation refers to chronic pancreatitis characterized by interlobular (perilobular) fibrosis presenting clinically as chronic alcoholic pancreatitis, and two kinds of complication of chronic inflammation are described: one is that caused by progression of the pancreatitis itself and the other by involvement of various anatomical structures. The former is represented by protein plugs and stones and by tumor-forming pancreatitis, the latter by involvement of the splenic vein, duodenum, intrapancreatic bile duct and colon.

Because the pancreatic and peripancreatic anatomy is very complex, inflammation in the pancreas can easily spread to various anatomical structures in and around the pancreas and various kinds of complication can arise. In this chapter, the term 'chronic inflammation' refers to so-called chronic pancreatitis characterized not only by intralobular fibrosis but also by interlobular (perilobular) fibrosis (fig. 1) [1], usually presenting clinically as chronic alcoholic pancreatitis.

Complications Caused by Progression of Pancreatitis Itself

Protein Plugs and Stones (Pancreatolithiasis)

Protein plugs consist of condensed pancreatic juice (or secretion) composed of protein suspected to be of zymogenic origin, and composed of erythrocytes, desquamated epithelium of the small duct and mucoproteins originating from the epithelium. As it becomes more condensed, collagen is produced as one of the components. Histologically, pancreatic stones show a

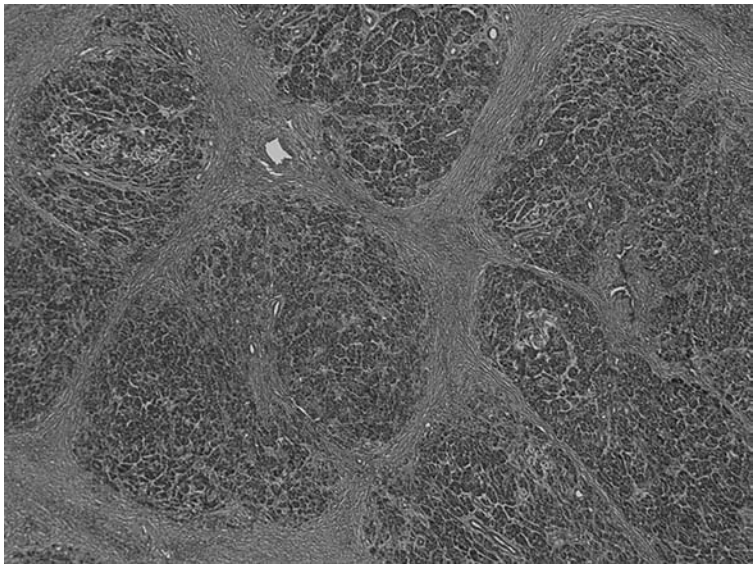

Fig. 1. Chronic alcoholic pancreatitis characterized by interlobular (perilobular) fibrosis. HE. ×40.

lamellar structure with calcification. There are two types: true stone, located in the pancreatic duct, and false stone, seen in necrotic parenchymal areas with calcification.

Klöppel et al. state in their hypothesis that the necrosis-fibrosis sequence is the basic pathogenetic event in the evolution of chronic pancreatitis [2], and that protein plugs and pancreatic stones are formed as the result of progression of chronic pancreatitis and then become causes of duct obstruction as well as further progression of the disease.

Tumor-Forming Pancreatitis

Tumor-forming pancreatitis (TFP) (fig. 2) [3] is a clinical term that our study showed is divided into two subtypes. One shows tissue repair with a background of chronic alcoholic pancreatitis, and the other consists of lymphoplasmacytic infiltration and fibrosis without a background of chronic alcoholic pancreatitis. The former, with a background of chronic alcoholic pancreatitis, is described in this section.

Tumorous lesions can be seen as a pathological repair process after ductal and parenchymal injury and consist of three layers, observed from the center of the lesion to the periphery (fig. 3).

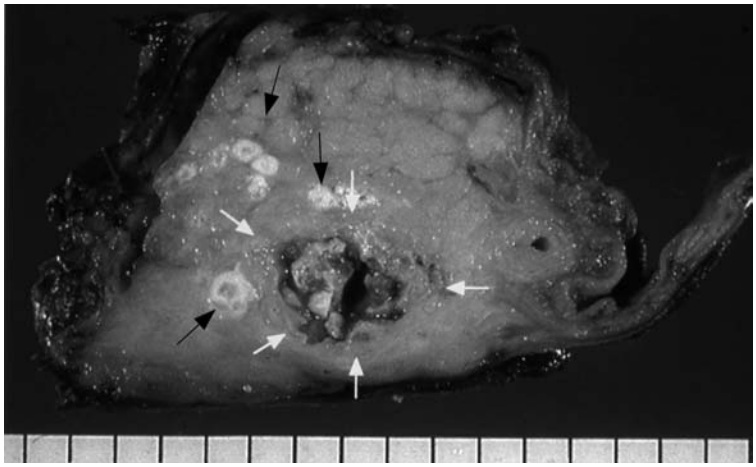

Fig. 2. Tumor-forming pancreatitis. Pancreatoduodenectomy specimen. The tumorous lesion (white arrows) showed an abscess-necrotic layer at the center and transition to surrounding fibrosis at the periphery. Black arrows indicate protein plugs. The background pancreas is very firm and shows chronic pancreatitis.

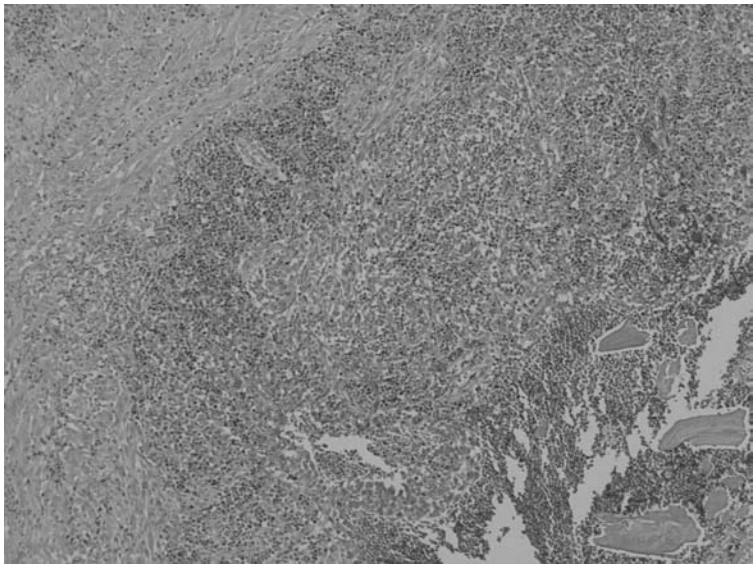

Fig. 3. Tumorous lesion in tumor-forming pancreatitis. The lesion consisted of an abscess-necrotic area with fibrogranulation and fibrous layers, from the center (lower right) to periphery (upper left). HE. ×40.

The first is an abscess-necrosis layer, consisting of condensed pancreatic juice, protein plugs, stones, neutrophils and small pieces of desquamated ductal epithelium. This core layer is hence considered to originate from the pancreatic duct.

The second is a fibrogranulation layer, and includes capillaries with swollen endothelium, lymphocytes, plasmacytes, and hemosiderin deposition. These tissues are inter-mixed with fibrosis.

The third is a fibrous layer, with successive transition to the surrounding fibrosis in the non-swollen pancreatic tissue. The thickness of each layer differs in each case. Some tumorous swelling involves several pancreatic ducts, which means that swelling lesions might conglomerate to form a larger one. A background of tumorous swelling shows a chronic pancreatitis pattern in which exocrine pancreatic tissue is rather well-preserved irrespective of pancreatitis.

To conclude: TFP is a 'stage' of tumor-forming in the natural course of 'chronic pancreatitis' with rather well-preserved exocrine tissue. Tumorous swelling presents histological findings of the tissue repair process for centriductal acute inflammation, caused by protein plugs and pancreatic stones.

Complications due to Involvement of Anatomical Structures by Inflammation

Involvement of Inflammation of the Splenic Vein

Involvement of the splenic vein [4] often occurs. The clinical manifestation of splenic vein obstruction is splenomegaly and varicose veins in the stomach (many in the gastric fundus) and esophagus, generally called localized (or left-sided) portal hypertension.

In our histopathological study of surgical and autopsy specimens from 12 patients with chronic alcoholic pancreatitis, fibrosis in the pancreatic parenchyma continuously extended to the wall of the splenic vein in 11 specimens; 5 patients showed organized thrombus formation with recanalization, 4 presented with phlebosclerosis and in 2 there were no apparent changes. In 2 of the 5 patients with organized thrombus formation in the splenic vein, localized portal hypertension had been clinically diagnosed: these patients had shown splenomegaly and varicose veins in the fundus of the stomach, but not liver cirrhosis.

Two mechanisms can lead to thrombus formation within the splenic vein. One is an extrinsic factor, a direct mass effect or cellular infiltration into the venous wall. The other is an intrinsic factor, an inflammatory process involving the vein. In the peripancreatic area, once fibrosis persistently compresses the splenic vein wall over a prolonged period (which might be considered a mild

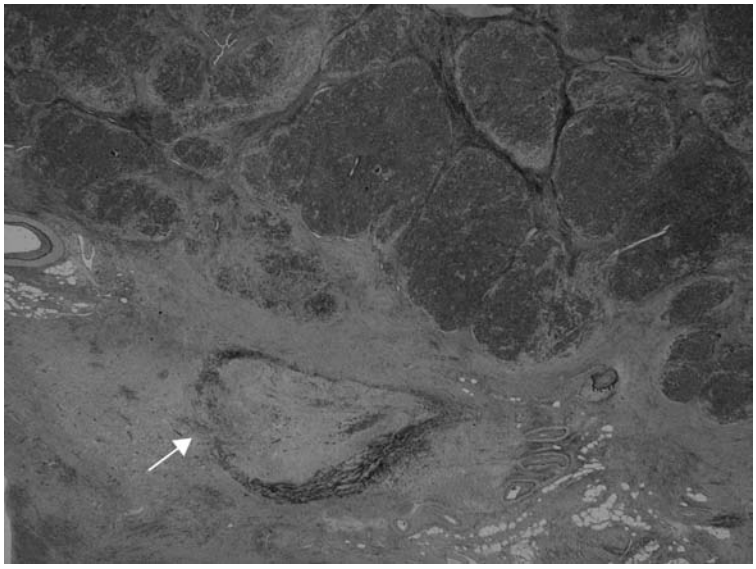

Fig. 4. Phlebosclerosis in chronic pancreatitis. Interlobular fibrosis was continuous to the peripancreatic lesion and involved the splenic vein (arrow). EVG. ×12.5.

mechanical trauma), endothelial cells become injured, finally leading to phlebosclerosis and thrombus formation. This fibrotic extension involving the splenic vein is important for the mechanism of thrombus formation.

Involvement of Inflammation to the Duodenum

Diffuse involvement in the duodenum (fig. 5) is rare and its clinical manifestation is characterized by duodenal stenosis. Our single case presented so-called groove pancreatitis in a broad sense, which is a rare form of segmental chronic pancreatitis involving a so-called 'groove' area between the head of the pancreas, the duodenum, and the common bile duct [5].

In chronic pancreatitis with duodenal stenosis, the following histopathological findings can be found in the duodenum and adjacent pancreatic head tissue: cyst formation, myoid stromal proliferation, spindle cell and smooth muscle fiber proliferation, and Brunner's gland hyperplasia.

Cysts can be observed in the muscularis propria, submucosa, and mucosa of the duodenum, with or without extraduodenal-peripancreatic extensions, and show various shapes and sizes: oval, slit-like, irregular, and collapsed. We have managed three surgically-resected cases showing duodenal stenosis in patients

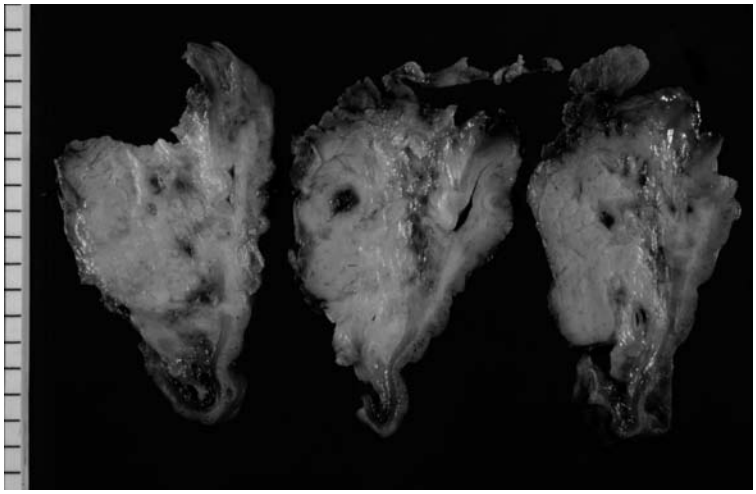

Fig. 5. Chronic pancreatitis involving the duodenum. Chronic pancreatitis in the head showed groove pancreatitis in the broad sense, also spreading to the duodenal wall. The duodenal wall was markedly thickened.

with chronic alcoholic pancreatitis [6]. The cysts were 15, 17 and 23 mm in diameter. In general, the majority are pseudocysts, the wall of which consists of granulation tissue surrounding the cyst and including slight hemosiderin deposition, spindle cells positive for α-SMA (smooth muscle actin), a marker of myofibroblasts, and smooth muscle proliferation. Cysts are thought to be derived from a ductal component of the ectopic pancreatic tissue, because some are occasionally accompanied by an epithelial lining with or without ductal structures.

Dense myoid stromal proliferation, spindle cell and smooth muscle fiber proliferation and pancreatic acinar tissue shows the characteristic histological pattern described as myoadenomatosis or pancreatic hamartoma.

Brunner's gland hyperplasia is typically seen coexisting with proliferation of smooth muscle fibers of the muscularis mucosae. Each gland is surrounded by these muscle fibers.

In the case of duodenal stenosis, cysts are seen especially in association with granulation tissue, including marked proliferation of α-SMA-positive spindle cells and smooth muscle fibers. Proliferation of myofibroblasts and smooth muscle fibers are observed not only in the tissue surrounding the cyst, but also in the submucosal layer of the duodenum.

Generally, myofibroblasts are known to play an important role in wound healing and subsequent contraction of the tissue. Therefore, proliferation of myofibroblasts and smooth muscle fibers around the cysts, especially the

former, may indicate the healing process of localized inflammation of duodenal wall cysts. This proliferation may also play a role in duodenal stenosis.

Recently, such pancreatitis has been referred to under the unifying name of paraduodenal pancreatitis [7] that shows the characteristic histopathological and clinical findings described in this small chapter.

Involvement of Inflammation of the Intrapancreatic Bile Duct

In TFP, the intrapancreatic bile duct shows luminal stenosis and fibrous thickening of the wall, caused by either circumferential or nodular protruding fibrosis.

Involvement of Inflammation of the Colon

Though cases with chronic pancreatitis involving the colon are very rare (fig. 5), the frequency of colonic 'stenosis' has been reported to range from 1.5% [8] to 2–3%. According to the literature [8], the most frequently involved site (52%) is the splenic (left colonic) flexure. Histopathological findings are rarely described. In our one case, a 65-year-old male patient underwent distal pancreatectomy due to suspicion of pancreatic cancer involving the transverse colon. Histologically, pericolonic and colonic inflammation and fibrosis were seen. Fibrosis was seen from the pericolonic area to the submucosal layer of the colon, accompanied by abscess in the subserosal layer.

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Autoimmune Pancreatitis

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Abstract

Autoimmune pancreatitis (AIP) is a distinct disease entity that has been characterized clinically, serologically, radiologically and pathologically. These characteristic features were first reported by Japanese authors. However, histopathological reports on Japanese AIP patients are rare. Histopathologically, AIP in Japanese patients is defined as lymphoplasmacytic sclerosing pancreatitis (LPSP), with the following characteristic features: (a) dense circumferential lymphoplasmacytic infiltration within and around the pancreatic ducts, especially the medium-sized, interlobular and main pancreatic ducts, and (b) prominent sclerotic collagen bundles with lymphoplasmacytic infiltration. Plasmacytes show various degrees of positivity for IgG4 on immunohistochemistry.

From a historical perspective, a number of cases reported by Japanese researchers have been very interesting contributions to understanding the histopathology and pathophysiology of conditions or diseases related, or suggested to be related, to autoimmune pancreatitis (AIP). Examples are (a) the first attempt to administer corticosteroids and the first demonstration of the effectiveness of corticosteroids in a patient with 'abdominal mass (complicated by) with Sjögren syndrome', which is currently called AIP [1]; (b) the first detailed description of lymphoplasmacytic sclerosing pancreatitis (LPSP) with cholangitis [2]; (c) the first presentation of characteristic findings of narrowing of the main pancreatic duct (MPD) on endoscopic retrograde pancreatography (ERP) [3] and the proposal of the concept of AIP [4], and (d) the first description of a high serum level of IgG4 in patients with AIP [5].

Thus, a small number of histopathological findings of so-called AIP were presented in Japan, and then compared to those of AIP in other countries. There have been few detailed histopathological reports on Japanese AIP [6].

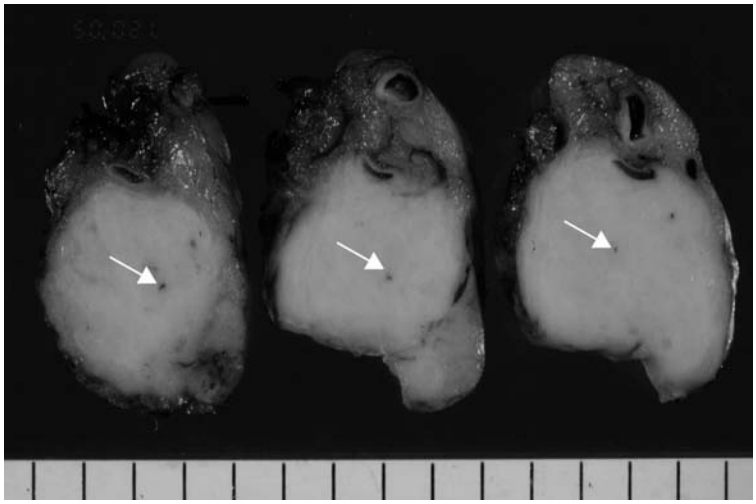

Fig. 1. Gross serial cut surfaces demonstrated whitish-grey mass with relatively clear margins at the interface with non-affected parenchyma. The main pancreatic duct (arrow) was stenotic or narrowed, but remained identifiable throughout the mass lesion.

Gross Findings

The affected lesion shows a firm swelling tumorous mass. The pancreas head is more frequently affected than the body or tail. When narrowing of the MPD or large pancreatic duct is long or severe, distal parenchymal tissue might become markedly atrophic. The cut surface demonstrates a whitish-yellow mass with varying degrees of loss of normal lobular structure (fig. 1). The MPD is stenotic or narrowed, but usually remains identifiable despite involvement in the mass lesion. The intrapancreatic bile duct is usually involved when the pancreatic head is affected. The peripancreatic or regional lymph nodes are sometimes swollen. The entire pancreas is reported to be affected clinically. To our knowledge, however, such cases have not been resected or studied histopathologically.

Histopathological Findings

Characteristic histopathological features are summarized as follows: (a) dense circumferential lymphoplasmacytic infiltration within and around the pancreatic ducts, especially the medium-sized, interlobular and main pancreatic ducts, and (b) prominent sclerotic collagen bundles with lymphoplasmacytic infiltration.

Fig. 2. The ductal lumen of the larger pancreatic duct was narrowed by the infolding wall, typically causing a star-like or slit-like appearance. HE. ×200.

Although neutrophilic infiltration in a Japanese AIP patient has not previously been reported in the literature, one of our cases showed LPSP with eosinophilic infiltration (unpublished observation). The ductal lumen was narrowed by the infolding wall, typically a star-like or slit-like appearance (fig. 2). Even when lymphoplasmacytic infiltration is seen in the ductal epithelium, it is not desquamated. Neither protein plugs nor pancreatic stones were identified histologically. However, such a clinical case was reported as showing pancreatic stone in long-term follow-up in an AIP patient.

Injury to the lobuli (parenchyma) shows a basically obstructive pattern. Lymphoplasmacytic infiltration and inter- and intralobular fibrosis are the main histological findings, usually accompanied by lobular atrophy, acinar atrophy or loss, lymphoid aggregates or hyperplasia to various degrees. Prominent sclerotic collagen bundles with lymphoplasmacytic infiltration are characteristically found (figs. 3, 4). On immunohistochemistry, plasmacytes show various degrees of positivity for IgG4.

This inflammatory process always involves the intralobular small venules and usually the interlobular veins, which is called obstructive phlebitis. In some cases the splenic vein shows phlebosclerosis, but there is no thrombus formation. The arteries and arterioles accompanying the veins and venules are usually intact. The intrapancreatic bile duct is often involved when the pancreas head is

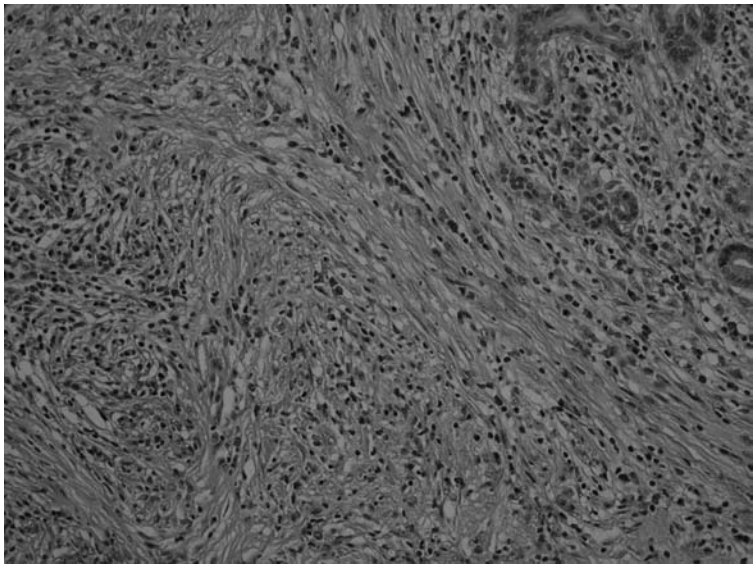

Fig. 3. Lymphoplasmacytic infiltration and sclerosis with parenchymal atrophy in the pancreatic lobuli. HE. ×100.

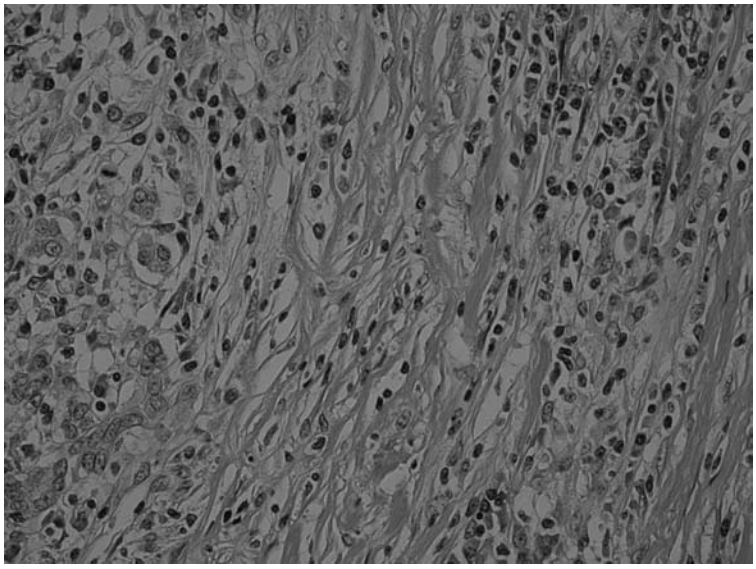

Fig. 4. Sclerotic collagen bundles and lymphoplasmacytic infiltration. HE. ×400.

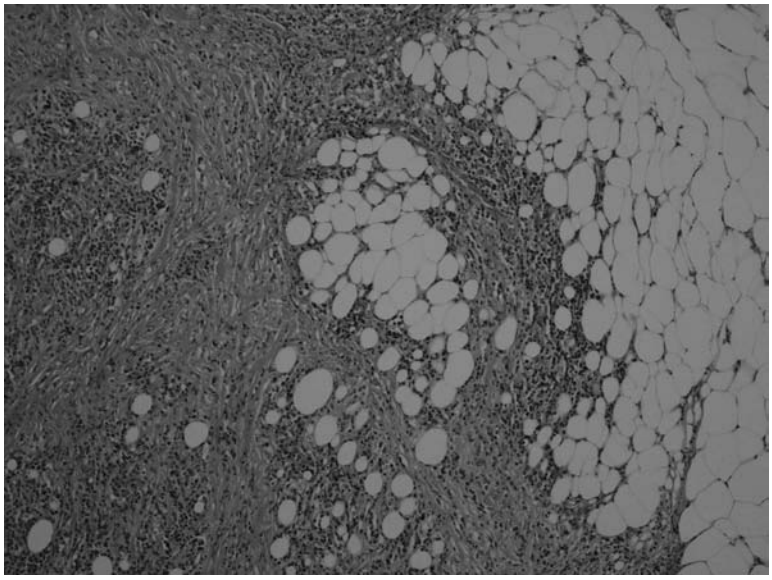

Fig. 5. Inflammatory process involves the peripancreatic fat tissue. HE. ×200.

affected. In some cases, the extrapancreatic bile duct shows the same changes as the pancreas does (mentioned below). The pancreatic islets usually do not show cell infiltrates except in extremely advanced cases. The inflammatory process also involves the peripancreatic fat tissue (fig. 5) and lymph nodes, but neither fat necrosis nor calcification is seen. Typically, enlarged lymph nodes are composed of lymphoplasmacytes in the peripheral sinus with the capsule becoming fibrously thickened. When the inflammatory tumorous lesion is localized, the pancreatic parenchyma distal to the lesion becomes markedly atrophic, and remarkable acinar atrophy, fibrosis, inflammatory cell infiltration and aggregated islets are seen in these regions. The background pancreatic tissue does not show any remarkable findings; there are no characteristic features of chronic alcoholic pancreatitis characterized by interlobular fibrosis.

Discussion

On the Relationship of AIP to Systemic Disease

AIP patients sometimes demonstrate extrapancreatic lesions such as intra- and extra-bile-duct sclerosing lesions (sclerosing cholangitis) [7], retroperitoneal

fibrosis and sialadenitis. These diseases may arise synchronously or asynchronously to AIP and show histological findings similar to those of AIP, namely lymphoplasmacytic infiltration and fibrosis with IgG4-positive plasmacytes. Especially sclerosing cholangitis presents the same histology as AIP: centroductal lymphoplasmacytic infiltration and sclerosing fibrosis with IgG4-positive plasmacytes and obstructive phlebitis. Therefore a comprehensive concept of IgG4-related sclerosing disease [8] or multifocal fibrosclerosis is proposed, and AIP can be considered a pancreatic manifestation of the concept.

On the Histopathology of AIP

Klöppel et al. classified AIP into two subgroups [9, 10]: either with or without the presence of granulocytic epithelial lesions (GEL). GEL is defined as granulocytic infiltration into the ductal epithelium, corresponding to 'crypt abscess' in ulcerative colitis. Klöppel et al. selected AIP cases from surgically resected cases of benign inflammatory pancreatic disease without pseudocysts, calculi, pancreas divism, duodenal wall cysts or associated pancreatitis, or a history of alcohol abuse, cholelithiasis or choledocholithiasis.

However, Notohara et al. at the Mayo Clinic summarized inflammatory pancreatic cases showing dense inflammatory cell infiltration around the pancreatic ducts, and classified them as either lymphoplasmacytic sclerosing pancreatitis (LPSP) or idiopathic duct-centric chronic pancreatitis (IDCP) [11]. Although this group does not use the terms 'AIP' or 'IgG4-positive plasmacyte' in their report, they summarized the cases similar to AIP showing characteristic features of narrowing of the MPD on ERP. IDCP is characterized by prominent lobular inflammation consisting of edema and infiltrating neutrophils, lymphocytes and plasmacytes. 'LPSP' is the same as the term described by Kawaguchi [2], who used it to describe inflammatory pancreatic lesions with cholangitis. The term 'LPSP' has been used by two different authors to indicate the same lesion, and Japanese AIP cases may correspond to 'LPSP'.

Currently, there are some issues that remain to be resolved regarding the histology of AIP: whether 'AIP with GEL' and 'IDCP' are the same lesion, whether 'AIP without GEL' and 'LPSP' are the same lesion, and whether 'LPSP' is 'true AIP'. It is also problematic that there is one case showing features of both LPSP and IDCP among the cases reported by Notohara, and it remains unclear whether eosinophilic pancreatits is 'AIP with GEL' [12]. Furthermore, we have one case of LPSP with eosinophils (mentioned above).

Based on the major clinicopathological features of AIP described by Japanese researchers, we conclude that AIP should be considered one distinct clinicopathological disease entity satisfying the features described in the introduction to this chapter.

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Regeneration of the Pancreas

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Abstract

The potential for regeneration of pancreatic tissue in the adult human has generally been regarded as minimal. However, in chronic pancreatitis, isolated lobuli are frequently seen in fibrosis. These isolated lobuli have nodular architecture and bear resemblance to regenerative nodules of the cirrhotic liver. In experimental animals, regeneration of the acinar cells has been shown in the literature since Fitzgerald et al., followed by other experimental studies for pancreas exocrine and/or endocrine cell regeneration. Recently, expression of growth factors in pancreatic regeneration as platelet-derived growth factor-A (PDGF-A) and vascular endothelial growth factor (VEGF) was determined by immunohistochemical analysis, and a combination of epidermal growth factor and leukemia-inhibitor factor induces exocrine-endocrine transdifferentiation. Also, the morphological examinations of experimental animals clarified the potential endocrine and exocrine progenitors as tubular complex and acinoinsular and/or ductuloinsular transformation.

The regenerative potential of the human pancreas tissue has been regarded as minimal. However, with regard to the pancreatic duct epithelium, proliferative changes, including regeneration, hyperplasia and metaplasia, are common pathological features. In the damaged human pancreas, such as in acute or chronic pancreatitis, isolated lobuli are frequently seen in fibrosis (figs. 1, 2). These isolated lobuli have a nodular architecture and show a resemblance to regenerative nodules of the cirrhotic liver. Though sequential examination of regenerative changes in the human pancreas is almost impossible, regeneration of acinar cells has been shown in experimental animals by Fitzgerald et al. [1]. These authors reported regeneration of acinar cells in the rat pancreas after administration of DL-ethionine. In this chapter, I would like to show my previous experiment on the characteristic histological patterns of regeneration in the chemically-injured animal pancreas [2, 3], and the comparison between the

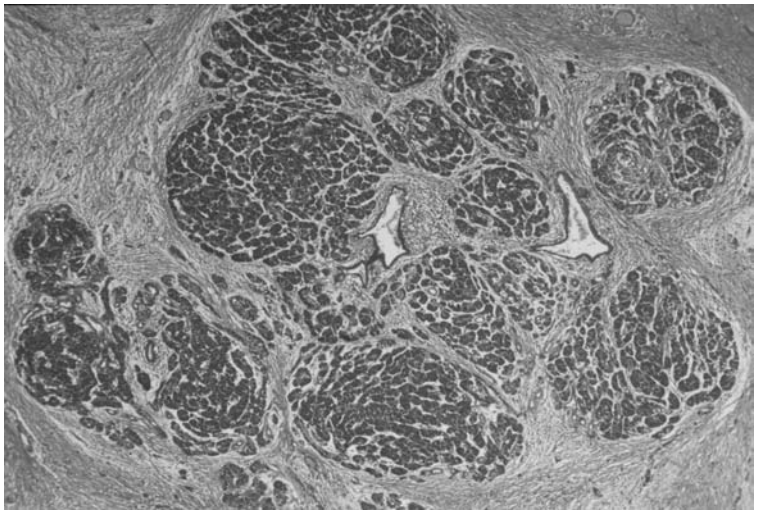

Fig. 1. Isolated lobuli in fibrosis in the case of chronic pancreatitis. HE.

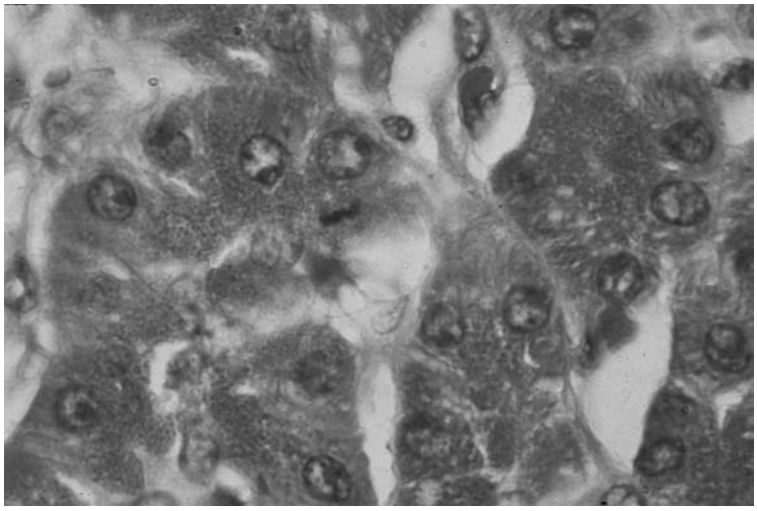

Fig. 2. The mitotic figure was occasionally observed in such lobuli. HE.

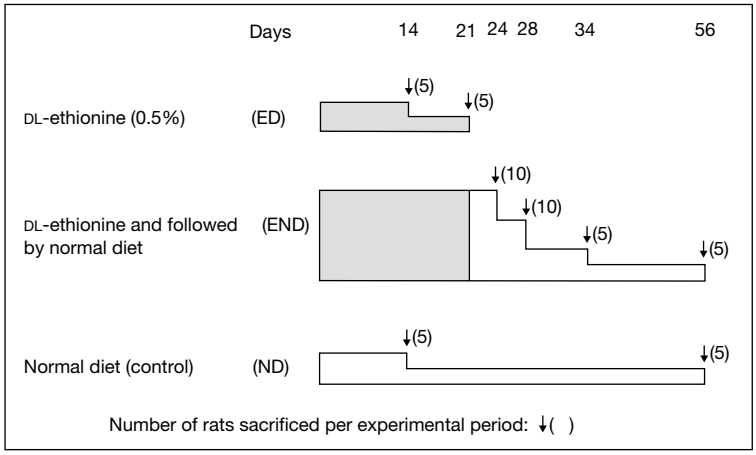

Fig. 3. Experimental design of pancreatic regeneration.

so-called cirrhotic human pancreas and the regenerative pancreas in experimental animals. Other experimental studies concerning pancreatic regeneration are also presented.

Experimental Pancreatic Regeneration

Methods in Brief

Sixty young male Wistar rats were divided into two groups. Ten rats were fed a synthetic control diet (ND group), and fifty rats were fed an experimental diet, containing 0.5% DL-ethionine (ED group). On day 14, five rats from each group and on day 21, five rats of group ED were sacrificed. After affirmation of necrosis and destruction of the pancreas, the remaining rats of group ED were changed to the normal diet (END group). On days 24, 28, 50 and 70, i.e. 3, 7, 29 and 49 days after cessation of the DL-ethionine supplemental diet feeding, the END group rats and the remaining rats of the ND group were sacrificed (fig. 3). To observe the regeneration of pancreatic tissue, histological and immunohistochemical examinations were performed, and changes in the γ-glutamyl transpeptidase(γ-GTP) activity in the regenerating pancreatic tissues were demonstrated histochemically. A part of the pancreas was submitted for electron microscopy to examine the fine structures of the regenerative cells.

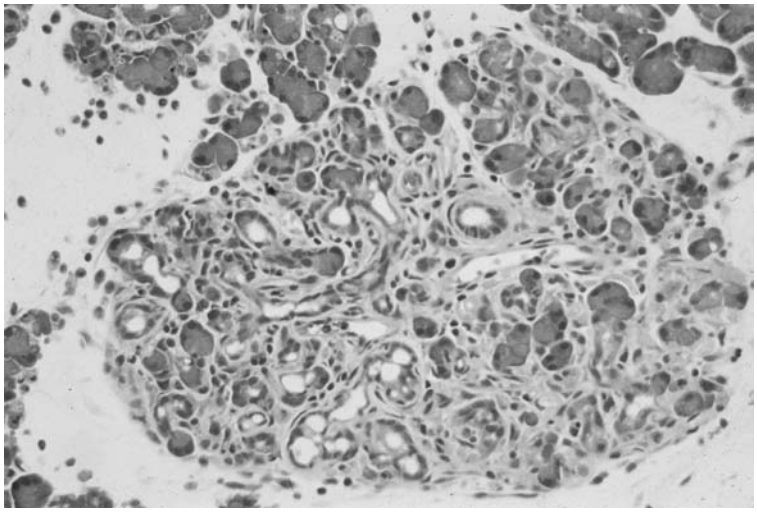

Fig. 4. Degenerative pancreas in rat of ED group. Ductular aggregation with inter- and intra-lobular fibrosis were noted. HE.

Results

Chronological Changes in the Exocrine Pancreas

On day 14, the pancreata of the rats fed the ethionine-supplemented diet (ED group) showed diffuse and/or focal necrosis and destruction of the acinar cells. On day 21, interstitial edema became gradually prominent and mild inter- and intra-lobular fibrosis appeared in the ED group (figs. 4, 5). Three days after the end of the ethionine-supplemented diet, most of the acinar cells in the END group had large oval-shaped nuclei with prominent nucleoli and frequent mitotic figures (fig. 6). Under the electron microscope, these acinar cells with basophilic cytoplasma were seen to be filled with compact rough endoplasmic reticuli and had large irregular shaped nuclei. On the day 7 after the end of DL-ethionine administration, the END group showed no edema, mononuclear cell infiltration or degeneration of the acinar cells. The mitotic index of the acinar cells on day 14 showed the same value as in the ED and ND groups. The peak of the mitotic index was observed in the END group. The index then gradually decreased, and consequently showed almost the same value in the END and ND groups on day 49 day after the end of DL-ethionine administration (fig. 7).

Chronological Changes in the Endocrine Pancreas

In the END groups, proliferation of small groups or isolated endocrine cells and the irregular-shaped hyperplastic islets (fig. 8) were occasionally found in

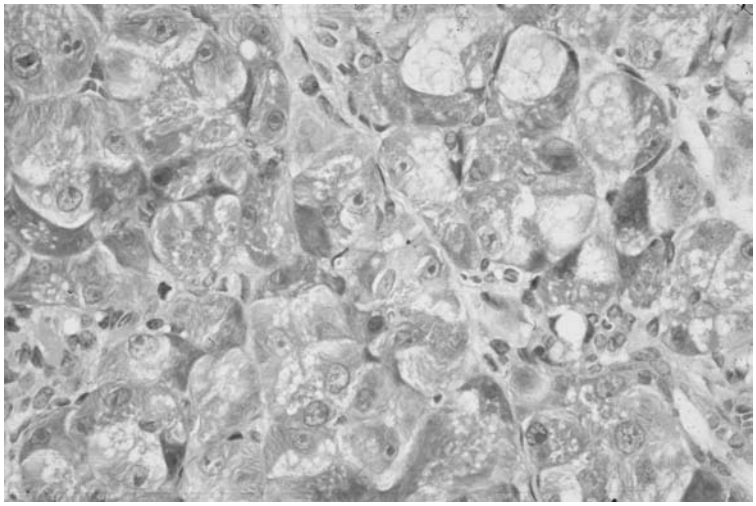

Fig. 5. Degenerative pancreas in rat of ED group. Most acinar cell cytoplasmas showed marked hydropic change and decrease of zymogen granules. HE.

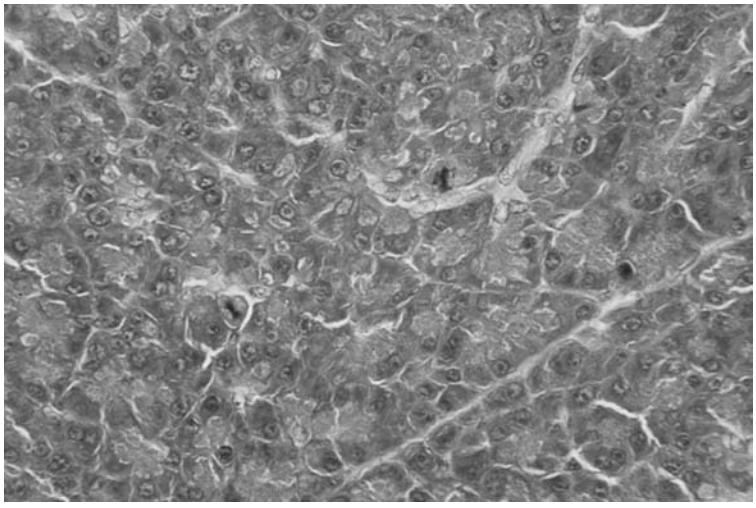

Fig. 6. Acinar cells in regenerated pancreatic tissue had large oval-shaped nuclei with prominent nucleoli, and mitotic figures were recognized. HE.

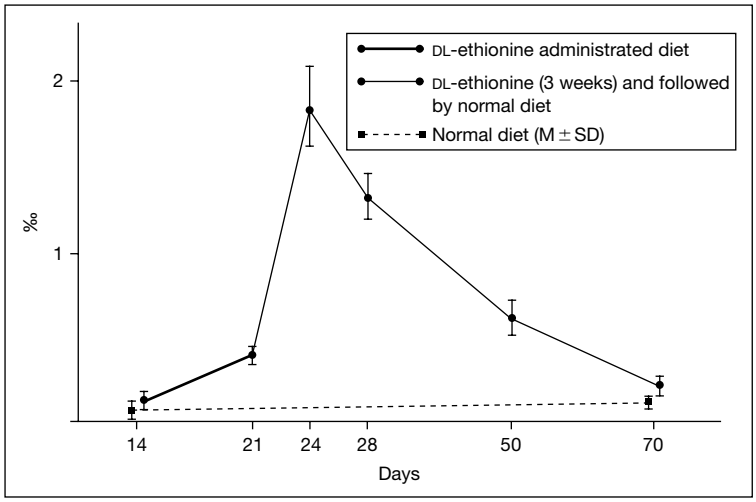

Fig. 7. Mitotic index (number of mitoses per 1,000 acinar cells; M ± SD).

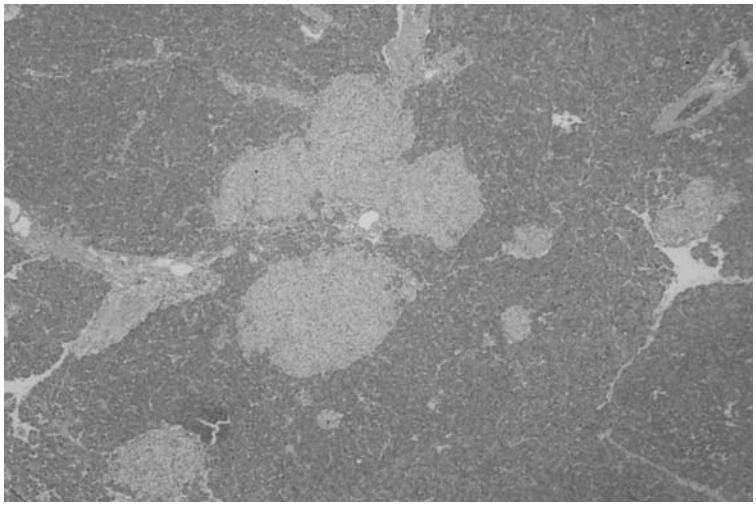

Fig. 8. Irregular hyperplastic islets were noted in regenerative pancreas. HE.

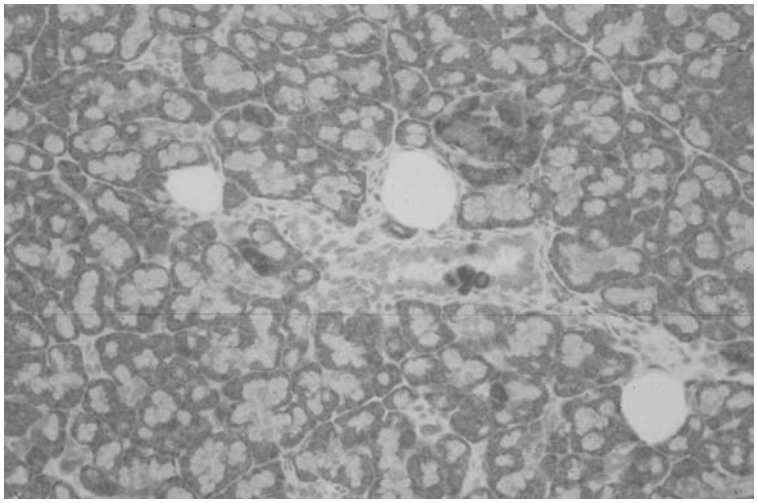

Fig. 9. Insulin-producing cells in islets, isolated in acinus and in ductular epithelium. Immunostain for insulin.

the regenerating pancreas. The endocrine cells in these hyperplastic and/or small islets were directly connected with acinar cells and ductular epithelia.

Immunohistochemical examinations showed that such small groups or isolated endocrine cells in acini and ductular epithelia coincided with insulin-producing cells (fig. 9).

The Activity of Pancreatic γ-GTP

The histochemically and electronmicroscopically observed activity of γ-GTP was positive in all the acinar cells of the ED group during the experiment. The γ-GTP was located in the cytoplasmic membrane and a part of the apical portion of the acinar cells (fig. 10), as well as in the apical portion of the ductular epithelium, but not in the endocrine cells. Electron-microscopic examination revealed that the γ-GTP activity was located along with the cytoplasmic membrane and demonstrated as electron dense material (fig. 11). On the other hand, the ED group showed reduced γ-GTP activity in all acinar cells along with cell degeneration and necrosis. On day 28 (7 days after the end of DL-ethionine administration), the acinar cells in the END group showed a greater γ-GTP activity than in the END group on day 24. Histochemically, on days 34 and 56, the acinar cells in the END group showed γ-GTP activity of almost the same intensity as in the ND group.

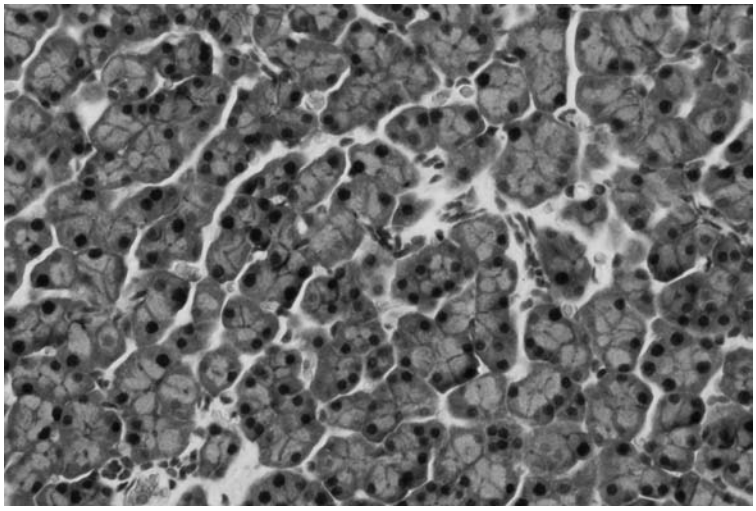

Fig. 10. Localization of γ-GTP in the acinar cells.Histochemical γ-GTP.

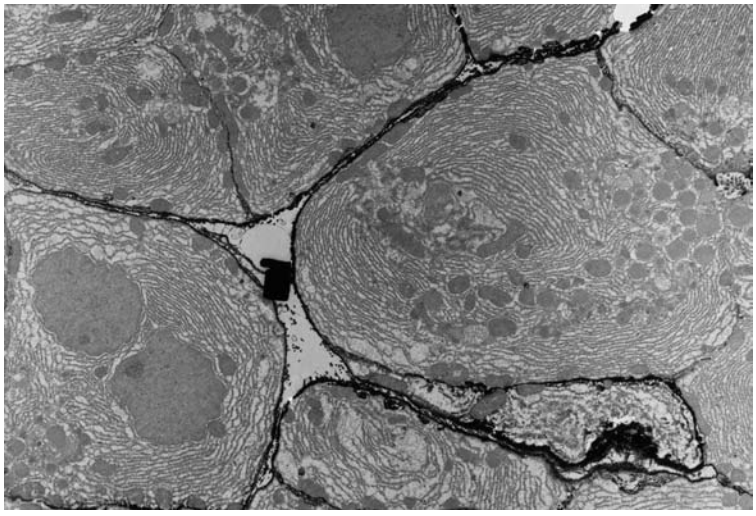

Fig. 11. Electron microscopic cytochemical demonstration of γ-GTP activity in the rat pancreas.

Conclusion and Summary

DL-Ethionine is the antagonist of methionine, which is one of the essential amino acids, and is known to cause pancreatic acinar cell necrosis in rats and other experimental animals by either intraperitoneal injection or oral administration. The mechanism of pancreatic damage is presumed to be abnormal metabolism in protein synthesis. There are also other chemical agents, such as 1-aminocyclopentane carboxylic acid (ACPC) and puromycin, which can cause pancreatic acinar cell necrosis and degeneration.

These chemical agents have therefore been used for experiments on pancreatic acinar cell regeneration. On the other hand, the chemical agents which produce pancreatic endocrine cell destruction, such as alloxan [4] and streptozotocin [5–7], have been used for various experiments on pancreatic endocrine regeneration, and it is also known that these chemical agents produce diabetic states in rats and guinea-pigs. Furthermore, studies on regeneration of pancreatic acinar cells and endocrine cells after partial or subtotal pancreatectomy of some experimental animals, have been reported [8]. My previous experiment on the characteristic histological patterns of regeneration in the chemically-injured animal pancreas suggested that pancreatic acinar cell regeneration followed by necrosis and destruction by DL-ethionine administration occurred as early as 3 days after the termination of DL-ethionine administration. Pancreatic endocrine cells also regenerated in an early phase, and these cells were found in ductal and/or ductular epithelia, as well as in isolated cases in the interstitium. Histochemically recognized γ-GTP activity in such pancreata was restored rapidly to the control value in parallel with the histopathological restoration. Electronmicroscopic observations supported this view, suggesting that functional restoration of pancreatic exocrine cells begins at an early stage and finishes within a shorter period. Recently, expression of some growth factors was proposed in close relation to pancreatic regeneration. Expression of platelet-derived growth factor-A (PDGF-A) and vascular endothelial growth factor (VEGF) in regeneration of rat pancreas was determined by immunohistochemical analysis [9], and epidermal growth factor and leukemia-inhibitor factor induced exocrine-endocrine transdifferentiation in vitro [10]. Furthermore, Renuka et al. [11] suggested that the increased muscarinic M1 and M3 receptor subtypes stimulated insulin secretion and islet cell proliferation during pancreas regeneration. Also, morphological examinations of experimental animals clarified the potential endocrine and exocrine progenitors as tubular complex [12] and acinoinsular and/or ductuloinsular transformation.

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Pathology of Pancreas in Collagen Diseases

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Abstract

Pancreatic vasculitis in collagen diseases, acute pancreatitis in systemic lupus erythematosus (SLE), pathologic findings of pancreas in Sjögren's syndrome (SS) and systemic sclerosis (SSc) are described. Vasculitis in collagen diseases presented necrotizing arteritis of the polyateritis nodosa (PAN) type, located in small and medium-sized arteries. The incidence of pancreatic arteritis was 71% in PAN, 53% in SLE, 50% in rheumatoid arthritis and 17% in SSc. The incidence of arteritis in the pancreatic head was larger than that in the pancreatic body and tail. The incidence of acute pancreatitis in SLE ranged from 4.5 to 12.5%, with a predominance in females. Various factors have been considered for the cause of pancreatitis, including drugs, vasculitis and thrombi. Pathologic findings of the pancreas in SS were no inflammation or mild inflammatory reactions. Recently, autoimmune pancreatitis was proposed, and about a fourth of the cases of autoimmune pancreatitis overlapped with SS. However, pancreatic pathology in SS led to the conclusion that SS usually does not produce autoimmune pancreatitis by the disease itself. In SSc, fibrosis occurs in various organs, especially the skin and esophagus. However, the data of D'Angelo et al. indicated that SSc does not produce pancreatic fibrosis by the disease itself.

The pancreas is an organ with a rare involvement in collagen diseases, and most pathological studies of the pancreas in collagen diseases have been focused on vasculitis in collagen diseases, acute pancreatitis in systemic lupus erythematosus (SLE), and pathological findings of the pancreas in Sjögren's syndrome (SS) and systemic sclerosis (SSc). Therefore, in this review, I describe the above-mentioned studies and include the data of my research group for pancreatic vasculitis in collagen diseases.

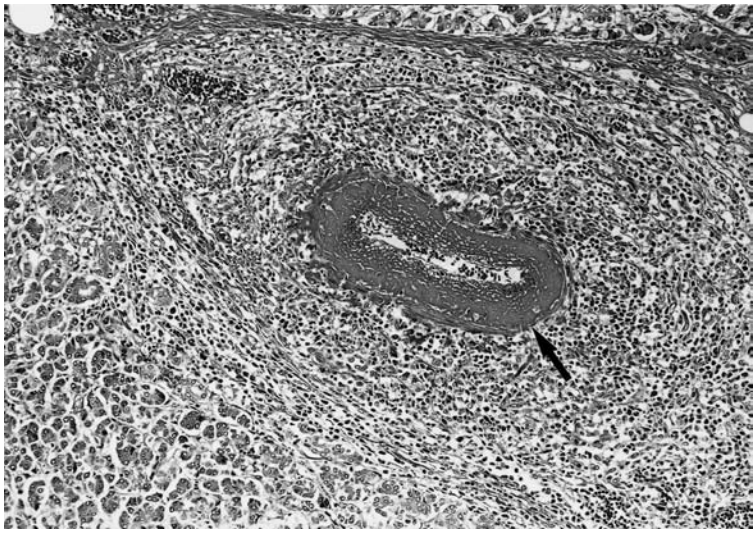

Fig. 1. Polyarteritis nodosa in the pancreas. Necrotizing arteritis associated with fibrinoid necrosis (arrow) in a medium sized artery. Hemorrhage and necrosis in pancreatic tissues around the affected artery are present. HE. ×100.

Pancreatic Vasculitis in Collagen Diseases

Concerning pancreatic vasculitis in collagen diseases, necrotizing arteritis has been reported in the cases with polyarteritis nodosa (PAN) and SLE. Kojima reported that in PAN the incidence of necrotizing arteritis was 60% (12/20 autopsy cases) [1]. In SLE, the incidence of such arteritis has been reported as 7.4% (2/27 autopsy cases) and 6.2% (1/16 autopsy cases) [2–3]. Although a few reports of pancreatic vasculitis in collagen diseases have been published, systematic examination of the pancreatic vasculitis in collagen diseases was not performed. In 1987, Yoshimine, a member of my research group for vasculitis, examined vasculitis in the histological sections from pancreatic head, body and tail in 51 autopsy cases with PAN, SLE, rheumatoid arteritis (RA) or SSc [4]. The incidence of necrotizing arteritis in each disease was 71% in PAN, 53% in SLE, 50% in RA and 17% in SSc. The arteritis presented necrotizing arteritis of the PAN type (fig. 1) and was located in the small and medium-sized arteries. The incidence of arteritis in the pancreatic head was larger than that in the pancreatic body and tail. The arteritis produced hemonecrosis in the pancreatic parenchyma surrounding the severely injured arteries (fig. 1), but not massive necrosis in the parenchyma of the pancreas.

Acute Pancreatitis in Systemic Lupus Erythematosus

Acute pancreatitis can be the initial manifestation of SLE. First reported by Dubois in 1953, it presents severe epigastric pain radiating to the back, nausea, vomiting, elevated serum amylase level, and dehydration [5]. The disease is a serious problem, because severe acute pancreatitis becomes one of the lethal disorders in SLE patients. The incidence of acute pancreatitis in SLE was 4.5% [6], 8.2% [7] and 12.5% [3]. The age of the patients ranged from 12 to 50 (average 24) years, with marked predominance of females (male:female = 3:26) [8]. The cause of the acute pancreatitis has been considered to be drugs (steroid, azathioprine and thazide diuretics), vasculitis, thrombi in the pancreatic arteries, hypovolemia, ischemia, cholecystitis, alcoholism, carcinoma, and viral infections [5].

Pathologic Findings of Pancreas in Sjögren's Syndrome

In 1965, Bloch et al. studied clinical and pathological fingings in 62 cases with SS [9]. In their study, abnormalities in the pancreas at necropsy were acute atrophy and disorganization of the pancreatic parenchyma, areas of acinal tissue replaced by vascular connective tissue and heavy cellular infiltration, and oncocytic changes in several acini [9]. Later, in 1981, Nakamura et al. studied pathological findings in 6 autopsies with SS, and reported that acinal atrophy and oncocytic changes of acinal and duct cells were common findings, found in 4 of 6 cases [10]. Intestinal lymphocytic infiltrates were found in 3 cases, and others were interstitial fibrosis, acinal ectasis with eosinophilic plug, and parenchymal fatty infiltration [10].

Recently, autoimmune pancreatitis was proposed as a new clinical entity [11]. The disease was designated as idiopathic chronic pancreatitis with a possible etiologic factor of autoimmunity. SS overlapped with autoimmune pancreatitis in about a quarter of the cases [12]. However, in most cases with SS, no inflammation or mild inflammatory reaction is seen in the pancreas, and the case reports of pancreatitis are few. This leads to the conclusion that SS usually does not cause autoimmune pancreatitis by itself.

Pathologic Findings of Pancreas in Systemic Sclerosis

In SSc, fibrosis occurs in various organs, especially the skin and esophagus. Concerning the pancreatic fibrosis in SSc, D'Angelo et al. studied 58 autopsies with SSc, coupled with the examination of 58 autopsy cases as controls [13]. In the SSc autopsies, fibrosis occurred in 10 cases (17%), but the incidence of the fibrosis in SSc was almost the same (19%) as the incidence in control cases [13].

These findings indicated that SSc does not cause pancreatic fibrosis by itself. Although case reports of pancreatitis in SSc have been published in the literature, it is thought that overlapping of pancreatitis in SSc is exceedingly rare [14].

Conclusion

I reviewed vasculitis in collagen diseases including the data of my research group, acute pancreatitis in SLE, and pathological findings of the pancreas in SS and SSc. I think that this review is useful for the recognition of disease entities and the pathological diagnosis of pancreatic disease in collagen diseases.

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Multinucleated Giant Cells in Various Pancreatic Diseases

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Abstract

According to the classification of pancreatic carcinoma, rare neoplasms containing numerous multinucleated giant cells are categorized as giant cell carcinoma in anaplastic ductal carcinoma. Osteoclast-like giant cells in the giant cell carcinoma are considered of reactive histiocytic origin, whereas the stroma is considered as a neoplastic component. However, multinucleated giant cells are occasionally found even in usual pancreatic diseases. In 62.5% of usual ductal adenocarcinomas, and in 34.5% of chronic pancreatitis cases, we found giant cells. Various histological and immunohistochemical types of giant cell were found. Epithelial atypical giant cells are positive for epithelial markers and negative for mesenchymal markers; they are bizarre atypical cells in the cancer nest. This type was seen in pleomorphic-type anaplastic carcinoma and in 55% of usual ductal adenocarcinoma. Coexpressed-type atypical giant cells are positive for both epithelial markers and vimentin. Non-expressed-type atypical giant cells are pleomorphic giant cells negative for epithelial markers and CD68. Coexpressed- and non-expressed-type giant cells are considered to be epithelial neoplastic cells with a non-cohesive invasive growth pattern. This type was seen in 10% of usual ductal adenocarcinomas. Mesenchymal-type giant cells are negative for epithelial markers and positive for mesenchymal markers (vimentin and CD68). These giant cells are considered of reactive histiocytic origin. In the giant cell carcinomas and usual ductal carcinomas, they are found in the lumen of the tumor gland or stroma. In chronic pancreatitis, they are accompanied by protein plugs, pancreatic stones, abscesses, and fat necrosis. This type was seen in 15% of usual ductal adenocarcinomas and 34.5% of chronic pancreatitis cases.

Although the majority of pancreatic cancers are ductal carcinomas, rare neoplasms containing numerous multinucleated giant cells have been found. In the Japanese classification of pancreatic carcinomas, giant cell carcinoma is categorized as anaplastic ductal carcinoma. However, multinucleated giant cells were occasionally found even in usual pancreatic lesions. There is a variety of

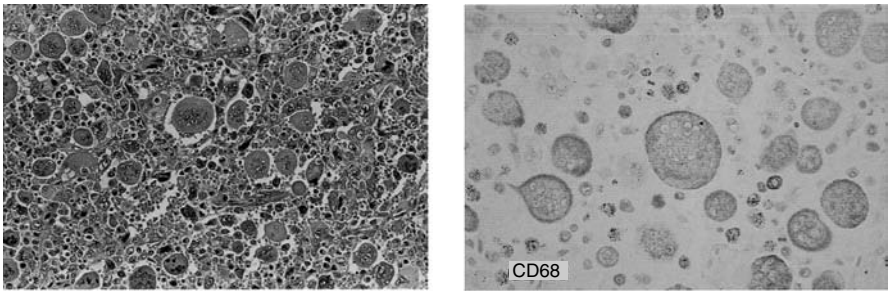

Fig. 1. Osteoclastoid-type giant cell carcinoma. CD68 is positive in osteoclast-like giant cells and some histiocyte-like mononuclear cells, but negative in pleomorphic large cells and atypical mononuclear cells.

giant cells. On the one hand, bizarre multinucleated giant cells are considered to represent pleomorphic forms of the malignant epithelial cells. On the other, macrophages and foreign-body giant cells may occasionally be drawn into areas of hemorrhage, abscess and necrosis. These giant cells arise from the fusion of macrophages. In these cases, the giant cells represent a reactive infiltration of macrophages.

We present some pancreatic tumors accompanied by numerous multinucleated giant cells and some types of multinucleated giant cells in various pancreatic diseases.

Pancreatic Tumors with Numerous Giant Cells

Extraskeletal osteoclast-like giant cell tumors have been described infrequently in a variety of organs. Among them, the pancreas is relatively frequent. Giant cell carcinoma of the osteoclastoid type is classified as anaplastic carcinoma. Associations with mucinous cystic tumors have also been reported.

Giant Cell Carcinoma (Anaplastic Carcinoma)

Histologically, the giant cell carcinoma is composed of osteoclast-like giant cells, pleomorphic giant cells and mononuclear cells. Some of the mononuclear cells appear to be histiocytic, whereas others are atypical. Typical glandular structures of adenocarcinoma are occasionally present (fig. 1).

The osteoclast-like giant cells and histiocytic mononuclear cells are positive for histiocytic markers and negative for epithelial markers. The pleomorphic giant cells and atypical mononuclear cells are negative for both epithelial and histiocytic markers. Some atypical mononuclear cells are positive for some cytokeratins and vimentin. Overt adenocarcinoma cells are positive for epithelial markers and negative for histiocytic markers. Although K-ras activation is

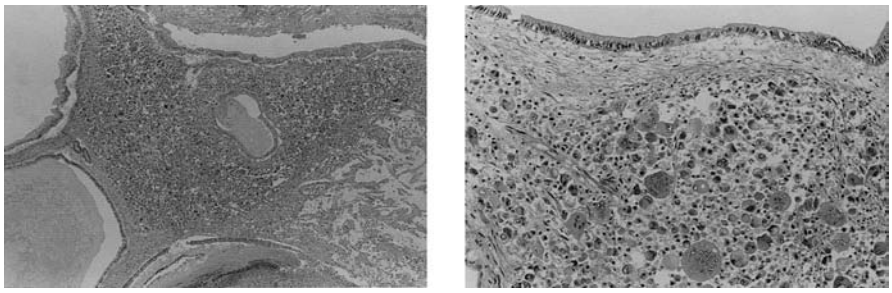

Fig. 2. Mucinous cystadenocarcinoma associated with osteoclast-like giant cells. Mucinous epithelium lining cystic spaces and osteoclast-like giant cells in the stroma.

considered as an early event in tumorigenesis of the pancreatic ductal epithelium, according to some reports [1–4], pleomorphic giant cells and some atypical mononuclear cells had K-ras gene mutations; however, the osteoclast-like giant cells lacked this mutation. A ductal neoplastic origin of such sarcomatous elements is suggested. Osteoclast-like giant cells and histiocytic mononuclear cells are considered to be of reactive histiocytic origin.

Osteoclast-Like Giant Cell Tumors Associated with Mucinous Cystic Tumor

Mucinous cystic tumors usually appear as a unilocular or multilocular encapsulated cystic mass, located in the tail or body of the pancreas, and predominantly occurs in middle-aged woman. Microscopically, the tumor is composed of mucin-producing columnar epithelium and overlying ovarian-type stroma (fig. 2). The ovarian-type stroma is considered as a component of the neoplasm, not a reactive phenomenon. Immunohistochemical features have been demonstrated in the stromal cells, such as overexpression of p53 and relationship to some ovarian hormonal functions [5–8]. Osteoclast-like giant cells associated with ovarian-type stroma of the mucinous cystic tumors have been reported [5, 9]. These giant cells were positive for histiocytic markers.

Osteoclast-like giant cells are hence considered to be reactive inflammatory cells, although stromal cells are neoplastic cells.

Multinucleated Giant Cells in the Ductal Carcinoma and Chronic Pancreatitis

Multinucleated giant cells are occasionally found even in the usual pancreatic lesions. According to our previous report [10], multinucleated giant cells were found in 62.5% of tubular adenocarcinomas, and 34.5% of chronic pancreatitis cases (table 1). However, multinucleated giant cells varied from

Table 1. Multinucleated giant cells in various pancreatic diseases

	n	Epithelial type	Coexpressed type	Mesenchymal type
I. Chronic pancreatitis	29	0	0	10 (34.5%)
II. Pancreatic carcinomas				
Mucinous cystic tumor				
Mucinous cystadenocarcinoma	1	0	0	1
Invasive ductal carcinomas				
Tubular adenocarcinoma	[40	22 (55%)	4 (10%)	6 (25%)]
WeII differentiated type	3	1	0	1
Moderately differentiated type	30	16	2	4
Poorly differentiated type	7	5	2	1
Mucinous carcinoma	2	1	0	0
Anaplastic carcinoma				
Pleomorphic type	1	1	0	0
Osteoclastoid type	4	0	0	4

Epithelial type: Keratin (+); EMA (+); Vimentin (−); CD68 (−).
Coexpressed type: Keratin (+); EMA (+); Vimentin (+); CD68 (−).
Mesenchymal type: Keratin (−); EMA (−); Vimentin (+); CD68 (+).

benign-looking histiocytic to bizarre neoplastic giant cells. They were classified histologically and immunohistochemically into several types.

Epithelial Atypical Giant Cell (fig. 3a)

We classified the giant cell as the epithelial type if it was positive for epithelial markers (keratin or EMA) and negative for mesenchymal markers (vimentin and CD68). Histologically, these giant cells showed marked cellular pleomorphism with sometimes irregular bizarre nuclei and abundant mitotic figures. These were found in the cancer nest and a transitional form from glandular structure was seen. This type was seen in pleomorphic-type anaplastic carcinomas and even in 55% of usual ductal adenocarcinomas.

Coexpressed Atypical Giant Cell and Non-Expressed Atypical Giant Cell (fig. 3b)

We classified the giant cell as the coexpressed type if it was positive for both epithelial markers (keratin or EMA) and vimentin. These giant cells were also pleomorphic atypical cells. These were found in the invasive area which showed a non-cohesive growth pattern, and these cells were individually detached from one another. This type was recognized in 10% of usual ductal

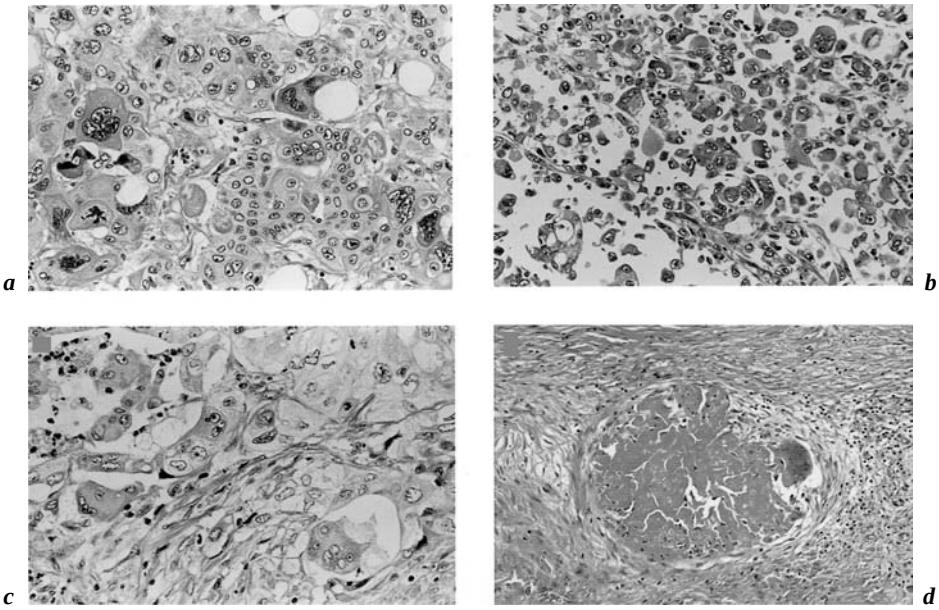

Fig. 3. Multinucleated giant cells in various pancreatic diseases. ***a*** Epithelial atypical giant cells in a moderately differentiated tubular adenocarcinoma. This type expressed keratin and EMA. ***b*** Coexpressed atypical giant cells in a poorly differentiated tubular adenocarcinoma. This type reacted with both epithelial and vimentin antibodies. ***c*** Mesenchymal giant cells in a moderately differentiated tubular adenocarcinoma. ***d*** Mesenchymal giant cell in chronic pancreatitis. This type (***c***, ***d***) reacted with CD68.

adenocarcinomas. All were far advanced autopsy cases. We considered this type as epithelial neoplastic origin. Epithelial neoplastic giant cells are common in ductal carcinomas with poor differentiation. These cells are usually positive for epithelial markers; however, coexpression of vimentin and cytokeratin is occasionally seen. Coexpression of vimentin and cytokeratin was reported in some conditions [11–13]. In cultured epithelial cells, when extensive cell-cell contact was achieved, the cells synthesized high levels of cytokeratins and low levels of vimentin. In contrast, when cell-cell contact was minimal, the cells synthesized very low levels of cytokeratins and high levels of vimentin. The results suggest that vimentin synthesis responds to alterations of cell spreading [12]. In clinicopathological study of gastric carcinoma, vimentin expression was related to infiltrative growth, lymph node involvement and vascular invasion [13].

In the anaplastic carcinoma, there were pleomorphic atypical giant cells which were negative for epithelial markers and CD68, and some of which were

positive for vimentin. These giant cells were considered to be of similar type as the coexpressed type.

Mesenchymal Giant Cell (fig. 3c)

We classified the giant cell as the mesenchymal type if it was negative for epithelial markers (keratin and EMA) and positive for mesenchymal markers (vimentin and CD68). These giant cells had multiple uniformly oval nuclei and abundant cytoplasm without atypical features. Osteoclast-like giant cells and foreign-body giant cells were included in this type. It was seen in 15% of usual ductal adenocarcinomas and 34.5% of chronic pancreatitis cases. In the tubular adenocarcinomas, these giant cells were found in the lumen of tumor the gland or stroma. In the giant cell carcinomas of osteoclastoid type, these giant cells were scattered between the stromal cells. In the mucinous cystadenocarcinoma, they were scattered in the ovarian-like stroma. In chronic pancreatitis, they were foreign-body giant cells associated with protein plugs, pancreatic stones, abscesses and fat necrosis. We considered this type of reactive histiocytic origin.

Conclusion

Multinucleated giant cells were occasionally found even in usual pancreatic ductal carcinomas or chronic pancreatitis. However, there are various histological and immunohistochemical types of multinucleated giant cells, ranging from benign-looking histiocytic to bizarre neoplastic.

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Age-Related Lesions of the Pancreas, Relevant to Branch Duct Type IPMT/IPMN and Differential Diagnosis of MCT/MCN

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Abstract

There are two types of epithelial metaplasia of the pancreatic duct: mucous cell metaplasia and squamous cell metaplasia. The former is often involved in papillary protrusion compatible with mucous cell hyperplasia, which occurs at multiple sites and shows age-related increases in frequency, approaching 100% in the elderly. Mucous cell metaplasia/hyperplasia is usually associated with naked islets of Langerhans and branch duct remnants due to parenchymal/acinar loss. Cystic dilatation of the branch pancreatic ducts in post-mortem pancreatography is also increased with age-related frequency. The epithelia of the cystic dilated duct are composed of mucous cell metaplasia/hyperplasia. Therefore, age-related pathological conditions of the pancreas consist of mucous cell metaplasia/hyperplasia, cystic dilatation of the branch ducts and naked islets of Langerhans, which occur in this order. Some hyperplasias show similar clinical pictures to intraductal papillary-mucinous tumor (IPMT), leading to a series of, or relevant to, both ductal lesions.

According to the description of the intraductal papillary-mucinous tumor (IPMT)/intraductal papillary-mucinous neoplasm (IPMN) by the AFIP (Armed Forces Institute of Pathology), 'this is an intraductal pancreatic tumor formed of papillary proliferations of mucin-producing epithelial cells that have some gastroenteric differentiation [1].' Mucinous cystic tumors (MCTs)/mucinous cystic neoplasms (MCNs), however, are cystic pancreatic tumors formed of epithelial cells producing mucin; there is evidence of gastroenteropancreatic differentiation and an 'ovarian-type stroma [1].'

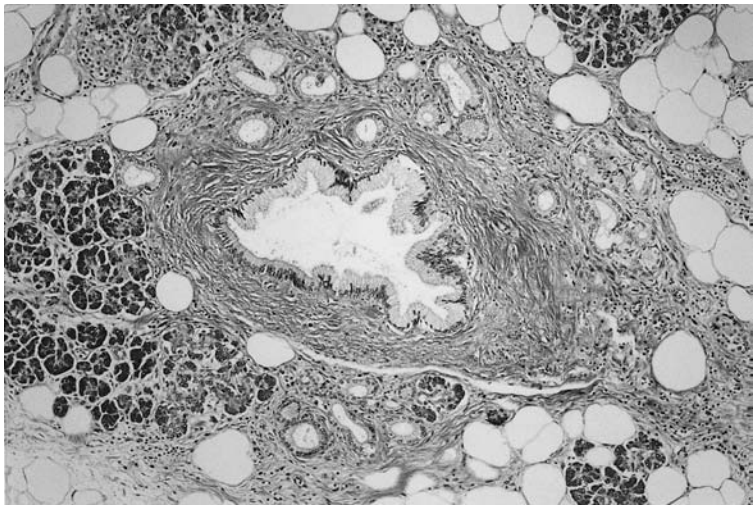


Fig. 1. Mucous cell metaplasia/hyperplasia. Tall columnar/mucinous epithelia arranged in mildly dilated and papillary proliferation in the lumen of the branch pancreatic duct. HE. ×100.

In the pancreas, especially in the elderly, IPMT-like non-tumorous epithelial changes occur [2]. Kimura et al. [3] reported that small non-neoplastic cystic lesions were found in nearly half of the 300 patients studied and the prevalence increased with age.

In this chapter, age-related lesions/findings of the pancreas are described and compared between IPMTs and MCTs.

Age-Related Pathological Findings in the Pancreas

Metaplasia/Hyperplasia of Pancreatic Ductal Epithelium

There are two types of epithelial metaplasia of the pancreatic duct: mucous cell metaplasia, or goblet cell metaplasia, and squamous cell metaplasia, including basal cell metaplasia. The former is composed of a tall columnar/mucinous epithelium, non-papillary or flat in a layer, often arranged in papillary protrusion, compatible with mucous cell hyperplasia and found in all sizes and levels of the pancreatic ducts, but predominantly in the branches and smaller ducts (fig. 1). The latter is composed of small nests of squamous cells, seldom in keratinization and malignant change, and found also in all sizes and levels of the pancreatic ducts, especially in the branching-off portion (fig. 2) [4].

Recently, Hruban et al. [5] proposed the terminology of pancreatic intraepithelial neoplasia (PanIN), which was a new nomenclature and classification sys-

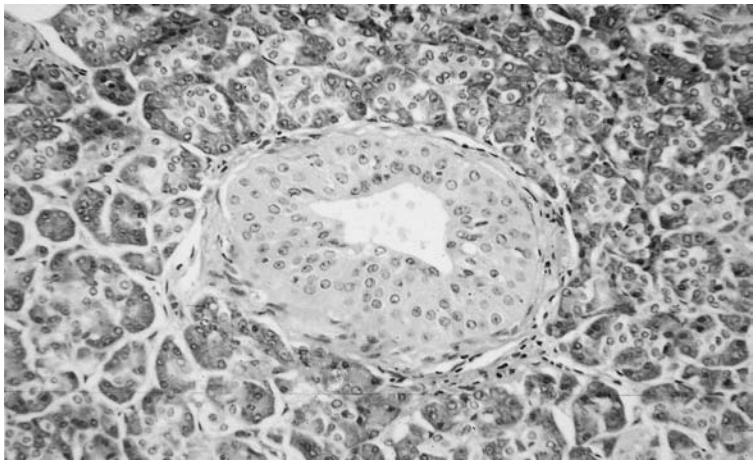


Fig. 2. Squamous cell metaplasia. A nest of mature stratified squamous or pseudostratified transitional epithelium was found just beneath the columnar epithelium. HE. ×250.

tem for pancreatic duct lesions and putative precursor lesions of invasive ductal carcinoma (IDC) in the smaller-caliber pancreatic ducts. The revised definition of PanIN is defined as non-invasive epithelial neoplasms arising in any part of the pancreatic ducts, including the main duct, while PanIN was usually <5 mm in diameter [6]. Most of the mucous cell metaplasia/hyperplasia in our previous study corresponded with PanIN/L-1A and PanIN-1B (mucinous duct proliferation without atypia), and less often PanIN-2 (mucinous duct neoplasia with atypia), but never PanIN-3 (severe dysplasia/carcinoma in situ). PanIN-1 and -2 are thought to be common incidental findings in the general population [7, 8].

Mucous cell metaplasia/hyperplasia occurs at multiple sites and shows an age-related increase in frequency; it is first detected in the first decade of life and increases thereafter, finally becoming very close to 100% in elderly persons. The average frequency of mucous cell metaplasia/hyperplasia is 52.6% (206 of 392 cases). The frequency by site for the head, body, and tail is 155 cases, 120 cases, and 129 cases, respectively. The pancreatic ducts with metaplasia/hyperplasia tend to dilate through both secreted mucinous fluid retention and cell proliferation/hypertrophy. Mucous cell metaplasia/hyperplasia is usually associated with naked islets of Langerhans due to parenchymal loss and the remnants of branch ducts, which appear as in elastosis, in upstreaming lobules (fig. 3) [4, 9, 10], because epithelial hyperplasia may cause obstruction/stenosis of the branch pancreatic ducts. Detlefsen et al. [11] cite that narrowing of the duct lumen can be caused by papillary proliferations of hypertrophic epithelium and may hamper secretion and cause fibrosis of the drained lobule. According

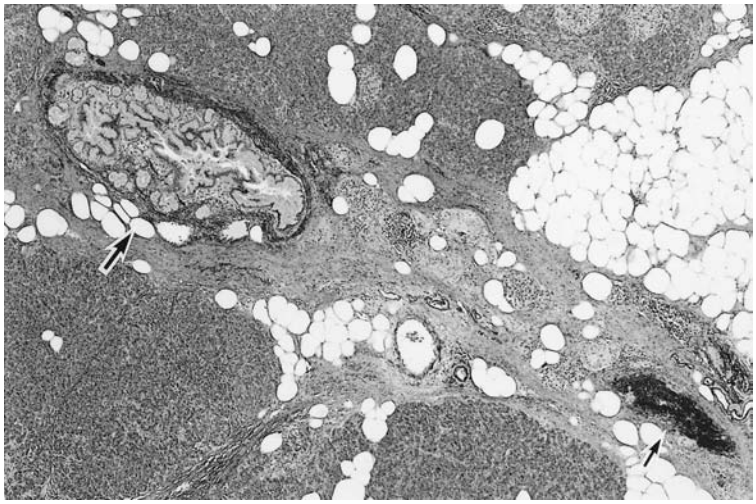


Fig. 3. Mucous cell hyperplasia in the branch duct and parenchymal loss in the upstreaming lobule. Branch duct with hyperplasia (large arrow) was dilated and associated with parenchymal loss, resulting in naked islets of Langerhans. A remnant of the pancreatic duct (small arrow) was found. Elastica van Gieson. ×41.

to our studies [9, 10], however, not only fibrosis but also fat replacement occur in the upstreaming lobule.

Cystic Dilatation of Branch Ducts, or 'Bubbles'

According to a postmortem pancreatography series by Komatsu [12], cystic dilatation of the branch pancreatic ducts was found in 37 of 85 cases (43.5%), showing age-related increases in frequency, and was found in multiple sites in all but 5 cases (fig. 4a). The lesion was also called a 'bubble'. The frequency by site was 28 cases in the head, 27 cases in the body and 16 cases in the tail. The size of the cystic dilatation ranged between 15.4 × 8.2 and 6.8 × 0.8 mm on postmortem X-ray.

The epithelia of the cystic-dilated duct are composed of tall columnar/mucous cells, with or without papillary protrusion, similar to those of mucous cell metaplasia/hyperplasia described above, and also associated with naked islets of Langerhans in the surrounding tissue (fig. 4b).

Both cystic dilatation of the branch duct and naked islets of Langerhans begin to be detected in about the fourth decade, about 20 years later than mucous cell hyperplasia, and also show age-related increases in frequency, as shown in figure 5.

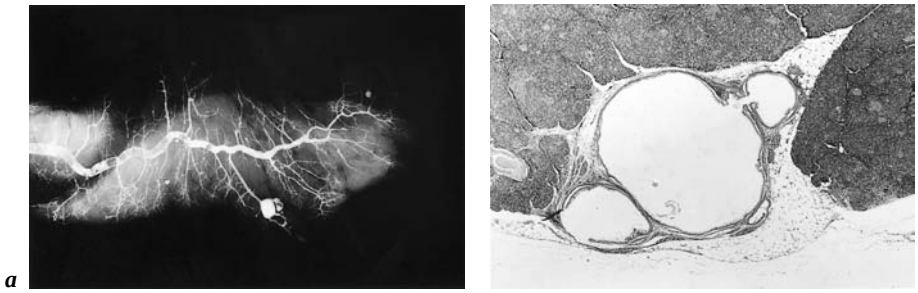


Fig. 4. Cystic dilatation of the branch pancreatic duct (44-year-old male). ***a*** Pancreatography at autopsy showing three cystic dilatations in the peripheral pancreatic ducts. ***b*** The cystically dilated duct in the center was lined/replaced by tall columnar/mucinous cells and associated with parenchymal loss and naked islets of Langerhans in the surrounding tissue. HE. Low magnification. (From [9] with permission.)

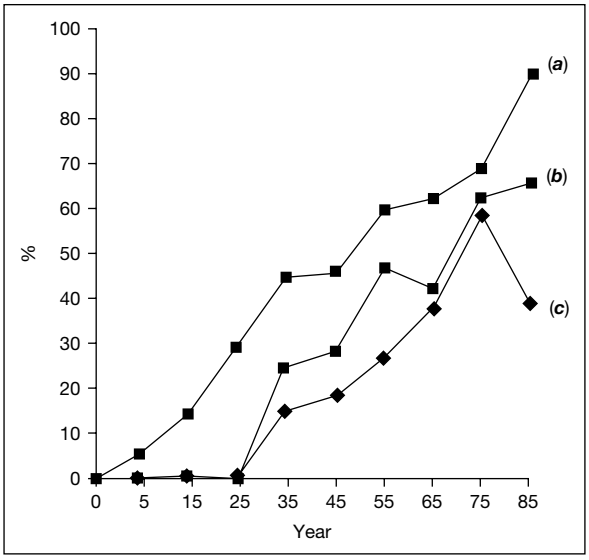


Fig. 5. Age-related frequency of (***a***) mucinous cell metaplasia/hyperplasia, (***b***) cystic dilatation of branch pancreatic duct and (***c***) naked islets of Langerhans.

Relationships between Hyperplasia, Cystic Dilatation of Branch Duct, and Naked Islets of Langerhans

Hyperplasia of the pancreatic duct epithelium begins in the first decade, and both cystic dilatation of the branch pancreatic ducts and naked islets of

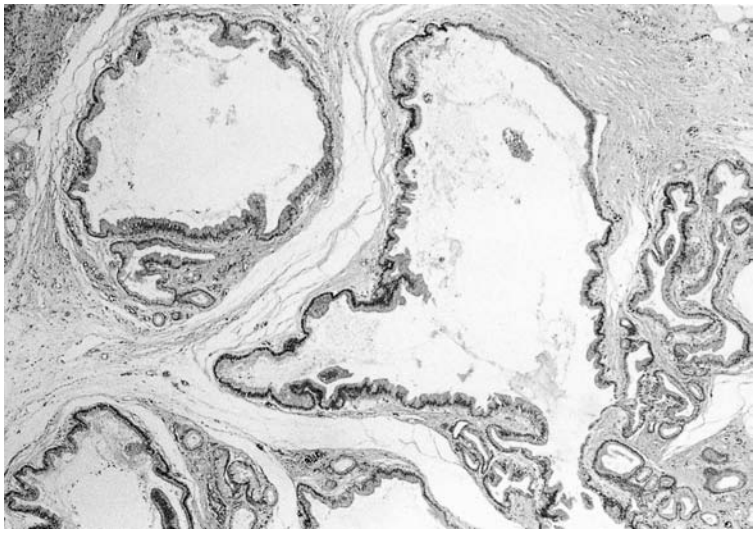


Fig. 6. Multiple cystic dilatations of branch ducts aggregating due to parenchymal loss. HE. ×20.

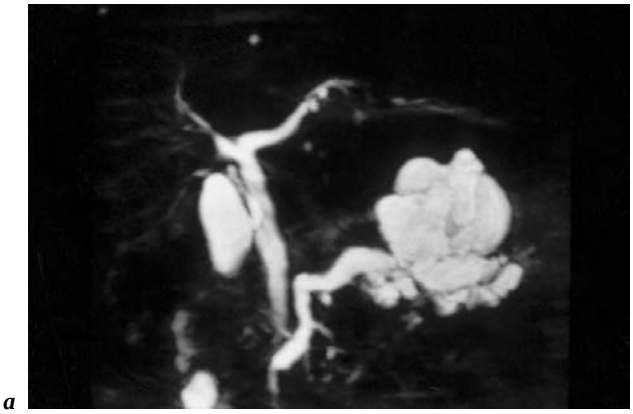
Langerhans occur about 20 years later, as described above. These lesions all show age-related increases in frequency. Hence, epithelial metaplasia/hyperplasia of the pancreatic ducts occurs first, then these ducts tend to dilate due to retention of mucus production and epithelial hypertrophy/proliferation, which also causes ductal narrowing [11], finally leading to cystic dilatation and surrounding or upstreaming parenchymal atrophy.

Therefore, age-related pathological conditions of the pancreas consist of mucous cell metaplasia/hyperplasia, cystic dilatation, or 'bubble', of the branch ducts and naked islets of Langerhans, which occur in this order.

Structure of Branch Duct Type IPMTs/IPMNs, and Differential Diagnosis to MCTs/MCNs

Structure of IPMTs/IPMNs

Numerous cystic dilatations of branch pancreatic ducts with epithelial hyperplasia appear together due to parenchymal atrophy/loss, resulting in a 'multilocular cyst' appearance (fig. 6); this causes confusion between IPMTs and MCTs [13]. Such cystic dilatations of the branch duct with hyperplasia are very similar to the branch duct type of IPMT (fig. 7) as follows: papillary



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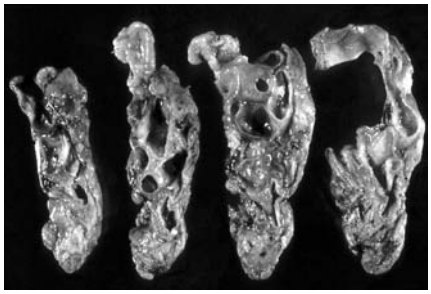

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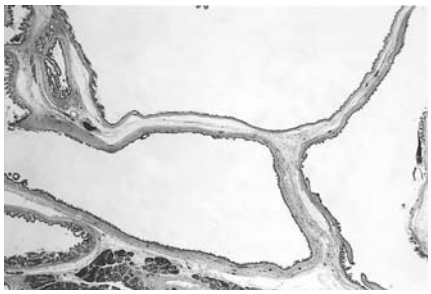

c

Fig. 7. IPMT in the branch duct (68-year-old female). ***a*** MRCP showing cystic/branch-like dilatation of the branch duct and mild dilatation of the main pancreatic duct. ***b*** Cut surface, consisting of cystic dilatation of pancreatic ducts. ***c*** Low magnification showing cystic dilatation of branch duct with parenchymal loss, resulting in 'multilocular cyst' appearance. HE. ×12.5.

arrangement, luminar mucin retention, surrounding parenchymal atrophy and highly frequent occurrence in elderly subjects: except for cellular atypism [2], and some hyperplasia that happen to show clinical pictures similar to IPMTs. Hence, both lesions are considered a series of, or relevant to, the ductal lesions.

Difference in Hyperplasia and Adenoma

With regard to the pathological determination of intraductal proliferation, the difference between hyperplasia and IPMT is very complicated. In a study of benign small intraductal tumors found incidentally at autopsy, Ratcliffe et al. [14] discusses the difficulty in morphologically distinguishing these branch-type IPMTs from ductal papillary hyperplasia found in normal and chronically

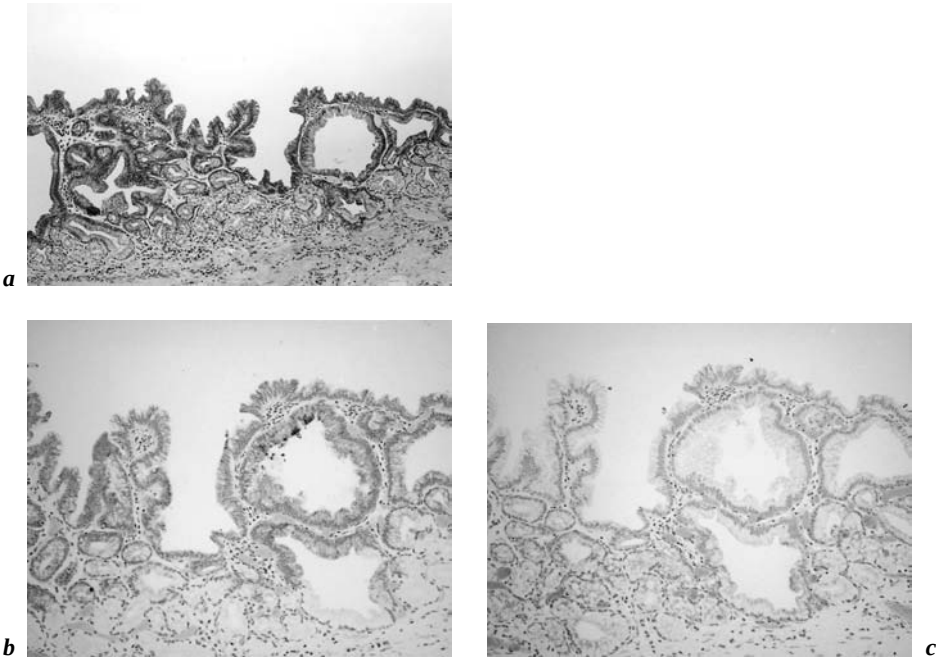

Fig. 8. Well developed papillary hyperplasia. ***a–c*** Hyperplasia showing double structures, consisting of papillary proliferation overlying pyloric gland-like tubuli beneath. ***a*** HE. ×125. ***b*** Immunostained with MUC5AC. ×200. ***c*** Immunostained with MUC6. ×200.

inflamed pancreatic tissue and in association with a pancreatic tumor. However, in our opinion, well developed papillary hyperplasia of the pancreatic duct shows a characteristic two-layered structure with papillary growth overlying pyloric gland-like tubuli beneath, whereas adenoma, even if fully developed, consists of only simple papillary growth without pyloric gland-like tubuli (fig. 8). This structure is described by the WHO [15] as follows: mucinous cell hypertrophy in medium-sized ducts may be associated with pyloric gland metaplasia in small glands surrounding the larger duct. The former two-layered structure has a mucinous characteristic similar to the gastric pyloric area mucosa: foveolar epithelium and pyloric glands which stain positive for galactose oxidase-cold thionine Schiff-paradoxical concanavalin A (GOCTS-PCS) [16], while the latter does not. Immunohistochemically, the overlying papillary proliferation is positive for MUC5AC, while the pyloric gland-like tubuli beneath stain with MUC6 (fig. 8b, c). Hence, epithelial hyperplasia and adenoma differ in structure, mucin histochemistry and MUC immunohistochemistry.

Table 1. Differences between branch type IPMTs and MCTs

	Branch type IPMT	MCT
Age	elderly	middle-aged
Gender	male	female
Location	head	body and tail
Structure	preserved/undeviated	deviation/exophytic protrusion
Capsula	absent (pancreatic duct wall)	present
Ductal communication	present	usually absent
Macroscopic view	cystic (dilatation of duct)	cystic
Epithelium	mucinous	mucinous
Stroma	fibrous	ovarian-like stroma

IPMT = Intraductal papillary-mucinous tumor; MCT = mucinous cystic tumor.

Differential Diagnosis between IPMT and MCT

MCTs are unilocular or multilocular cystic tumors, which are composed of mucinous epithelium and ovarian-like stroma. In cases of multilocular cystic tumor, there was no apparent interlocular communication. The MCT sometimes communicated with the main pancreatic duct [17].

MCTs tend to occur in middle aged women, and are located in the body and tail of the pancreas, while IPMTs tend to occur most frequently in elderly men, and are located in the head, similar to mucous cell metaplasia/hyperplasia [4], as shown in table 1. Hence, MCTs are different from IPMTs.

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Mucinous Cystic Neoplasms of the Pancreas: A Morphological and Immunohistochemical Study

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Abstract

Mucinous cystic neoplasms (MCNs) of the pancreas are rare, mucin-producing cystic tumors. Generally, pancreatic MCNs occur mostly in middle-aged women and are located in the body and the tail of the pancreas. MCNs can be unilocular or multilocular cystic tumors with fibrous capsules and cysts, and are filled with mucinous fluid. Histologically, these cysts are lined by a tall columnar and mucin-secreting epithelium with various grades of atypia. A cellular stroma composed of spindle cells, known as ovarian-like stroma (OLS), is observed in the cyst wall and septum. Immunohistologicaly, OLS shows positivity for vimentin and α-smooth muscle actin and strong nuclear staining with estrogen and progesterone receptor. The aim of this study is to clarify the clinicopathologic features of pancreatic MCNs, especially the OLS and diacrisis from intraductal papillary-mucinous neoplasms.

Mucinous cystic neoplasms (MCNs) are rare but well-known disease entities that occur in the pancreas, liver, and the retroperitoneum [1, 2]. MCNs of the pancreas are mucin-producing unilocular or multilocular cystic tumors with a fibrous capsule and are lined by mucin-secreting epithelium associated with an underlying subepithelial ovarian-like stroma (OLS). OLS is the subepithelial component resembling the ovarian stroma, that is composed of bundles of densely packed spindle cells. Immunohistologically, the OLS shows strong positivity for α-smooth muscle actin (SMA) and vimentin, and weak, focal positivity for desmin [3]. Both estrogen receptors (ER) and progesterone receptors (PgR) are expressed in the nuclei of OLS cells. The grade of dysplastic changes should be classified as slight (mild), moderate, and severe. In accordance with the

World Health Organization (WHO) classification, also reported in the Armed Forces Institute of Pathology (AFIP) fascicle, pancreatic MCNs are divided into adenomas, borderline tumors, and non-invasive and invasive carcinomas.

Clinical Features

MCNs occur predominantly in middle-aged women, and in male patients are very rarely reported in the literature [4]. These tumors are often discovered as an abdominal mass. Patients are usually asymptomatic or show nonspecific symptoms [5, 6]. They have epigastric pain or intermittent or continuous discomfort associated with an enlarged palpable abdominal mass. Generally, MCNs have no communication with the pancreatic ductal system. However, pancreatic duct communication was confirmed in several pancreatic MCN by both examination of resected materials and preoperative pancreatography [7].

Gross Appearance

On gross examination, most pancreatic MCNs are in the tail, or the body to tail of the pancreas, and rarely in the body. The tumors are unilocular or multilocular cystic tumors and tend to contain a large cavity in the center with small cavities in the periphery (fig. 1). The tumors consist of a thick fibrous capsule with colorless and smooth external surfaces, and contain concentrated colorless, slightly brown or hemorrhagic mucinous fluid. The largest pancreatic MCNs measure up to 26 cm in the greatest dimension [6], and the smallest are 2–3 cm.

Microscopic Features

Histologically, the cyst walls and septa in cystic tumors are composed of three layers: mucinous epithelial cells, the OLS, and a thin or thick fibrous capsule. The lining epithelia of MCNs are composed mostly of tall columnar, mucin-secreting cells, and they exhibit a range of atypia from benign-looking to atypical cells. The epithelial cells show a variable degree of papillary growth. Adding a supplementary explanation, Albores-Saavedra et al. [6] has reported that many of the tall columnar cells are similar to those of the major pancreatic ducts, while others resemble those of the colon or of the superficial gastric epithelium. Goblet cells, which may form invaginations, are seen in nearly all tumors, and Paneth cells occur in 10% of tumors.

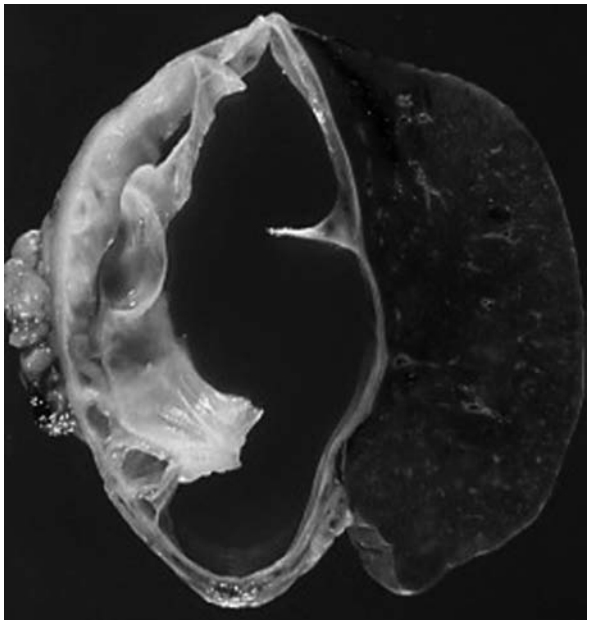

Fig. 1. Mucinous cystic neoplasms of the pancreas. A multilocular cyst is located at the pancreatic body to tail. The cyst is apparent on the cut surface.

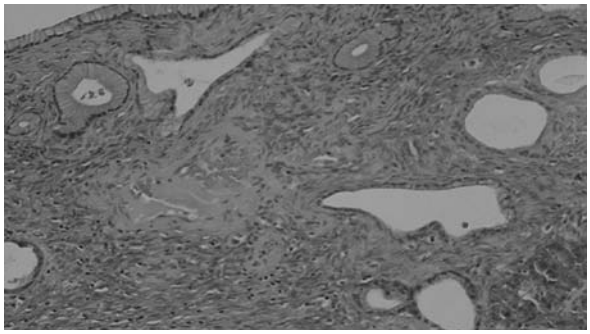

Fig. 2. The cystic wall is lined by tall columnar mucinous epithelium. The ovarian like stroma is composed of mostly of oval to spindle-shaped cells with scant cytoplasm arranged in bundles.

The OLS is composed mostly of oval to spindle-shaped cells with round to elongated nuclei without atypia and with a small amount of cytoplasm, resembling ovarian stroma (fig. 2). Immunohistologically, the OLS shows strong immunopositivity for vimentin and α-SMA and focal immunopositivity for

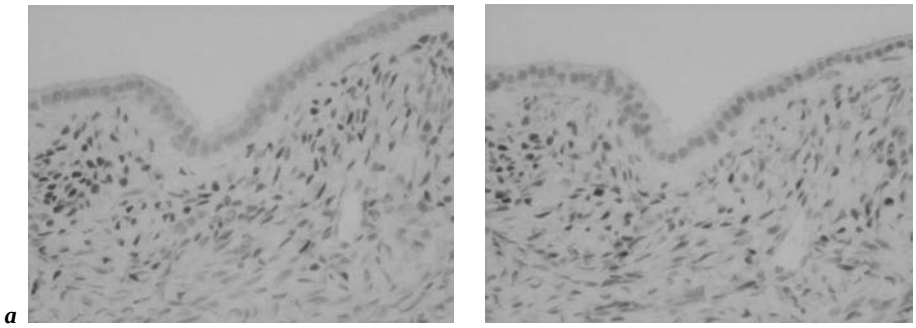

Fig. 3. Immunohistochemical study of OLS of pancreatic mucinous cystic neoplasms. ***a*** The OLS shows immunopositivity for estrogen receptor. ***b*** The OLS also shows immunopositivity for progesterone receptor.

desmin. Both ER and PgR are expressed in the nuclei of OLS cells (fig. 3). Sometimes, MCNs contain pseudosarcomatous mural nodules . Garcia et al. [8] reported a case of pancreatic mucinous cystic neoplasm with pseudosarcomatous mural nodules in which both the epithelial component and the pseudosarcomatous mural nodules were believed to be malignant. They considered the mucinous cystic component to represent a cystadenocarcinoma and the pseudosarcomatous mural nodules an anaplastic carcinoma. The epithelial origin of the mural nodules was confirmed on the basis of cytokeratin and EMA reactivity. The stroma component was mesenchymal in origin and the sarcomatous stroma portended an adverse clinical course. The presence of a sarcomatous element is related to the aggressiveness of MCNs [9].

Histological Grading (Benign/Borderline/Carcinoma)

According to the WHO classification [10], pancreatic MCNs are classified as adenomas, borderline tumors, noninvasive adenocarcinomas, and invasive carcinomas. Zamboni et al. [11] classified a series of 56 MCNs of the pancreas as 22 adenomas, 12 borderline tumors, 6 noninvasive carcinomas, and 16 invasive carcinomas.

Pancreatic and ovarian MCNs show similar morphologic features and biologic behavior [11], such as malignancy correlated with multilocularity and papillary projections or mural nodules, and loss of OLS. Compagno and Oertel [5] believed that all pancreatic MCNs should be regarded as potentially

malignant, regardless of the epithelial component, and that histology alone is not a definitive prospective indicator of behavior. However, pancreatic MCNs in general have an indolent clinical course and are less aggressive than conventional infiltrating pancreatic ductal adenocarcinomas. Based on the morphologic features and biologic behavior, such as malignancy correlated with multilocularity and papillary projections or mural nodules, pancreatic MCNs are similar to their ovarian counterparts [11].

Differential Diagnosis

Pancreatic MCNs were previously confused with intraductal papillary-mucinous neoplasms (IPMNs), because both tumors are mucin-producing cystic tumors. Massive mucin production is found in both, and papillary projection is a common histological characteristic. However, there are also many differences. MCNs and IPMNs differ sufficiently in clinical presentation, such as anatomic location, radiographic appearance and patient demographics. IPMNs are most frequently found in men in their sixties, originate in the head of the pancreas, and histologically are characterized by dilatation of the main or branch pancreatic ducts. Pancreatic MCNs occur mostly in middle-aged women, and are located in the body and tail of the pancreas. Moreover, the presence of OLS distinguishes MCNs from IPMNs.

Histogenesis of OLS

Pathogenesis of the pancreatic MCNs is unclear. Zamboni et al. [11] reported that the OLS exhibits a variable degree of luteinization, characterized by the presence of epithelioid cells with round to oval nuclei, and abundant clear or eosinophilic cytoplasm. Expression of ER and PgR in the OLS has also been reported [12] in most cases of pancreatic MCNs and hepatobilliary MCNs. In our study, the OLS showed strong immunopositivity for α-SMA and vimentin, and weak focal immunopositivity for desmin, findings which suggest a myofibroblastic character. These findings were similar to the descriptions of Thompson et al. [13]. However, with double staining of the OLS in our present study, immunoreactivity for ER and PgR were found in the nuclei of myofibroblasts. In addition to ultrastructural features, the OLS was seen with myofibroblasts and was composed of mainly spindle-shaped cells which on electron microscopy showed few granular endoplasmic reticula and numerous small pinocytotic vesicles along all membranes. α-SMA in the ovarian stroma has been related to stimulation of the latter by a variety of factors [14]. Cytoskeletal

protein expression can also be modulated in some cells by sex hormones. Thus, estrogen and progesterone were found to stimulate smooth muscle cells [14]. Moreover, the stroma of ovarian MCNs was strongly immunopositive for α-SMA and vimentin, as pancreatic MCNs, whereas normal ovarian stroma was immunonegative for α-SMA. In addition, double staining of the OLS showed immunopositivity for α-SMA and ER and for α-SMA and PgR [7]. Hence, we emphasize that the OLS represents myofibroblastic proliferation in response to tumor development, but not ovarian stroma, and that OLS might be dependent on hormonal activation, and the response to tumor development mentioned above might be one reason why MCNs develop predominantly in females.

In our series, pancreatic duct communication was confirmed in 7 (31%) of 22 pancreatic MCNs by both examination of resected materials and preoperative pancreatography. Therefore, we propose MCN criteria as follows: a cystic tumor lined by mucin-producing epithelium, usually supported by OLS, with or without pancreatic duct communication.

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The Spectrum of Serous Cystic Tumors of the Pancreas

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Abstract

Accurate diagnosis of cystic lesions of the pancreas is important due to their varied clinical course and behavior. Pancreatic cystic neoplasms fall predominantly into two groups: mucinous cystic tumors (MCT) and serous cystic tumors (SCT). While the MCT is a potentially malignant neoplasm that requires resection, the SCT has little or no malignant potential, and therefore should be treated conservatively. SCT of the pancreas is a rare neoplasm. With improvements in imaging methods, the morphologic spectrum and biologic diversity of pancreatic SCTs have broadened in recent years. Currently four subtypes of SCT may be distinguished. The classic and most common type is serous microscopic adenoma. It occurs predominantly in elderly females. It is characterized by a honeycomb-like appearance with a central stellate, occasionally calcified scar. The second type is serous oligocystic adenoma, which exhibits distinctly different macroscopic features from the serous microscopic adenoma. It is composed largely or exclusively of macrocysts (>2 cm), which are few in number. Because the cystic spaces are larger, the imaging appearance of serous oligocystic adenomas may be confused with MCTs, pseudocysts or solitary true cysts. The third type of SCT is solid serous adenoma. This tumor has a solid appearance with well-defined margins. The fourth type is the malignant serous cystadenocarcinoma. Several investigators have reported cases of SCT that are histologically indistinguishable from serous microscopic adenomas, but that show signs of malignancy. The cytological findings of all four types of SCT are similar. SCTs are composed of clear to eosinophilic cuboidal epithelium that is rich in glycogen, as demonstrated by PAS-positive, diastase-sensitive reactivity. The differentiation of SCTs from other cystic lesions is very important because of the great difference in their management. Despite their rarity, recognition of these subtypes of SCT will prevent misinterpretation of the radiologic and macroscopic features of cystic pancreatic lesions.

Serous Cystic Tumors

In general, 10–15% of pancreatic cysts are considered to be primary cystic neoplasms. Cystic pancreatic neoplasms comprise only 1% of all pancreatic

neoplasms and fall into two groups [1]. One group includes serous cystic tumors (SCTs), the other mucinous cystic tumors (MCTs). In 1978, Compagno and Oertel were the first to differentiate pathologically between SCTs and MCTs [2]. Recent molecular studies have revealed that SCTs can be distinguished from MCTs [3]. Accurate diagnosis of these neoplasms is important due to their varied clinical course and behavior. MCTs either carry a potential for malignant transformation or have already transformed into cystadenocarcinomas at the time of presentation [4]. In contrast to MCTs, SCTs are generally believed to be benign [2, 5]. The cellular origin of SCTs has not yet been established. There is no uniform consensus on whether they originate from acinar, centroacinar, or ductal cells. However, the immunohistochemical and ultrastructural features of SCTs suggest a centroacinar cell origin [6–9].

SCTs of the pancreas are uncommon tumors and are usually classified as microcystic adenomas [2, 10]. The serous microcystic adenoma has a characteristic spongy gross appearance as a result of numerous tiny cysts and a central scar [1, 2, 5, 10]. The epithelial cells are rich in glycogen, giving rise to the alternative name of glycogen-rich cystadenoma [2]. In recent years, lesions of the pancreas, including SCTs, have been detected more easily because of the advance in diagnostic imaging methods. Moreover the morphologic spectrum and biologic diversity of SCTs have expanded. Two newly described SCTs, known as serous oligocystic adenoma and solid serous adenoma, have been reported as variants of the more familiar serous microcystic adenoma [1, 8, 11, 12]. SCTs are frequently incidental findings. It is important to distinguish between these subtypes. Due to slow growth, most SCTs do not require resection unless the patient is symptomatic [13, 14]. Malignant transformation of SCTs is exceedingly unusual. However, there have been several reports of malignant SCTs called serous cystadenocarcinoma [15–18].

Serous Microcystic Adenomas

In 1978, Compagno et al. described microcystic adenomas as composed of innumerable tiny cysts [2]. Serous microcystic adenoma is the most common subtype of SCT, accounting for more than 70% of SCTs [19, 20]. Synonyms include microcystic adenoma, glycogen-rich adenoma, and simply serous cystadenoma [1, 2, 10].

Females are more commonly affected (70%). Tumors usually affect elderly individuals in their 6th and 7th decades (34–91 years). The patients may present with a palpable mass or vague abdominal pain. Asymptomatic cases have been found incidentally during imaging techniques, at laparotomy or at autopsy. Serous microcystic adenoma is a slow-growing tumor and it can become large

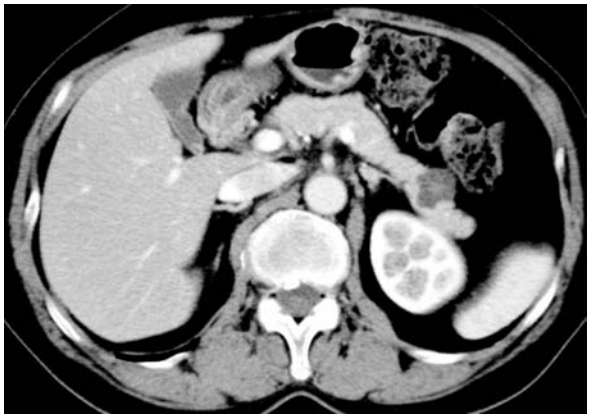

Fig. 1. Serous microcystic adenoma. Enhanced CT scan shows well-defined cystic lesion in the pancreatic tail. Note the central stellate scar.

enough to compress adjacent structures [10]. The tumors may occur in any part of the pancreas but have some predilection for the head. When located in the head of the pancreas, these tumors can compress the common bile duct and result in jaundice. Gastrointestinal hemorrhage and pancreatic duct obstruction have been reported [21]. Rare cases co-exist with other pancreatic neoplasms, such as pancreatic ductal adenocarcinoma and islet cell tumor [22–25]. Ultrasonography (US) and computed tomography (CT) show characteristic multiple small loculations. A central scar with 'sunburst' calcification is evident in some patients (fig. 1). Most cases are solitary, but in rare cases the tumor is multicentric [26, 27]. Compagno and Oertel report three cases which involved the entire pancreas and four cases which were found in the body and tail [2]. Association of SCT with von Hippel-Lindau (VHL) disease has been suggested [28]. VHL disease is an autosomal-dominant genetic disorder, characterized by the development of a variety of neoplasms.

Macroscopically, the diameter of the tumors is 1–25 cm (mean 6–10 cm). The tumors are spherical to ovoid, well-circumscribed masses. The distinctive feature of the tumor is its composition of many small cysts, which imparts a characteristic honeycomb-like cut surface. The cyst walls are paper-thin and translucent. The larger tumors often have a central stellate scar that is sometimes calcified and from which fine fibrous septa radiate to the periphery (fig. 2). The individual cysts are usually a few millimeters but some may be a few centimeters in size. The cysts contain clear serous fluid, with a low CEA level. No necrosis is noted.

Microscopically, the tumors are composed of multiple small cysts, which vary in size and shape (fig. 3) [10]. A micro-glandular pattern and a pattern

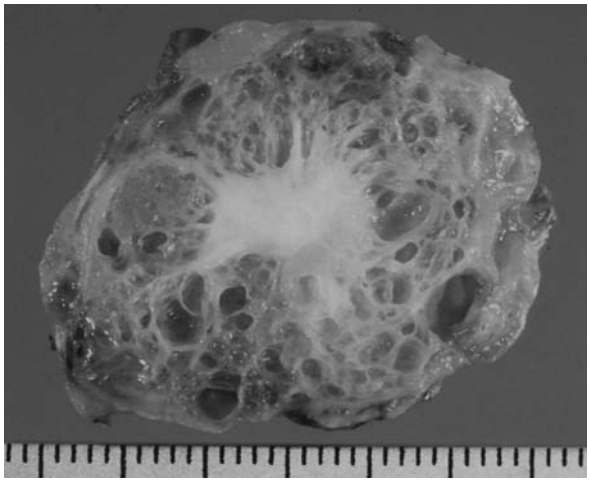

Fig. 2. Serous microcystic adenoma. The cut surface discloses a honeycomb meshwork of numerous small cysts and a central stellate scar.

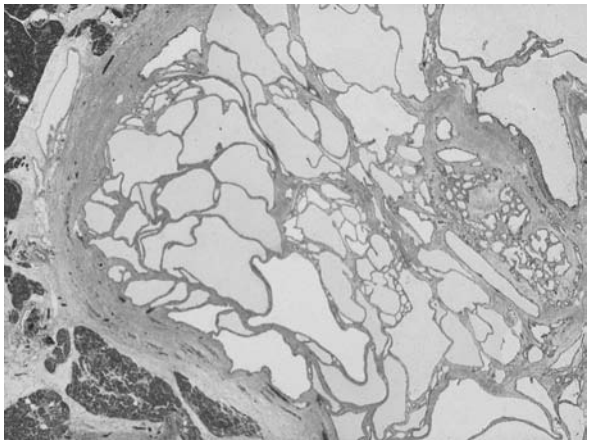

Fig. 3. Serous microcystic adenoma. Characteristic spongy appearance as a result of numerous cysts, which vary in size and shape. The tumor is well demarcated from the adjoining pancreatic tissue by a small fibrous band.

consisting of larger cysts are usually admixed. The cysts are lined by a single layer of small, flat to cuboidal cells (fig. 4). Some foci of the tumors may show low-papillary patterns, covered by cytologically bland epithelium (fig. 5) [29]. The tumor cells have clear to pale cytoplasm due to their abundant glycogen

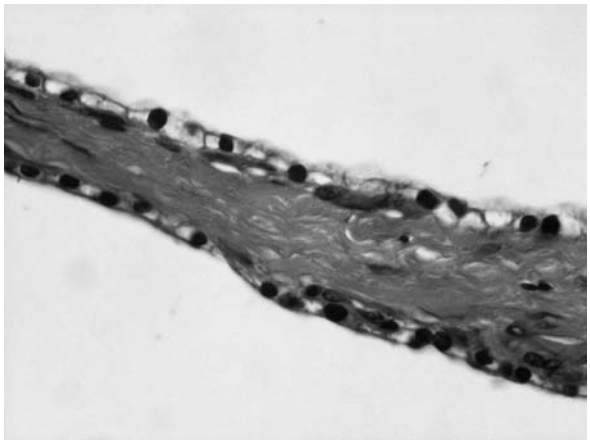

Fig. 4. Cysts are lined by a single layer of flattened epithelial cells with clear cytoplasm and well-defined cell margins. The nuclei are dark and centrally located. Note the thin underlying fibrous cyst wall.

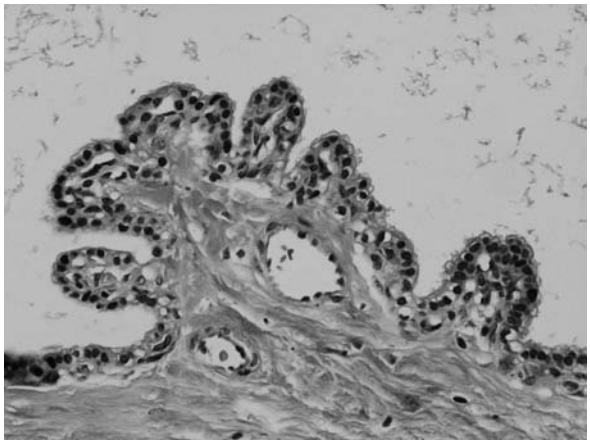

Fig. 5. Some tumor areas show a tendency to papillary tufting with a fibrovascular core.

content. Because of the presence of intracytoplasmic glycogen, Periodic acid-Schiff (PAS) staining is positive, and staining for PAS after diastase treatment is negative (fig. 6). In rare cases, the cytoplasm is eosinophilic and granular. They have only an insignificant amount of mucin. The nuclei are small, round-to-ovoid, somewhat hyperchromatic. Mitotic activity, cytological atypia and nuclear pleomorphism are absent. The cysts are separated by thin fibrous tissue,

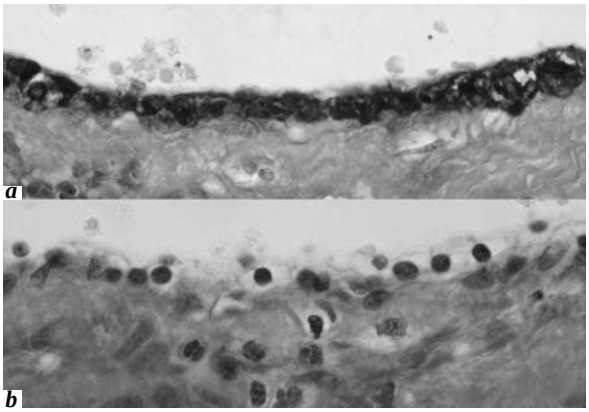

Fig. 6. PAS stain demonstrating large amounts of cytoplasmic glycogen (***a***), which is removed by diastase pretreatment (***b***).

which has numerous blood vessels. The prominent vascularization of these tumors can be appreciated by angiography. The trabeculae between the cysts may also show calcification, which appears as a radiating pattern radiographically. The trabeculae may contain entrapped Langerhans islets at the periphery of the tumor. An incomplete fibrous pseudocapsule separates the tumors from the adjacent pancreas. The characteristic cytology of SCT is that of cellular sheets of low cuboidal cells, clear cytoplasm, and intranuclear cytoplasmic inclusions [1].

Serous Oligocystic Adenoma

Although SCTs have been classified as microcystic adenomas, recent advances in imaging techniques have revealed more cases of serous oligocystic adenoma [25]. Serous oligocystic adenoma is the second common subtype of SCT, accounting for 7–30% of all SCTs [14, 19, 20, 27, 30, 31]. Synonyms include macrocystic serous cystadenoma, and serous oligocystic and ill-demarcated adenoma [1, 8, 30–33].

Serous oligocystic adenoma occurs equally in both sexes. Tumors usually affect individuals with a mean age in the fifth decade (26–73 years old), younger than patients with serous microcystic adenoma with a mean age in the seventh decade [31]. Tumors are often identified incidentally during US or CT. Some patients are symptomatic, complaining of abdominal pain or dyspepsia. Patients do not have VHL disease. Serous oligocystic adenomas can develop

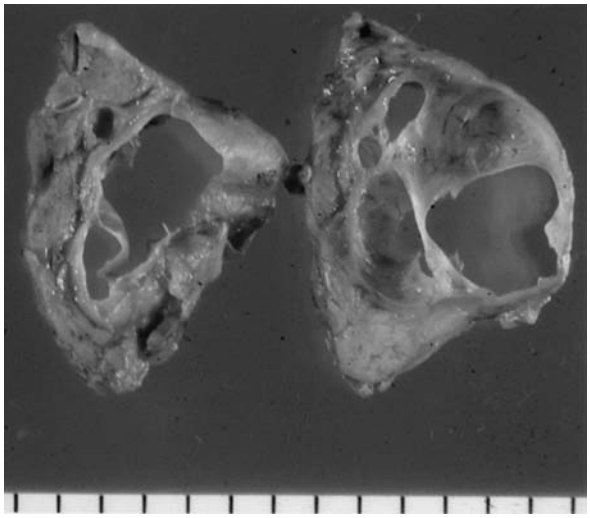

Fig. 7. Serous oligocystic adenoma. A few, relatively large cysts in the pancreatic tail.

anywhere in the pancreas, but they show a prediction for the pancreatic head [32]. Laboratory examination including tumor markers is within the normal range in all patients. Fine-needle aspiration is acellular and thus not informative for the cytological analysis [7]. Pre-operative diagnosis of serous oligocystic adenoma is difficult. Serous oligocystic adenoma exhibits radiologic and gross pathologic features distinct from those described for serous microcystic adenoma. It may be misdiagnosed as MCT that require resection, as a pseudocyst, or as a solitary true cyst. Recognition of an oligocystic variant of SCT is therefore important for physicians and pathologists alike [33]. At present, the diagnosis is still based on pathologic examination after surgical removal of the tumor [31]. These tumors show no malignant transformation [11].

The gross appearance of serous oligocystic adenoma is distinctly different from that of serous microscopic adenoma. The sizes of the tumors range from 1.5 to 15.0 cm. They are characterized by macrocystic ($>$2.0 cm in diameter) or oligocystic patterns (fig. 7) [30]. These are composed of fewer but larger cysts. Some tumors show a predominantly or exclusive unilocular pattern [33]. Minute microcysts are found surrounding the main cavity; some of these are not apparent on gross examination. Endoscopic US may be useful in detecting peripherally located millimeter-sized cysts in unilocular lesions [31]. These microscopic cysts support the idea that this tumor is a particular form of serous microscopic adenoma in which some cysts develop at the expense of the others

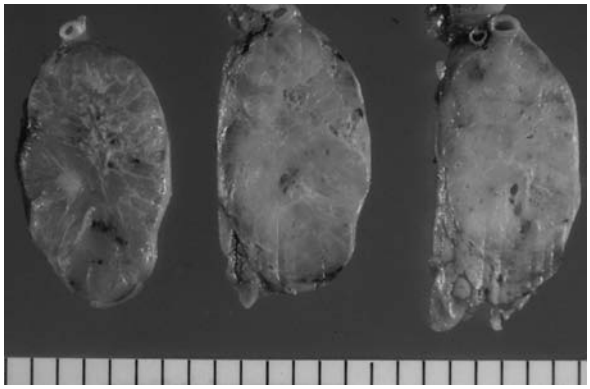

Fig. 8. Solid serous adenoma. Well-demarcated solid mass in the pancreatic body.

[30, 31]. Thick-walled cysts have a glistening inner surface and clear fluid content. Some cysts are filled with dark-brown fluid derived from old blood [33]. The cyst walls are smooth. No mural nodules, papillary projections, or calcifications are present [34]. A central stellate scar or network of fibrous septa is absent [30, 35]. These features are similar to those seen in mucinous cystic tumors. Serous oligocystic adenomas are often well-circumscribed from the surrounding pancreatic tissue, although some are poorly demarcated [32].

Microscopically, the serous oligocystic adenoma is easily distinguishable from mucinous cystic neoplasm, as the latter is lined by tall columnar mucin-rich cells with an ovarian-like stroma [36]. The histological features of serous oligocystic adenoma are quite similar to those of the serous microcystic adenoma. The cysts are lined with a single layer of cuboidal, PAS-positive epithelium. The lining epithelium shows neither mitoses nor cytological atypia. SCTs lack ovarian-like stroma. There is no communication between the cysts and the excretory duct system [30]. The histopathologic diagnosis is difficult when the epithelial lining is extensively denuded.

Solid Serous Adenoma

In 1996, Perez-Ordonez et al. described a case of solid neoplasm that appears to represent another morphologic variant of SCT [12]. Solid serous adenoma of the pancreas is a non-cystic variant of SCT [12, 37]. The tumor has a firm consistency and well-defined margins. Grossly, this tumor shows no recognizable cysts (fig. 8) [9]. Histologically, it has a solid or microglandular

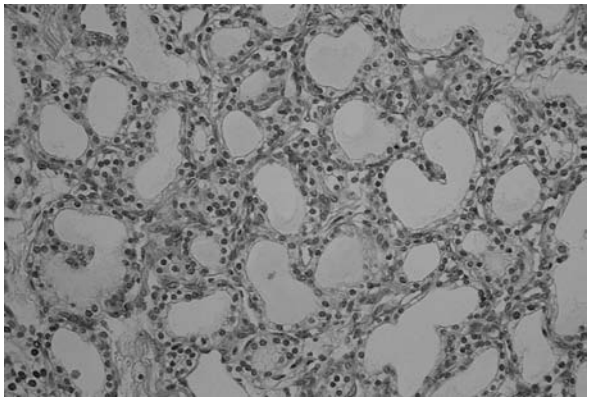

Fig. 9. Solid serous adenoma. Microglandular architecture with small cell nests.

architecture (fig. 9). The lesion is formed by clear cells. Otherwise the cytologic, histochemical, and immunohistochemical features of solid serous adenoma are indistinguishable from those of otherwise conventional SCTs. Recognition of this lesion is important because the vast majority of solid tumors in the pancreas are malignant [12]. The differential diagnosis of this tumor includes clear cell carcinoma and metastatic renal cell carcinoma.

Serous Cystadenocarcinoma

In contrast to mucinous cystic tumors of the pancreas, which are known to have considerable malignant potential, the serous variant is not considered potentially malignant. Operative resection for SCT has been suggested in patients with complications, but its progress can be followed carefully without surgery [2, 13]. However, there have been several reports of malignancy in serous cystic tumors of the pancreas [15–18, 22, 38]. These tumors have metastasized or have been associated with aggressive local growth. A malignant counterpart of the serous cystic tumor has been described as serous cystadenocarcinoma or microcystic adenocarcinoma.

Serous cystadenocarcinoma is essentially indistinguishable from serous cystadenoma, but shows signs of malignancy. Kamei et al. reported a case of multifocal SCT with atypical cells [22]. One of the seven multiple tumors described showed an increase in the nuclear/cytoplasmic ratio, irregular nuclear margins, and perineural invasion. Abe et al. described a case of SCT with invasive growth to the lymph node and adipose tissue [38]. Invasion of the surrounding tissue can result in a distant metastasis [15]. In one case, the

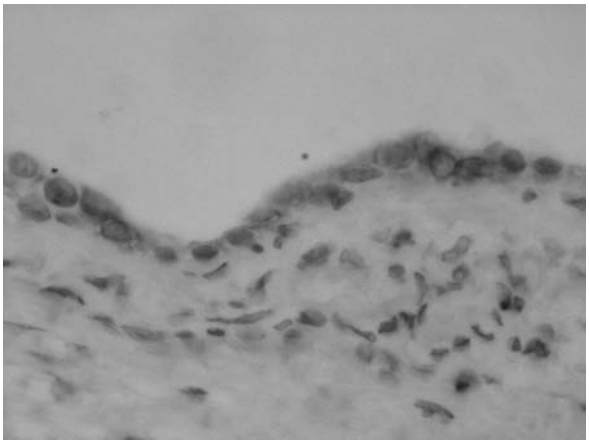

Fig. 10. Tumors cells showing cytoplasmic immunoreactivity with cytokeratin 7.

microscopic appearance was similar to that of microcystic adenoma, but metastases were present in stomach and liver [38]. The controversy on whether SCT has potential for malignancy provokes debate on whether surgical excision is necessary [38].

Immunohistochemical and Ultrastructural Findings

SCTs are positive for cytokeratins, detected by AE1/AE3 or CAM5.2, epithelial membrane antigen, CA19–9, neuron-specific enolase and α-inhibin [1, 9]. SCTs express cytokeratins 7, 8, 18, 19, MUC1, and MUC6, which characterize pancreatic duct cells including the centroacinar cells (fig. 10) [9, 33]. Cytokeratin 20, CEA, estrogen and progesterone receptors, and endocrine markers are unexpressed.

Ultrastructural studies have shown prominent apical microvilli, well-formed desmosomes, and contractile filaments [6, 8, 10]. Glycogen is abundant within cyst-lining cells. Tumor cells are relatively organelle-poor. These features are comparable to those of normal centroacinar cells [10].

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Intraductal Adenoma and Epithelial Hyperplasia of the Pancreatic Ducts

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Abstract

Background/Aims: It is still controversial that hyperplastic epithelial lesions of the pancreatic duct are always pre-neoplastic conditions. This is an attempt to reclassify 'hyperplastic lesions' apart from low-grade neoplastic lesions, such as intraductal papillary-mucinous adenoma or low-grade intraepithelial neoplasia. **Methods:** To compare the features of mucin phenotype, genetic alteration and proliferative activity between 'hyperplastic lesions' and low-grade neoplastic lesions by reviewing previous studies by ourselves and others. **Results:** The MUC1 and MUC2 double-negative phenotype should be considered as the characteristic expression pattern of 'hyperplastic lesions' in terms of equality to that of the normal pancreatic duct. K-ras mutation was not always observed in neoplastic lesions. Proliferative activity, represented by Ki-67 labeling index, of 'hyperplastic lesions' was significantly different from low-grade neoplasia. **Conclusions:** 'Hyperplastic lesion' should be treated as a pure term representing morphological features, separated from the connotation of low-grade neoplasia.

Hyperplastic and dysplastic epithelial lesions of the pancreatic ducts were first suggested to be histological precursory lesions of invasive ductal adenocarcinoma in 1954 [1]. Since then, these lesions have been reported using up to 70 different names, such as 'lesion', 'metaplasia', 'hyperplasia', 'dysplasia' and 'neoplasia'. Their biological natures have also been variously estimated, from secondary phenomena due to obstruction caused by the tumor itself to real pre-neoplastic conditions [2]. It might be natural that they were considered to be precancerous lesions, because they are frequently found in the pancreas with carcinoma [3, 4]. However, lesions showing the same morphological features were also frequently observed at autopsy in patients free from pancreatic diseases [5, 6].

Nowadays, proliferative epithelial lesions of the pancreatic duct with slight or no dysplasia are treated as one of the following categories: low-grade intraepithelial neoplasia, termed 'Pancreatic intraepithelial neoplasia (PanIN)-1A/B';

low-grade intraductal papillary mucinous neoplasm, known as 'intraductal papillary-mucinous neoplasm (IPMN) adenoma'; or just 'hyperplasia' in the strict sense. Considering that both PanIN and IPMN represent neoplastic lesions, the term 'hyperplasia' should be applied to lesions that do not progress into carcinoma. However, there is some doubt as to whether morphologically similar lesions should be divided into different concept of pathology. The following is a review and discussion of the studies of the above-mentioned 'hyperplastic lesions', namely low-grade PanIN and IPMN.

'Lesions Like Hyperplasia' Categorized as Neoplasia

PanIN and IPMN are intraepithelial neoplasias of the pancreatic duct. PanIN was proposed in 1994 as a precursor lesion of invasive ductal adenocarcinoma of the pancreas [7], while IPMN has been recognized since 1982 [8] as a distinct group of lesions with an indolent prognosis. So far, these two categories of disease have tended to be morphologically confused as intraepithelial lesions. In 2003, a meeting of international experts on the precursor lesions of pancreatic cancer was held, and the basic definitions of both IPMN and PanIN were again confirmed. The differential diagnosis between IPMN and PanIN was discussed as follows: IPMN arises in the main pancreatic duct or its major branches and usually produces lesions greater than 1 cm in diameter, while PanIN is involved in smaller pancreatic ducts less than 5 mm in diameter [9]. Both were divided into three histological grades, based on their nuclear and architectural atypia namely, from PanIN-1A/B, PanIN-2 to PanIN-3, and from adenoma borderline to carcinoma of IPMN, respectively. Among them, IPMN adenoma and PanIN-1A are the lowest grades of these entities. Their exact definitions are described as follows:

PanIN-1A: These are flat epithelial lesions composed of tall columnar cells with basally located nuclei and abundant supranuclear mucin. The nuclei are small and round to oval in shape. When oval, the nuclei are oriented perpendicular to the basement membrane. It is recognized that there may be considerable histological overlap between non-neoplastic, flat, hyperplastic lesions and flat, neoplastic lesions without atypia. Therefore, some may choose to designate three entities with the modifier term 'lesion' ('PanIN/L-1A') to acknowledge that the neoplastic nature of many cases of PanIN-1A has not been unambiguously established [10].

IPMN adenoma: The epithelium is comprised of tall columnar mucin-containing cells that show slight or no dysplasia; i.e. the epithelium maintains a high degree of differentiation of adenomas [11].

As the above shows, it was overlooked, even in the context of the definition of each disease, that these low-grade neoplasias might be confused with non-neopalastic hyperplastic lesions.

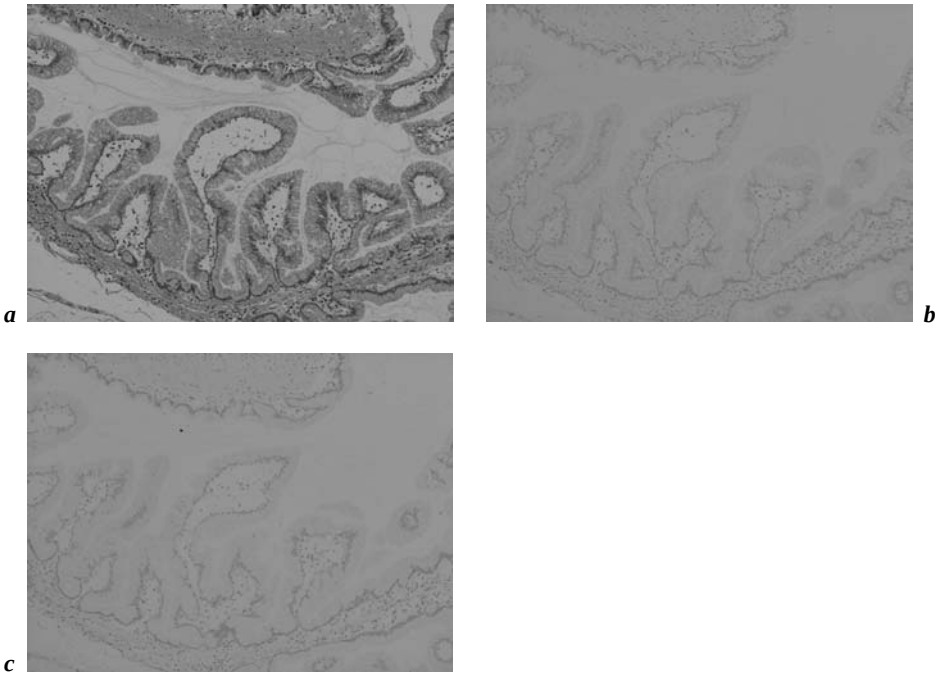

Fig. 1. Hyperplastic lesion in IPMN shows either the papillary part or the flat part by HE (***a***). Both parts reveal negative staining for MUC1 (***b***) and MUC2 (***c***).

Mucin Phenotype of 'Hyperplasia-Like Lesions'

Mucins are high-molecular-weight glycoproteins, with oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by O-glycosidic linkages [12–14]. During the past several years, core proteins for several human mucins have been identified [12, 13, 15–18]. Among them, MUC1 and MUC2 have been examined most frequently for the studies of pancreatic intraepithelial neoplasm. Some studies showed that intraepithelial neoplasias with MUC1+/MUC2− show an aggressive nature, while those with MUC1−/MUC2+ have an indolent nature [19, 20].

In the normal pancreas, MUC1 is expressed in the luminal surfaces of the ducts, with no intracytoplasmic or base basolateral membranous staining in the intralobular small ductules that are lined by small, cuboidal cells with minimal cytoplasm [21]. Meanwhile, no MUC2 labeling is noted in the normal pancreas [21].

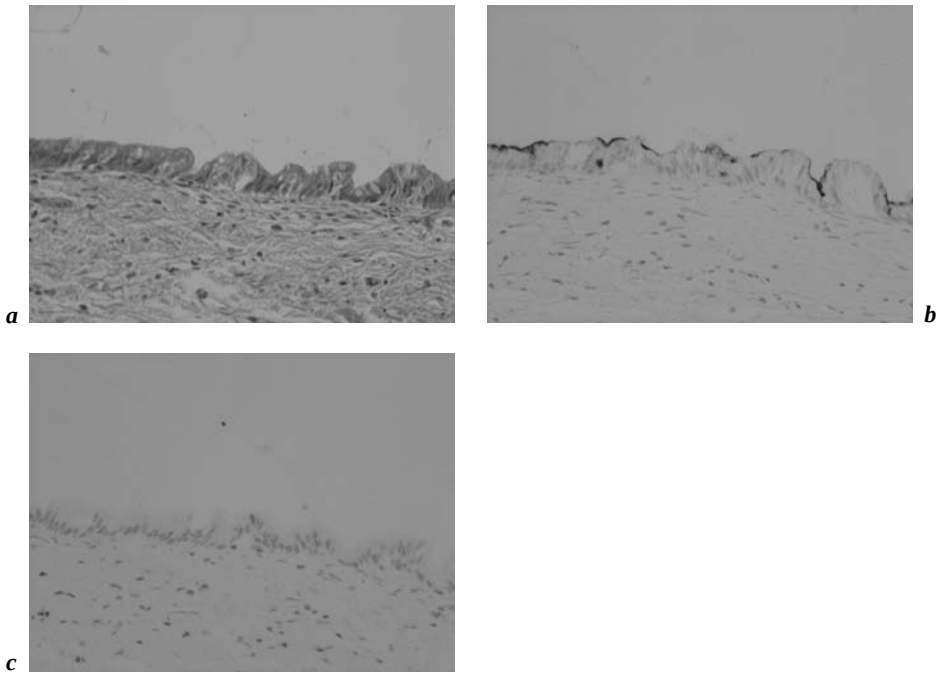

Fig. 2. Two cases with hyperplastic lesion in IPMN show positive staining for MUC1 (***b***) and negative stain for MUC2 (***c***). These lesions are of flat or serrated appearance (***a***).

According to our data, among 26 'hyperplasia-like lesions' from patients with IPMN, which is to be categorized as IPMN adenoma by WHO, (M:F 20:6, age 49–81, 18 branch ductile type and 8 main ductile type), MUC1−/MUC2−(fig. 1), MUC1+/MUC2− (fig. 2) and MUC1−/MUC2+ (fig. 3) were observed in 17, 2 and 7 cases, respectively. The degrees of atypia and accumulation of nucleus were the same among these lesions. MUC1, the membrane-bound type mucin detected in most epithelial tissues, was stained at the apical site of the epithelium, while MUC2, an intestinal-type mucin, was diffusely stained in the epithelium. MUC1+/ MUC2− lesions showed a serrated appearance without a papillary intestinal core, while MUC1−/MUC2+ lesions consisted of papillas with an intestinal core. The averages of their height were 0.34 ± 0.21 mm (0.1–0.8 mm), 0.14 ± 0.01 mm (0.13–0.15 mm) and 0.66 ± 0.54 mm (0.15–1.5 mm) in each lesion of MUC1−/MUC2−, MUC1+/MUC2− and MUC1−/MUC2+, respectively (Kruskal-Wallis test, $p = 0.13$). MUC1+/MUC2− lesions tend to be lower papilla, while MUC1−/MUC2+ lesions tend to be higher, albeit not statistically significant. We also examined the MUC1/MUC2 expression pattern among 12 PanIN-1 lesions from 6 patients with invasive pancreatic cancer (M:F

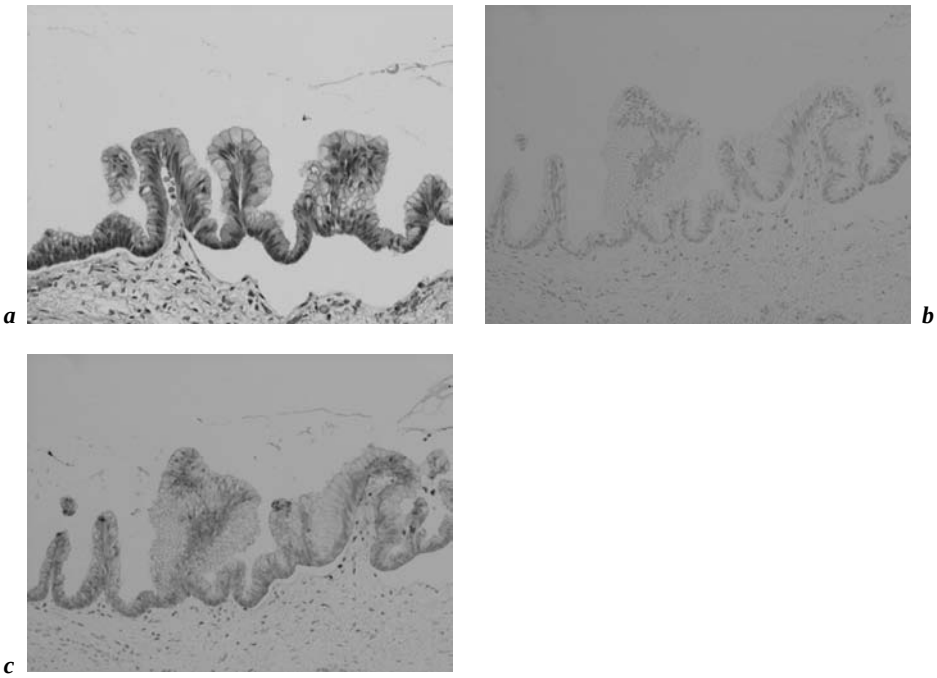

Fig. 3. Seven cases with hyperplastic lesion in IPMN show negative staining for MUC1 (***b***) and positive stain for MUC2 (***c***). These lesions show papilla with intestinal core (***a***).

2:4, age 56–68). Seven of them showed MUC1−/MUC2−, and 5 lesions showed MUC1+/MUD2− (fig. 4). None of them showed MUC1−/MUC2+.

Our data suggested the possible overlap of lesions, both between low-grade neoplasia and hyperplasia, and between IPMN and PanIN, as follows:

MUC1−/MUC2− intraepithelial lesions, showing the same pattern observed among normal pancreatic ducts, might fall into the category of non-neoplastic lesions, in spite of their indistinguishable morphological features.

IPMN adenoma with MUC1+/MUC2− might be close to PanIN-1 with MUC1+/MUC2−, having both the same MUC1/MUC2 pattern and low-height morphology.

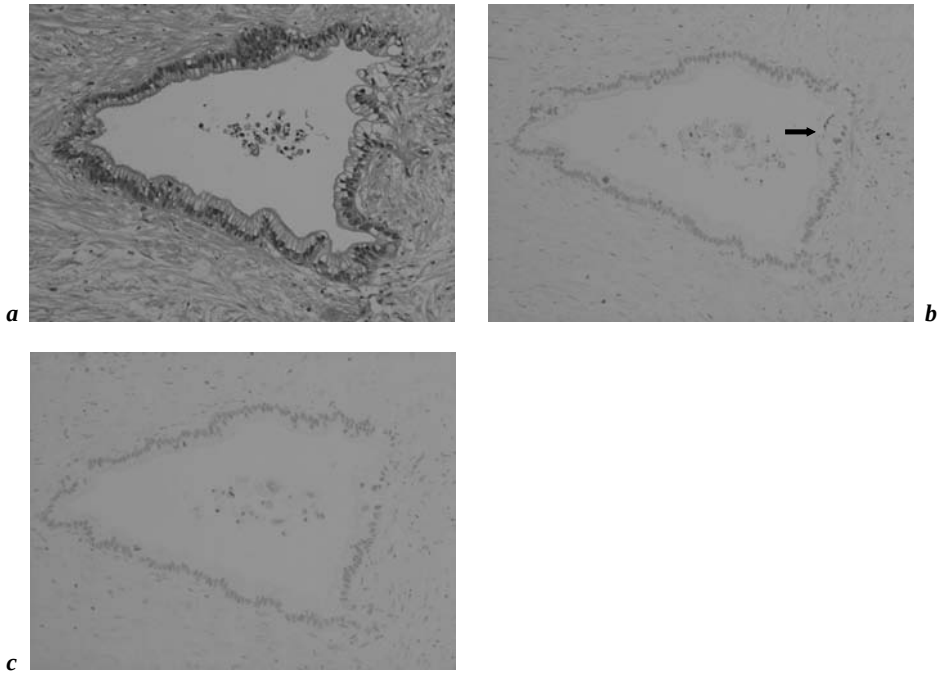

Fig. 4. Some PanIN-1 lesions (***a***) show partly positive staining for MUC1 (***b***, arrow), and totally negative stain for MUC2 (***c***).

Genetic Alteration in 'Hyperplastic Lesions'

The mutations in K-ras codon12 were thought to be most frequent and possibly the earliest mutation among the genetic alteration sequences of both ductal pancreatic adenocarcinoma and IPMN. 'IPMN adenoma' with slight or mild atypia may only have the K-ras gene aberration. However, it is still controversial whether K-ras mutation supports these neoplastic features. Some have said that it was observed in up to 100% of pancreatic adenoarcinoma, but was not detected in the non-neoplastic tissue of the pancreas [3, 22–24]. Others have said that it was also suggested that K-ras gene mutation occurs frequently in the multifocal hyperplastic foci of the pancreatic duct, and that the mutation may not have direct relevance to the carcinogenesis of pancreatic cancer [4].

When K-ras analysis was performed at the cellular level using sophisticated microdissection and molecular methods, K-ras mutations were detected in hyperplastic ductal changes in the pancreas with chronic pancreatitis [7], in disease free pancreas [4] and also in the normal epithelium of the pancreatic

specimens containing carcinomas [25]. In addition to this, ductal lesions in patients with chronic pancreatitis exhibit K-ras mutations without the additional indications of neoplastic transformation, such as severe dysplasia or mutated p53 protein. Therefore, for diagnostic and therapeutic purposes, apart from being namable as neoplasia, the detection of K-ras mutations should be supplemented by the demonstration of additional genetic alterations or clinical signs of malignancy [26].

Among the most commonly observed types of hyperplasia, such as mucinous cell hyperplasia, ductal papillary hyperplasia and adenomatoid hyperplasia, the incidences of K-ras codon12 mutations were reported to be 55%, 61% and 48%, respectively [25, 27]. These differently defined hyperplastic lesions should be placed into the same category, with regard to the genetic alteration. In addition to this, these studies also suggest that the K-ras mutations observed among these lesions are not sufficiently supportive to confirm their neoplastic nature.

Proliferative Activity of 'Hyperplastic Lesions'

The proliferation rate can be determined by the labeling index (LI) of antibody against Ki-67, which is a nuclear protein that correlates with cellular proliferation. Many studies have investigated the relationship of Ki-67 labeling with prognosis in a variety of different types of neoplasia.

The overall Ki-67 LI was said to be 0 in normal pancreatic ducts in the normal pancreas [28]. We examined Ki-67 LI among 22 lesions of low-graded IPMN from 14 patients (M:F 13:1, age 35–81) and 12 lesions of PanIN-1 from 6 patients with pancreatic cancer (M:F 2:4, age 56–68) [29]. They were $3.43 \pm 2.93\%$ and $2.80 \pm 2.36\%$, respectively. A t test comparing the Ki-67 LI between them showed no significance ($p = 0.5732$), i.e. both low-grade lesions of PanIN and IPMN showed less proliferation activity. Though they show similar proliferative activities at this grade of atypia, a different prognosis was proposed for each category of lesion. It is still questionable whether they should be differently categorized, in regard to the proliferative activity. Is it possible for them to be unified as just 'hyperplastic lesions' around the tumor?

Concluding Remarks

The concept of 'hyperplasia' should be left as a pure term representing morphological features. Repeated consensus meetings have certainly contributed to the standardization of pathological diagnosis. However, it is still open to question as to how to differentiate 'hyperplastic lesions' from low-grade intraepithe-

lial neoplasia, not only because of their morphological similarity, but also because of the continuous discussion of their neoplastic nature. Further investigations, adding to the studies above, will ultimately lead to some conclusion as to the true nature of 'hyperplasia-like lesions'.

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Carcinoma in situ, Invasive Ductal Carcinoma of the Pancreas, and Intraductal Papillary-Mucinous Neoplasm

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Abstract

Pancreatic intraepithelial neoplasia (PanIN) is a progression model of invasive ductal carcinoma (IDC) of the pancreas concerning genetic alterations, and its adoption requires conditions of invisible ductal lesions without obvious duct dilation. Intraductal components (ICs) of conventional pancreatic cancer (PC) are thought to include intraductal spread of carcinoma in situ (CIS) and intraductal extension of IDC (cancerization of ducts: ductal invasion and colonization). CIS is usually low papillary or flat, and its histology changes to tubular adenocarcinoma with desmoplasia in the case of invasion. The invasive component shows more enlarged individual tumor cells and more atypia than IC. Most conventional PCs are found with various diagnostic imaging techniques by direct or indirect findings of the main pancreatic duct such as stenosis, interruption, or secondary dilation of the main pancreatic duct. PC is a mostly fatal disease that requires new treatment. In order to improve the poor prognosis associated with this disease, we must detect small PCs at a curable stage and recognize the precursors of PC.

The change from a normal to a cancer cell is caused by accumulation of genetic alterations. Specific genetic alterations are thought to be associated with each progression step. Since tumors associated with oncogenes and tumor suppressor genes were discovered, cancer has also been thought of a genetic disease. Lesions with the ability to progress to cancer by accumulating genetic alterations can be called precursors of cancer.

Most pancreatic cancers (PCs) that are diagnosed and treated are conventional PCs with poor prognosis. To improve this very poor prognosis, we must clarify the risk factors of PC. It is especially important to know what kinds of lesion progress to PC, i.e. to clarify the characteristics of PC precursors.

In this section, intraductal lesions that were thought to be precursors of conventional PC and related molecular findings are discussed.

Importance of Early Detection of Conventional PC

Conventional PCs are invasive ductal carcinomas (IDCs) with poor prognosis that account for more than 80% of pancreatic tumors. Early detection of small T1 tumors of the pancreas has become possible due to recent advances in diagnostic imaging. However, most T1 pancreatic tumors are already advanced tumors and their treatment results are not favorable compared with other digestive T1 cancers [1]. Although discovery rates are improving, resection rates are not. Many PCs are not inoperable when found, while the five-year survival rate for carcinoma in situ (CIS) is almost 100%. Therefore, it is very important to diagnose and resect pancreatic tumors at the curable in situ stage (Stage I) [2–4].

Therefore, screening examinations for early detection, identifying high risk groups, and identification of PC precursors are important areas to be addressed immediately.

Precursors of Conventional PC and PanIN Classification

In Japan, historically, atypical intraductal lesions have been studied in detail using resected or autopsied pancreata. Kuzuka and colleagues documented in their 1,174 autopsied pancreata study that atypical ductal lesions were found in 0.7 and 29.2% of pancreata without and with PC, respectively [5]. An atypical epithelium was often observed in pancreatic ducts and ductules away from the tumor in resected pancreata with PC. However, an atypical epithelium is rarely found in pancreata without PC. Therefore, it has been assumed that PC occurs from an atypical epithelium. Moreover, the reason why tiny PCs were rarely found is that an atypical epithelium occurring from peripheral ducts or ductules immediately progresses to an invasive carcinoma, i.e. conventional PC is thought to occur de novo and immediately invade the pancreatic parenchyma.

Recently, the pancreatic intraepithelial neoplasia (PanIN) classification proposed by Klimstra DS and Longnecker DS [6] was examined as a progression model of conventional PC concerning both histological changes and genetic alterations in caliber pancreatic ducts [7, 8]. Histological diagnoses used in Japan such as mucinous metaplasia/hyperplasia, papillary hyperplasia, atypical hyperplasia, and CIS are almost the same as those of the PanIN classifications: PanIN-1A, PanIN-1B, PanIN-2, and PanIN-3. In terms of molecular

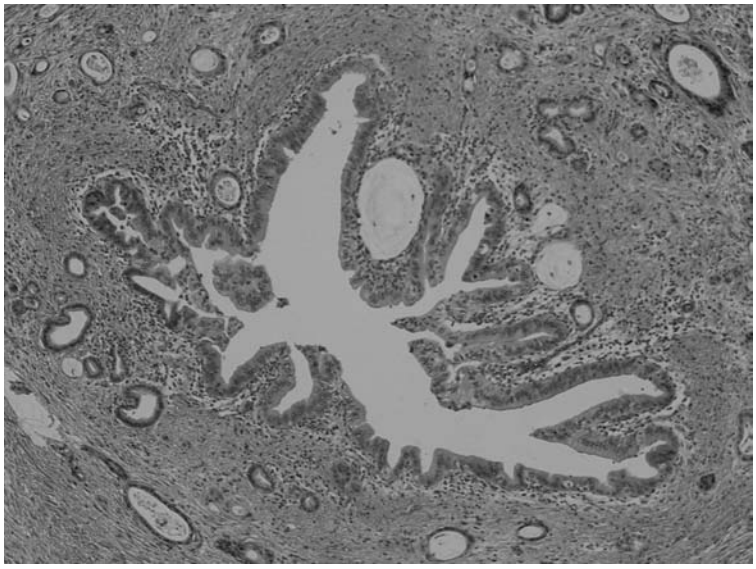

Fig. 1. CIS morphologically shifting to invasive carcinoma.

biology, a point mutation of K-ras gene is found in PanIN-1A, genetic alterations of Her2/neu (c-erbB-2) and p16 occur in PanIN-2, and genetic alterations in p53 and DPC4/smad4 occur in PanIN-3. Moreover, there are many CISs (corresponding to PanIN-3) adjacent to the invasive tumor. It has been proved that there are no differences in genetic alterations between some CIS components and invasive carcinoma components [9]. Therefore, CIS is recognized as a precursor of IDC of the pancreas.

ICs of Conventional PC

We often find intraductal components (ICs) inside PC, observing mural elastic fibers of the pancreatic ducts with elastica van Gieson (EVG) staining. ICs of conventional PC are thought to include both intraductal spread of CIS and intraductal extension of PC (cancerization of ducts: ductal invasion and colonization). CIS is usually low papillary or flat, and its histology changes, for example to tubular adenocarcinoma with desmoplasia in the case of invasion. Individual tumor cells of the invasive component are more enlarged and are more atypical than that of IC (fig. 1). In contrast, cancerization of ducts, especially intraductal extension of PC (ductal colonization), is similar to the invasive component in terms of histology. There were no differences between

cancerization of the ducts and the invasive component in either size or atypia of tumor cells [10–12].

Objective Lesions of the Pancreatic Duct for the PanIN Classification

As objective lesions of the pancreatic duct for the PanIN classification is a caliber pancreatic duct which is undetectable radiographically, it is basically impossible to find PanIN lesions preoperatively. The PanIN classification cannot be used for radiographically detectable ductal lesions which are routinely diagnosed and treated because PanIN lesions are only incidentally found away from the tumor of resected pancreata with PC. A classification system for PanIN developed in 1999 was recently revised at a meeting of international experts on PC precursors [7, 8]. In the new PanIN classification, PanIN lesions are diagnosed not by location of ductal lesions (whether peripheral or central ducts) but by the size of the duct, i.e. ductile conditions for PanIN lesions require that lesions are usually invisible lesions without obvious changes such as radiographical duct stenosis, interruption, or secondary dilation of the duct [8].

Conventional PC and Abnormalities of the Main Pancreatic Duct

In daily screening, conventional PC is found mainly by direct and/or indirect findings on the main pancreatic duct such as stenosis, interruption, and secondary dilation. Matsukuma and colleagues documented that T1 tumors were found in 10 (7.1%) of 141 IDC cases, and most patients with T1 tumors had some kind of clinical symptoms, as well as poor prognoses [1]. Abnormal findings of the main pancreatic duct were seen in 27 cases (93.1%) of 29 T1 tumors in the study by Furukawa et al.[13] and in 22 cases (95.6%) of 23 T1 tumors on ERCP (endoscopic retrograde cholangiopancreatography) in that by Ohhashi et al. [14]. In the study by Yamasaki et al., ICs preserving mural elastic fibers of the pancreatic duct circumferentially were found in 37 cases (69%) of 54 PCs, and 9.0% of these ICs were in the main pancreatic duct, 64.0% in the large pancreatic duct, and 27.0% in the small pancreatic duct [10]. Therefore, it is important not to miss abnormalities of the main pancreatic duct on pancreatography.

IPMN and Conventional PC

Both intraductal papillary-mucinous neoplasm (IPMN) and conventional PC occur from the pancreatic ductal epithelium, but both tumors are thought to

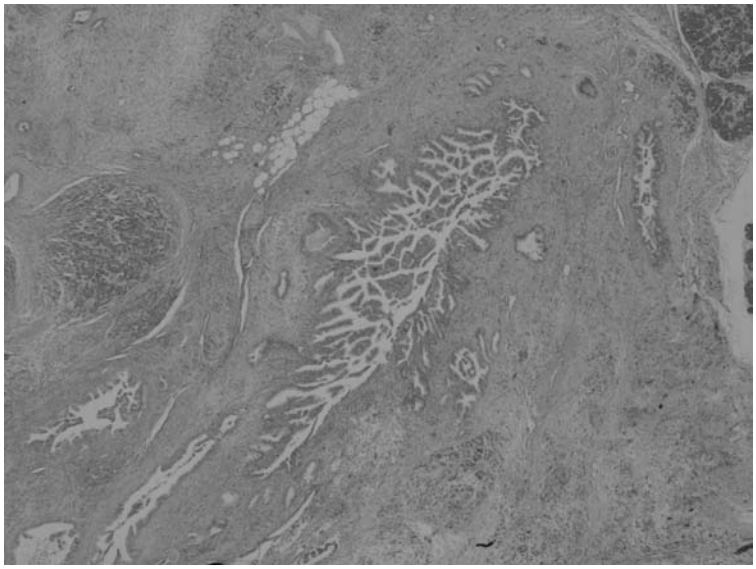

Fig. 2. PC with papillary ICs in a dilated main pancreatic duct. IC without copious mucin production are (low) papillary.

be completely different in terms of size of the originating duct, style of progression, genetic alterations, and prognosis. However, some IPMNs gradually and/or immediately progress to invasive carcinoma with malignant transformation through accumulation of genetic alterations, i.e. some conventional PCs occur via IPMN (fig. 2) [15]. Furthermore, there are some PCs with papillary ICs in the main pancreatic duct and large-branch pancreatic ducts. These ICs without copious mucin production are papillary or low papillary (fig. 2). It is sometimes problematic to diagnose whether conventional PC or an invasive carcinoma derived from IPMN; these PCs can be thought of as intermediary carcinomas between conventional PC and IPMN.

ICs to be Differentiated from Precursors of Conventional PC

Non-Aggressive CIS

The five-year survival rate of CIS is almost 100%. However, it is not clear how many cases of CIS progress to invasive carcinoma. Actually, some CISs immediately progress to invasive carcinoma. In contrast, some CISs stay in the pancreatic duct for a long period and do not invade the pancreatic parenchyma.

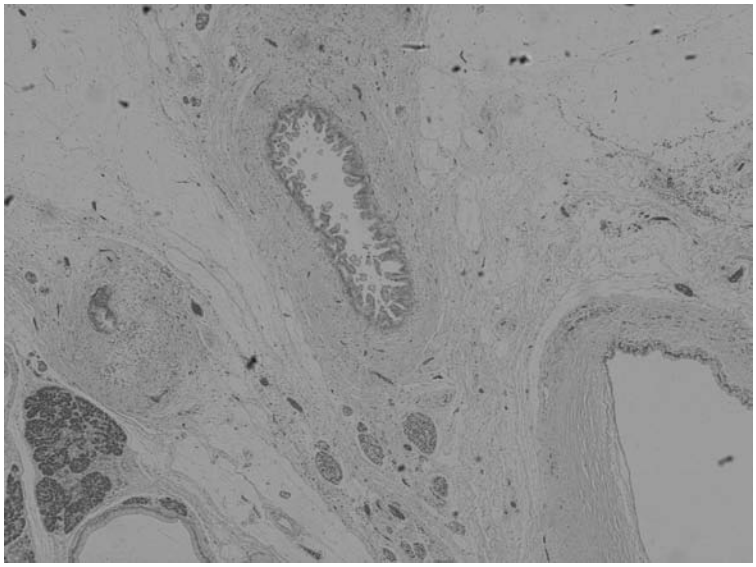

Fig. 3. Non-aggressive CIS. CIS staying in the pancreatic ducts for long periods will not necessarily lead to conventional PC acquiring high malignant potentiality.

CIS staying a long time in the pancreatic ducts will not necessarily lead to conventional PC acquiring a high malignant potentiality (fig. 3). Therefore, for an analysis of early features of conventional PC, small PCs with ICs are more suitable than CIS without invasion.

CIS-Like IPMT

Whereas the PanIN classification excludes IPMN, the two tumor types are sometimes difficult to distinguish from each other [16]. CIS-like IPMN is histologically similar to CIS, but its development is like that of IPMN; it spreads intraductally and hardly invades the pancreatic paremchyma (fig. 4). CIS-like IPMN should be also distinguished from precursors of conventional PC.

Cancerization of Ducts

Both secondary ductal invasion and colonization of invasive carcinoma (cancerization of ducts) are treated as mimickers of PanIN-3 and are excluded for objective pancreatic lesions for PanIN [8]. An infiltrating carcinoma in close proximity to a duct lesion and an abrupt transition from a highly atypical lesion to the normal duct epithelium both suggest the possibility of cancerization of the duct (fig. 5). In these cases, serial (step) sections may be helpful to

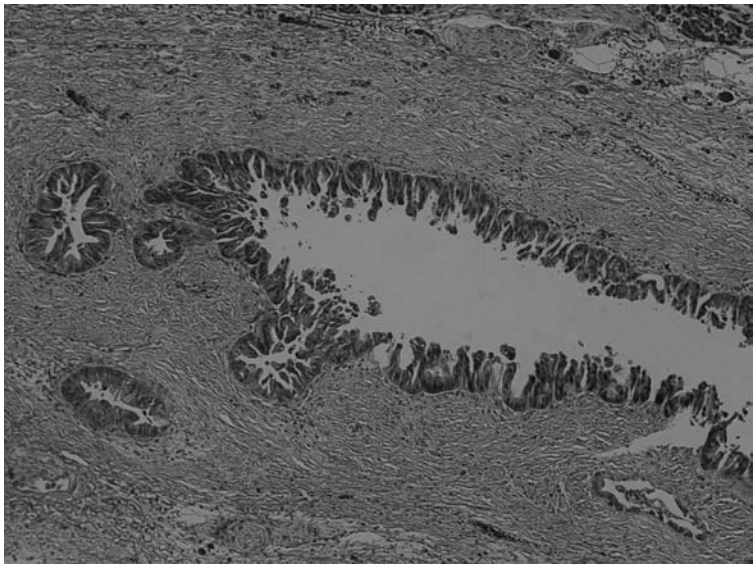

Fig. 4. CIS-like IPMN. CIS-like IPMN is histologically similar to CIS but its development is like that of IPMN; it spreads intraductally and hardly invades the pancreatic paremchyma.

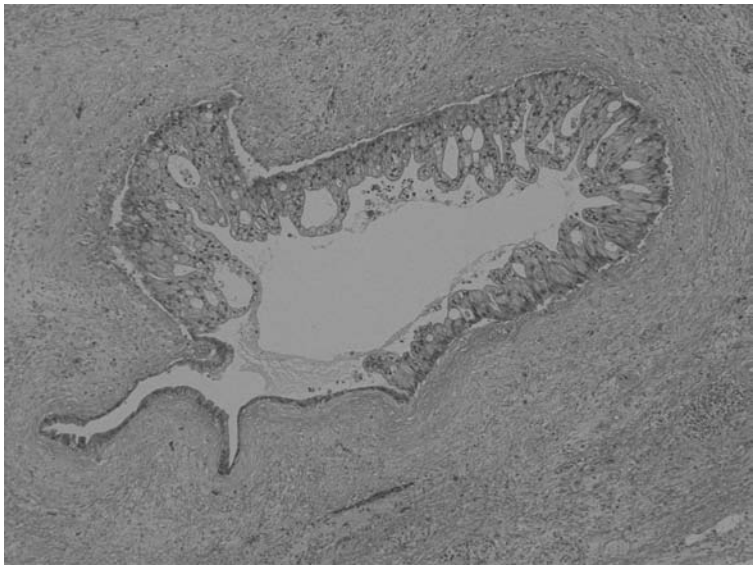

Fig. 5. Cancerization of ducts. An infiltrating carcinoma with an abrupt transition from a highly atypical lesion to the normal duct epithelium.

define the relationship between the duct lesion and the infiltrating carcinoma. However, it is often difficult to determine whether carcinoma initially spreads intraductally (CIS), or whether an infiltrating carcinoma secondarily involves the ducts (cancerization of ducts).

Summary

Possible precursors of conventional PCs were discussed. To improve overall survival rates of whole pancreatic tumors, it is important to diagnose conventional PCs with poor prognosis at the early curable stage. Identification and clarification of precursors of conventional PCs will facilitate diagnosis and therapy for conventional PCs in terms of carcinogenesis, establishment of molecular markers for early detection, and discovery of molecular targets for conventional PCs.

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Intraductal Components of Invasive Ductal Carcinoma of the Pancreas

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Abstract

Invasive ductal carcinoma (IDC) of the pancreas arises in the ductal epithelium and extends into the pancreatic tissue via stromal invasion. Carcinoma in situ (CIS) is believed to be a precursor lesion to invasive carcinoma. IDC is composed of intraductal components and infiltrating components. The intraductal components of IDC are considered to represent both CIS/colonization of ducts and the intraductal invasion/cancerization of ducts. Colonization of ducts is defined as intraductal carcinoma in another location to which CIS has spread. A tubular pattern of intraductal components indicates intraductal invasion, while a low papillary pattern indicates CIS/colonization of ducts. Intraductal components of IDC can be identified by visualizing mural ductal system elastic fibers on elastica van Gieson (EVG)-stained sections. Each carcinoma component shows different biological behavior. IDC is a highly invasive neoplasm that also spreads noninvasively through the pancreatic ductal tree. Intraductal spread is a characteristic feature of well-differentiated IDC and the number of intraductal carcinoma foci is correlated with the grade of tumor differentiation. The large branch ducts are the main routes of intraductal spread, which suggests IDC might be derived from the epithelium of large branch ducts. IDC can also spread through the ducts beyond the tumor mass. Intraductal spread is confined to the vicinity of the tumor mass (within 2.0 cm), and it can involve the pancreatic resection margin. It is emphasized that the pancreatic margin of resection should be at least 2.0 cm from the macroscopical tumor mass. The proliferative activity is lower in the intraductal components than in the corresponding infiltrating ones. The presence or absence of intraductal components is not correlated with age, sex, tumor location, tumor size, or stage. IDC with intraductal components tends to be associated with longer survival compared with IDC without intraductal components.

IDC of the pancreas is believed to originate from the ductal system [1]. Pancreatic duct cell changes may occur in the normal pancreas or in association with IDC [2]. Major duct epithelium changes are distinguished in squamous

metaplasia, mucinous cell hypertrophy, papillary hyperplasia, atypical hyperplasia and CIS. The prevalence of squamous metaplasia and mucinous cell hypertrophy is not significantly different between cases with and without IDC [3–5]. Papillary hyperplasia is more prevalent in the vicinity of the IDC and the larger ducts [6]. Papillary hyperplasia can be secondary to the IDC duct occlusion or it might be due to a primary neoplastic change [3]. In surgically resected IDC specimens, atypical hyperplasia or intraductal carcinoma foci are observed in and around the tumor mass [1–8]. These intraductal carcinoma foci associated with IDC have also been described as CIS. Because of these facts, CIS and atypical hyperplasia are considered to be precursor lesions for IDCs [9].

Recent molecular studies on intraductal lesions and associated infiltrating components of IDC indicate that accumulation of specific genetic alterations underlies the multistage processes of tumorigenesis and progression in IDC [10]. The oncogene K-ras mutation is an early event in the pathogenesis of IDC. A number of tumor suppressor genes have been found to play a critical role in the progression of pancreatic carcinogenesis [5]. Yamano et al. described genetic divergence, as well as genetic progression, in the clonal evolution of pancreatic cancer by studying allelic loss of intraductal lesions and infiltrating components of IDC [11]. Along these lines, pancreatic intraepithelial neoplasia (or PanIN), which is a new nomenclature and classification system for pancreatic duct lesions, has been proposed [12].

Definition

Infiltrating components of IDC of the pancreas show duct-like structures combined with tubular neoplastic glands [4]. Therefore, in well-differentiated IDC, distinguishing between the intraductal component and the space circumscribed by the infiltrating component can be extremely difficult [2]. The incidence of intraductal components of IDC has been reported to be 59% by other authors [3, 4, 6–9, 13]. We showed that 69% of IDC cases were accompanied by intraductal components with the following methods [14]. It is known that pancreatic ductal systems contain elastic fibers in the wall, and that ductal mural elastic fibers are preserved even in some pathological states [14–17] (fig. 1). Intraductal components of IDC can be clearly identified by visualizing mural elastic fibers and emphasizing the contour of ductal systems on elastica van Gieson (EVG)-stained sections (fig. 2). Ducts showing infiltrating components should be carefully excluded (fig. 3). Only ducts which are not continuous with infiltrating components of IDC and whose wall structures are completely preserved are selected. Carcinoma foci in these ducts are defined as intraductal components of IDC. The intraductal component of IDC is distinguished from

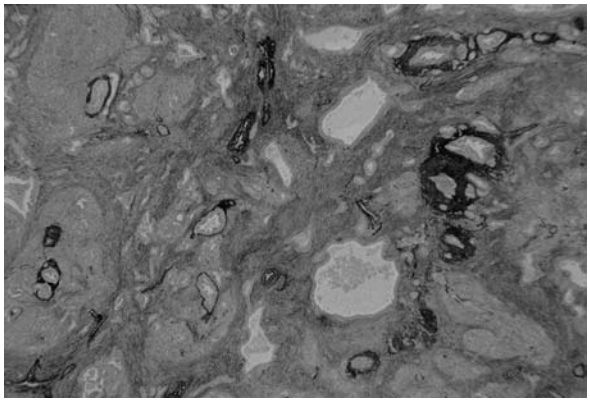

Fig. 1. Low power field of IDC (EVG). Note the well-preserved ductal mural elastic fibers.

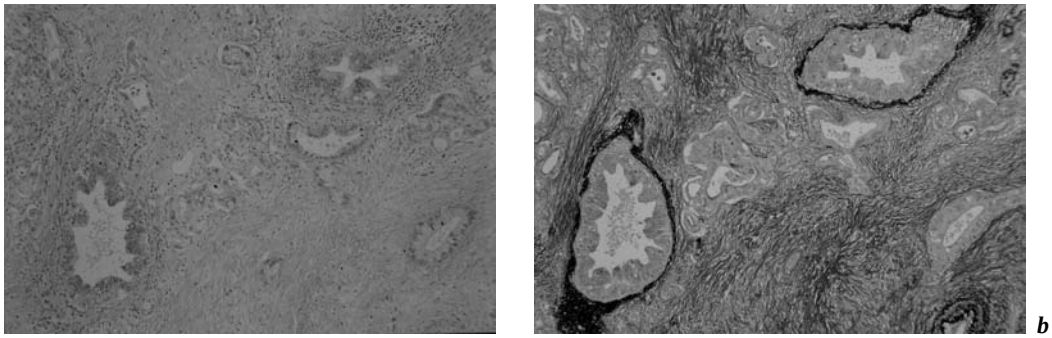

Fig. 2. The elastic fiber contour of the ductal system (***a*** HE, ***b*** EVG). Intraductal components rimmed with ductal mural elastic fibers and infiltrating components without ductal mural elastic fibers.

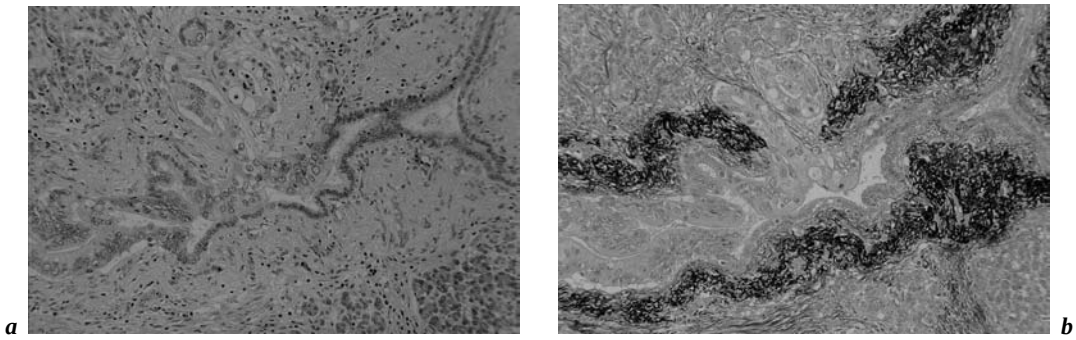

Fig. 3. Intraductal invasion (***a*** HE, ***b*** EVG). The ductal mural elastic fibers are destroyed locally by infiltration of IDC.

atypical hyperplasia by the application of generally accepted published criteria [1, 2, 4, 12]. Duct-like structures that are not rimmed with elastic fibers imply infiltrating components of IDC.

Carcinoma in situ and Intraductal Invasion

Intraductal components of IDC include both CIS and also infiltrating components of IDC [12, 14, 18]. It is often difficult to determine whether the carcinoma initially spreads through the duct without invasion (CIS/colonization of ducts), or infiltrating components secondarily involve the ducts (intraductal invasion/cancerization of ducts) [4, 12, 14]. Colonization of ducts is defined as intraductal carcinoma in another location to which CIS has spread. Although it may not always be possible to distinguish between CIS and intraductal invasion on a morphologic basis, serial sections are helpful in defining the relationship between the duct lesion and the infiltrating carcinoma component [12, 14].

The intraductal components of IDC are classified into three histological patterns as follows: low papillary (including flat), tubular (including solid and cribriform) and mixed (low papillary plus tubular) (fig. 4). The low papillary pattern is characterized by low papillary projections lacking a fibromuscular core or by irregular stratification and pleomorphism of epithelial cells. The tubular pattern is associated with desmoplasia. The incidences of the low papillary, tubular and mixed patterns are 39%, 56%, and 5%, respectively. IDC venous invasion shows the tubular type histological pattern, so a tubular pattern of intraductal components in IDC indicates intraductal invasion, while a low papillary pattern indicates CIS/colonization of ducts [18].

Cell proliferative activity is generally low in CIS compared to infiltrating carcinoma [19–21]. The proliferation index, evaluated using Ki67, is significantly lower in the intraductal components of IDC than in the associated infiltrating components (figs. 5, 6). The difference between the Ki67 labeling indexes in the intraductal and associated infiltrating carcinoma components suggests that these components show different biological behaviors [14]. The lower cell proliferative activity in intraductal components may explain why intraductal carcinoma spread is limited to the vicinity of the infiltrating focus. Wilentz et al. demonstrated that the pattern of Dpc4 expression in intraductal carcinoma components did not match that in the associated infiltrating carcinoma components [22]. Dpc4 is a tumor suppressor gene targeted in IDC. They concluded that loss of Dpc4 expression occurred biologically late in pancreatic tumor progression. These data imply that intraductal components associated with IDC represent CIS rather than intraductal invasion. The frequency of p53 protein overexpression is similar in both the intraductal and associated infiltrating

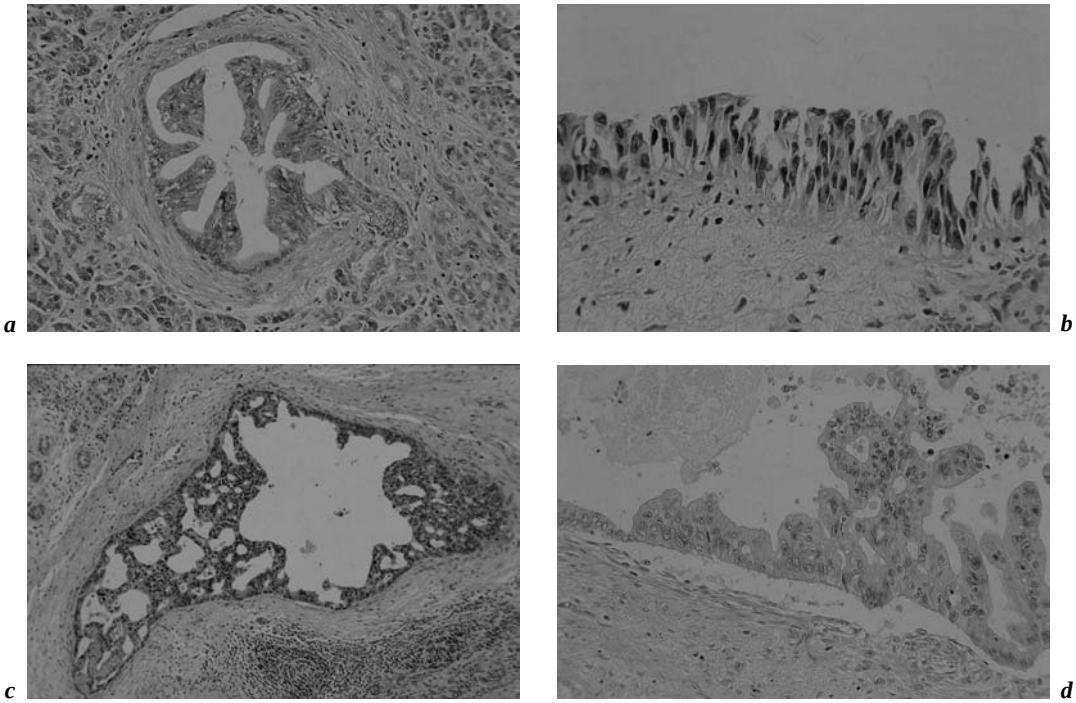

Fig. 4. Intraductal components of IDC. Low papillary (***a***), flat (***b***), cribriform (***c***) and mixed patterns (***d***).

components, suggesting that it represents an early genetic event in the process of pancreatic neoplasia [13, 23].

Clinicopathological Features

Intraductal spread of IDC is frequent in the well-differentiated type, common in the moderately differentiated type and occasional in the poorly differentiated type tumors [4, 24]. Some cases of well-differentiated tumors show marked intraductal extension, but none of the less-differentiated types show such marked intraductal extension. There is a significant correlation between the number of intraductal carcinoma foci and the grade of tumor differentiation [14].

IDC extends into the pancreatic tissue via stromal invasion. IDC also spreads through the pancreatic ductal tree. Intraductal spread is another important route of intrapancreatic extension in IDC. The intraductal carcinoma foci may be diffuse lesions involving the main pancreatic ducts, the large branch ducts and the small

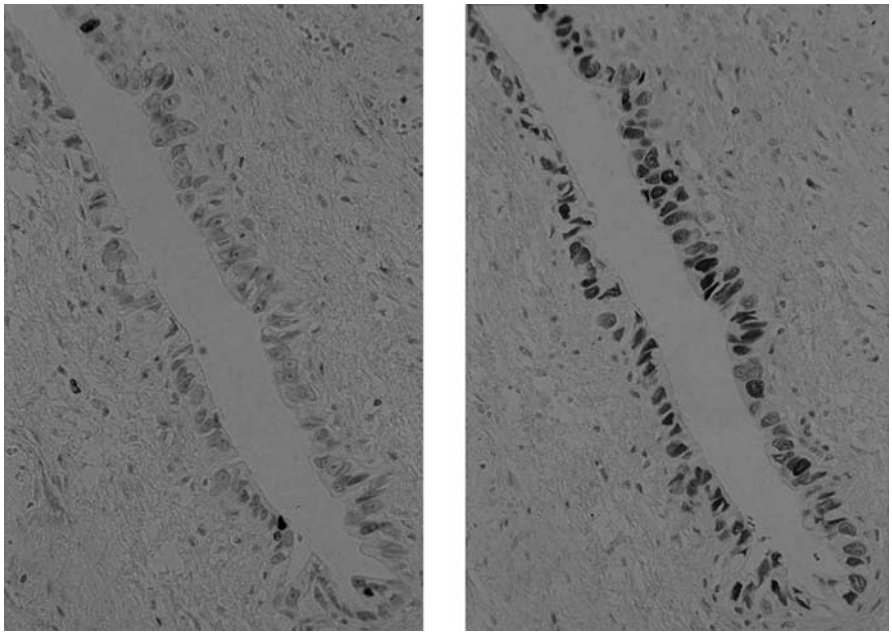

Fig. 5. Immunohistochemical features of IDC intraductal components. ***a*** The level of Ki67 immunoreactivity is low. ***b*** Most tumor cells show strong nuclear immunoreactivity for p53.

branch ducts, or they may occur focally in the pancreatic ducts. We observed the following distribution of intraductal carcinoma foci: 9.0% of foci were in the main pancreatic ducts, 64% in the large branch ducts and 27% in the small branch ducts [14]. The large branch ducts are the main routes of intraductal spread (fig. 7). The histogenesis of IDAP remains controversial. Duct cells, ductule cells and islets cells have been suggested as tumor progenitor cells [7, 25, 26]. The IDAP cells of the well-differentiated type are closely reminiscent of large interlobular duct cells [27]. Intraductal components of IDAP have a propensity to spread in large ducts rather than small branch ducts. These facts raise the possibility that IDAP might be derived from the epithelium of large branch ducts.

IDC can extend through the ducts beyond the tumor mass. The extension of the carcinoma as intraductal components has been cited as a particular problem in evaluating the surgical resection margin [24]. Intraductal carcinoma foci are observed even at the pancreatic resection margin. The intraductal carcinoma foci varied in location from the center (51%) or edge (35%) of the macroscopic tumor mass to outside the macroscopic tumor mass (14%). We reported that intraductal carcinoma foci were present outside the macroscopic tumor mass in 32% of IDAPs [14]. Intraductal spread of carcinoma is confined to the vicinity

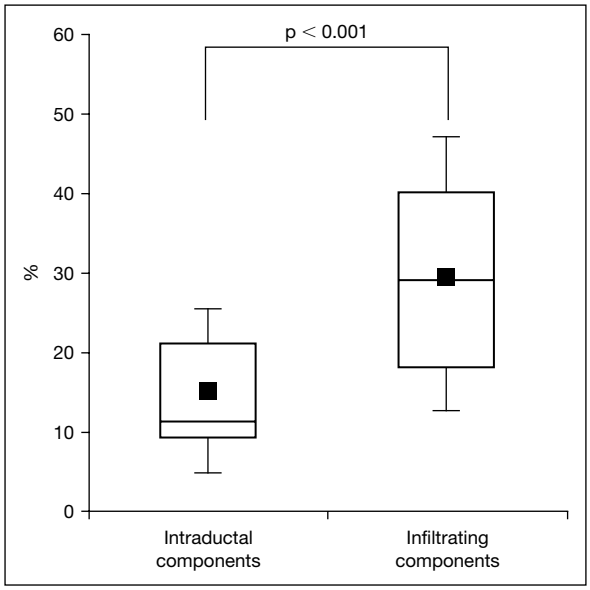

Fig. 6. The Ki67 labeling index is significantly lower in the intraductal components than in the associated infiltrating components.

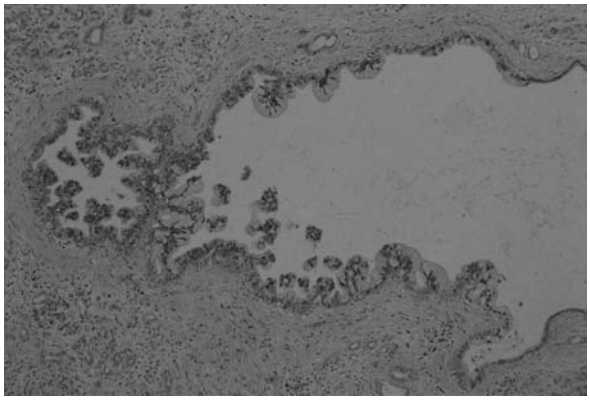

Fig. 7. Colonization along a large branch duct.

of the tumor mass in most cases [4]. The distance of intraductal carcinoma extension beyond the edge of the macroscopic tumor mass was limited to within 2.0 cm in our cases [14]. These findings emphasize that the pancreatic margin of resection should be at least 2.0 cm from the macroscopic tumor mass. In rare cases, the tumor may even spread along the main pancreatic duct into tumor-free

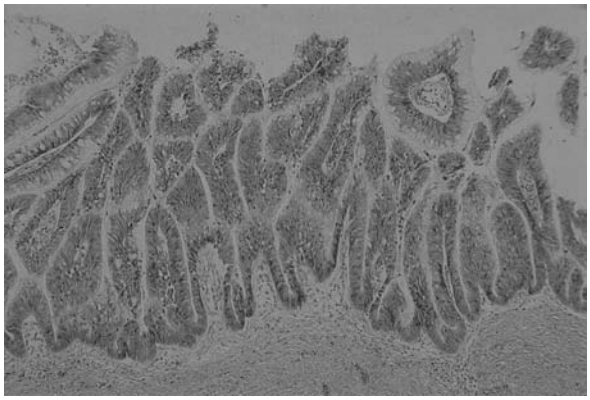

Fig. 8. IPMN showing a high papillary pattern.

tissue distant from the main tumor mass. None of the pancreatectomy specimens contained discontinuous foci of intraductal carcinoma [14, 24]. These facts suggest that multicentricity of ductal adenocarcinoma of pancreas occurs less frequently than was previously reported [24].

Ductal carcinoma of the pancreas is divided into two main categories: IDC and intraductal papillary mucinous neoplasm (IPMN) [4, 5]. IDC tends to invade extraductally from the early phase of its development. IPMT retains in situ or intraductal growth to a considerable extent before stromal invasion occurs [28]. Because of its poor prognosis, IDC should be differentiated from IPMN. Usually, this is not difficult because of the grossly solid and invasive tumor growth of IDC. There may occasionally be a problem distinguishing IDC with a marked intraductal extension from IPMN with clear invasion. In our study, three of 54 IDCs showed marked intraductal spread [14]. The intraductal components of these IDCs were confined in the immediate vicinity of associated infiltrating components. Moreover, intraductal components of IDC did not show a high papillary growth pattern, excessive secretion of mucin or association with the adenomatous components, which are characteristic of IPMN (fig. 8).

The presence or absence of intraductal components is not correlated with age, sex, tumor location, tumor size, or stage [14].

Prognosis

The presence or absence of intraductal spread of carcinoma is correlated with the grade of tumor differentiation ($p = 0.015$), but not with tumor stage

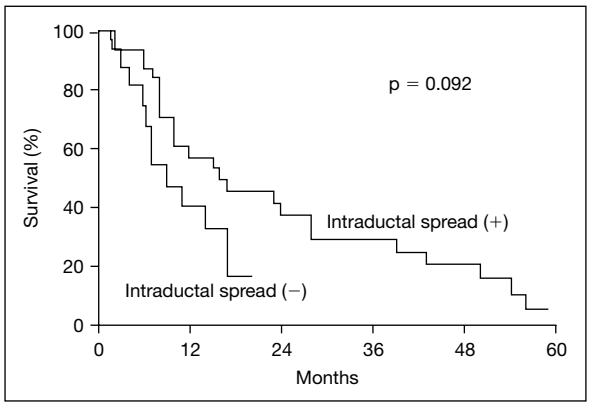

Fig. 9. Kaplan-Meier survival curves in patients with IDC according to the presence (n = 31) or absence (n = 16) of intraductal spread.

(p = 0.28) [14]. IDC with intraductal components tends to be associated with longer survival compared with IDC without an intraductal component. Thirty-one patients whose IDAP had intraductal components had a median survival of 12 months, compared to 8 months for 16 patients whose IDC did not have intraductal spread (p = 0.09) (fig. 9). Fukushima et al. showed that IDC with intraductal components was associated with long-term survival of patients (p = 0.002) [29]. It is known that histologic grade is essentially a prognostic factor [27]. The longer survival of patients with intraductal spread might reflect the fact that this group has a higher fraction of well-differentiated tumors.

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Fine Needle Aspiration Cytology of Noninvasive Ductal Carcinomas of the Pancreas

Differences from Invasive Ductal Carcinoma

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Abstract

Invasive ductal adenocarcinoma (IDA) of the pancreas (IDAP) originating from the ductal gland has a poor prognosis worldwide. To improve the prognosis, treatment for noninvasive carcinoma stages is needed. Noninvasive carcinomas are principally intraductal papillary-mucinous carcinomas (IPMC) and pancreatic intraepithelial neoplasm 3 (PanIN-3). Small papillary-cohesive clusters, with mainly small regular (about 10 μm) nuclei, clearly defined cell borders, a mixture of some goblet cells, and a monoclonal aspect are cytologically observed in both IPMC and IPMN, while euchromatin and nuclei malignancy are observed only in IPMC. PanIN-3 cells have small papillary-cohesive and compact clusters, dense and meager cytoplasm without prominent anisocytosis and without cytoplasm >21 μm at the shortest diameter. The nuclei are individually well enveloped in well preserved cytoplasm separately from each other, and are small regular nuclei mostly highly suspicious for malignancy. IDA cells have loose sheet-solid clusters, poorly preserved cytoplasm, nuclei that tend to adhere to each other, a combination of large nuclei (short diameter >15 μm) with hyperchromatin, and a monoclonal aspect. To preoperatively differentiate noninvasive IPMC/PanIN-3 from IDAP, these would be clinically very useful.

Precancerous IDAP lesions are mainly PanINs and IPMNs. PanIN has been recently established as a term for intraductal tumors by the WHO [1]. The prognosis of IDAP is still poor, and its occurrence is increasing worldwide. Detection in the early stage is difficult even now, and most cases are detected too late because PanIN-3 (severe dysplasia: SD/carcinoma in situ: CIS) has a

rapid cell cycle [2] and invades immediately. However, though rare, several cases have been detected and undergone surgery in PanIN-3 stage [3] with a very good prognosis [3]. Consequently, to improve IDAP prognosis, detection and treatment in PanIN-3 stage is needed. IPMC is often found in the noninvasive carcinoma stage because it is slow growing, and the noninvasive stage extends over a long period of time, showing a distinct clinical entity. We have reported the cytologic features and differences between noninvasive IPMC and IDA [4]. It has been discovered that, in IDAP cases without clinical features of mucin-producing pancreatic tumors, cell clusters resembling noninvasive IPMC cells of the type with dense cytoplasms and scarce goblet cells have been found, and these have been PanIN-3 cells, while the cytologic pattern of PanIN-3 has been distinguishable from that of IDA [5]. Here, we clarify the cytologic features of noninvasive IPMC and PanIN-3, describe the differences from IDA, and consider the differences between noninvasive IPMC and PanIN-3.

Cytologic Features of Noninvasive IPMC and PanIN-3 and Differences from IDA

Cytologic Features of Noninvasive IPMC

IPMC is commonly clinically detected as a mucin-producing tumor of the pancreas. They were first reported by Ohhashi et al. [6]. This type of tumor has characteristic features, such as an enlarged papilla of Vater with a patulous orifice that secretes mucin and a dilated main pancreatic duct, and has, unlike ordinary pancreatic cancer, a favorable prognosis [6]. The entity was established by the WHO in 1996 as a noninvasive IPMN exocrine tumor of the pancreas [7]. Not only carcinomatous, but also adenomatous and hyperplastic mucin-secreting epithelia are now known to manifest these features [8]. IPMC grows intraductally for a relatively long time. However, the prognosis after stromal invasion is bad [9, 10]. Half of invasive carcinomas are mucinous noncystic carcinomas, and most of the others are ordinary tubular adenocarcinomas [11–13]. Cytologic features noninvasive IPMC were first reported in 1989 [14]. Later, it was reported that among sensitivity, specificity and overall accuracy of ultrasonography, ERCP and pancreatic juice cytology of mucin-producing tumors of the pancreas (11 cases), pancreatic juice cytology has achieved the best results [8]. Immunocytochemically, it has been reported that the detection rate using p53 protein (9 IPMC) showed an increase of 23% in comparison with only cytology [15], and the detection rate using telomerase (13 IPMC) showed an increase of 54% in comparison with only cytology [16]. Benign cases (IPM adenoma) were all negative for cytology, p53 protein and telomerase [8, 15, 16]. Namely, the specificity of benign cases was 100% using any of the above

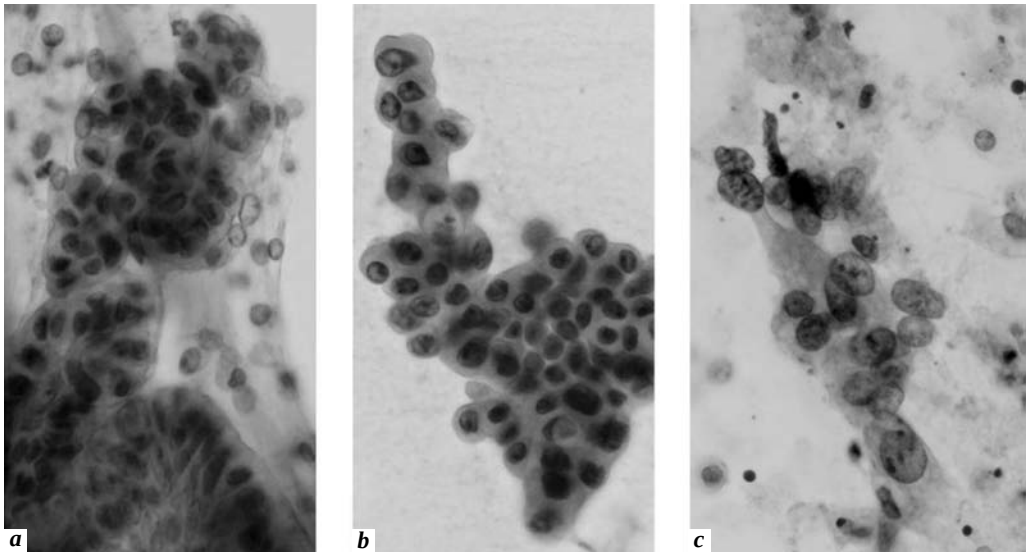

Fig. 1. Arrangement of benign IPMN (hyperplasia), noninvasive IPMC and IDA. ***a*** Benign IPMN is papillary and cohesive. Pancreatic ductal brushing, Papanicolaou stain. ×400. ***b*** Noninvasive IPMC shows a small papillary-cohesive cluster that is accompanied by outer protrusions of cells. Vinyl tube aspiration of the main duct of the resected pancreas, Papanicolaou stain. ×400. ***c*** IDA is loose, in sheets. Scrap smear, Papanicolaou stain. ×400.

methods. As for cytological differential diagnosis of noninvasive IPMC and IDA, it was reported to be impossible [17, 18]. It was subsequently reported that they are distingushable [4]. The following describes the cytologic features of noninvasive IPMC and the differences from benign IPMN (hyperplasia/adenoma) and IDA [4]. Then, we describe noninvasive IPMC with a special type of goblet cells. Lastly, invasive IPMC is described as cases in which the invasive component is a mucinous noncystic carcinoma and cases in which the invasive component is IDAP.

Cytologic Features of Noninvasive IPMC and Differences from Benign IPMN (Hyperplasia/Adenoma) and IDA

Arrangement

Benign IPMN (hyperplasia/adenoma) is papillary-sheet-palisading and cohesive (fig. 1a). Noninvasive IPMC shows small papillary-cohesive clusters that are often accompanied by outer protrusions of cells (fig. 1b). IDA is in sheet-tubular-solid form and loose (fig. 1c). As for arrangement of noninvasive

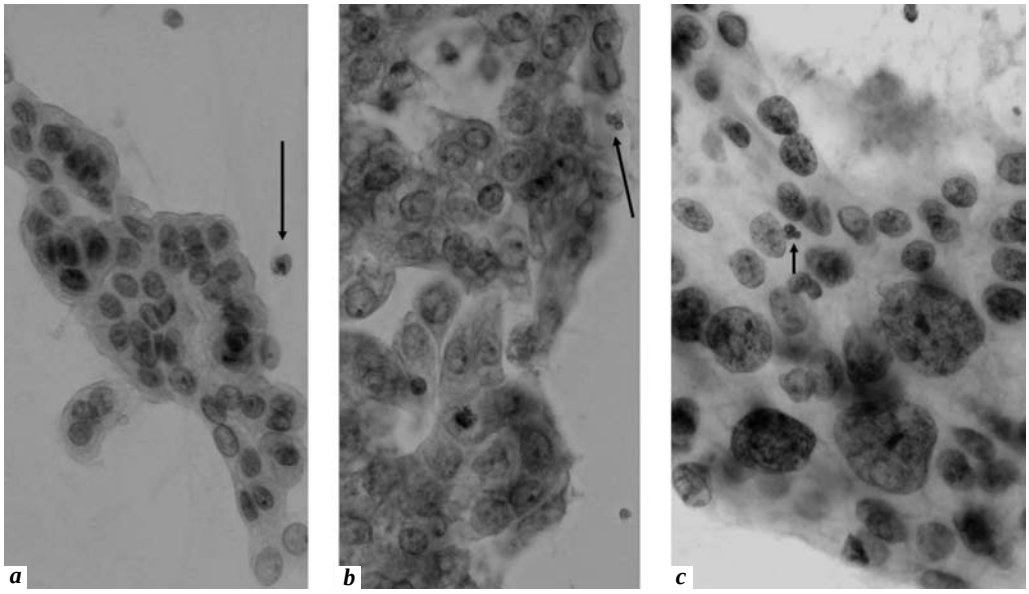

Fig. 2. Nuclei of benign IPMN (hyperplasia), noninvasive IPMC and IDA. ***a*** Benign IPMN has small nuclei but no hyperchromatin. Pancreatic ductal brushing, Papanicolaou stain. ×400. ***b*** Noninvasive IPMC shows regular small nuclei, although larger than those of benign IPMN, at about 10 μm in diameter without anisonucleosis (the standard makes neutrocytes including cytoplasm 10 μm, and each nucleus is measured from the proportion). Euchromatin shows severe atypia. Scrap smear, Papanicolaou stain. ×400. ***c*** IDAP has a combination of large nuclei (>15 μm in diameter) and hyperchromatin with prominent anisonucleosis. Scrap smear, Papanicolaou stain. ×400. Arrows show neutrocytes.

IPMC (1989–2001) [8, 14–18], there has been only a description of non-looseness [17]. Conversely, IDA has been reported as loose clusters [4, 19] and lacking cohesiveness [4, 19]. The papillary arrangement appears to be part of the basic histologic morphology of IPMT [7, 14], but not of IDA. These findings correspond with a report that higher grade adenocarcinomas tend to show non-cohesive clusters, whereas well-differentiated adenocarcinomas frequently exhibit cohesive and well-demarcated groups, resembling those in benign ductal fragments [20]. Consequently, cases with small papillary-cohesive clusters are classed as noninvasive IPMC.

Nuclei

Benign IPMN (hyperplasia/adenoma) has small, regular sized nuclei and no hyperchromatin (fig. 2a). Noninvasive IPMC shows small, regular nuclei, about 10 μm in diameter (the standard makes neutrocytes including cytoplasm 10 μm,

and each nucleus is measured from this proportion), and euchromatin suggesting malignancy (fig. 2b). IDA has a combination of large nuclear size (>15 μm at the short diameter) and hyperchromatin (fig. 2c). Namely, small, regular nuclei are observed in benign IPMN and noninvasive IPMC, but not in IDA [4] Conversely, a combination of large nuclei (>15 μm at the shortest diameter) and hyperchromatin (defining malignancy) is observed only in IDAP, but not in benign IPMN and noninvasive IPMC [4]. Euchromatin (suggesting malignancy) is common in noninvasive IPMC, but rare in IDA and not seen in benign IPMN [4]. Uneven chromatin distribution is common in noninvasive IPMC [4, 8, 17, 18] and IDA [4, 19–21], but is absent or rare in IPMN [4]. Coarsely granular chromatin (this is more advanced irregular chromatin distribution and is conclusive for malignancy) is rarely observed in noninvasive IPMC [4, 17, 18], and is common in IDA [4, 19, 20], but not in IPMN [4]. Prominent nucleoli have been reported in noninvasive IPMC [4, 8, 17, 18] and IDA [5, 19–21]. For these reasons, noninvasive IPMC seem to have been reported as being undistinguishable from IDA. Consequently, cases with small malignant nuclei with euchromatin and without anisonucleosis are suggested to be noninvasive IPMC.

Cytoplasm

Benign IPMN (hyperplasia/adenoma) (fig. 3a) and noninvasive IPMC (fig. 3b) have clearly defined cytoplasmic borders and small regular-sized cytoplasms. IDA has prominent anisocytosis, and poorly defined cytoplasmic borders (fig. 3c). A mixture of goblet cells is cytologically observed in benign IPMN (fig. 4a) and noninvasive IPMC (fig. 4b). This emphasizes the polyclonal-like finding. In contrast, these are cytologically hardly found in IDA (fig. 4c) [4]. IDA cells are monoclonal-like. In histological microscopic photographs, a polyclonal aspect is also recognized in IPMN [13, 22] and IPMC [7, 23], but not in IDA. Several researchers have classified IPMN into clear-, dark-, and compact-cell types based on cytoplasm condensation, epithelial form and expression pattern of MUC1, MUC2 and MUC5AC [24, 25]. Other researchers have also proposed that the papillae of IPMN could be divided into two types: intestinal and pancreatobiliary [26, 27]. In our experience [4], goblet cells are usually found in noninvasive IPMC. Consequently, cases with clearly defined cytoplasmic boundaries, a mixture of goblet cells and a polyclonal aspect suggest benign IPMN and noninvasive IPMC.

Conclusion

For all practical purposes, if a case with a clinically detected mucin-producing pancreatic tumor exhibits small papillary-cohesive clusters, only the features of mainly small (about 10 μm) regular malignant nuclei, euchromatin, clearly defined cell borders, a mixture of goblet cells and a polyclonal aspect, are more strongly suggestive of noninvasive IPMC.

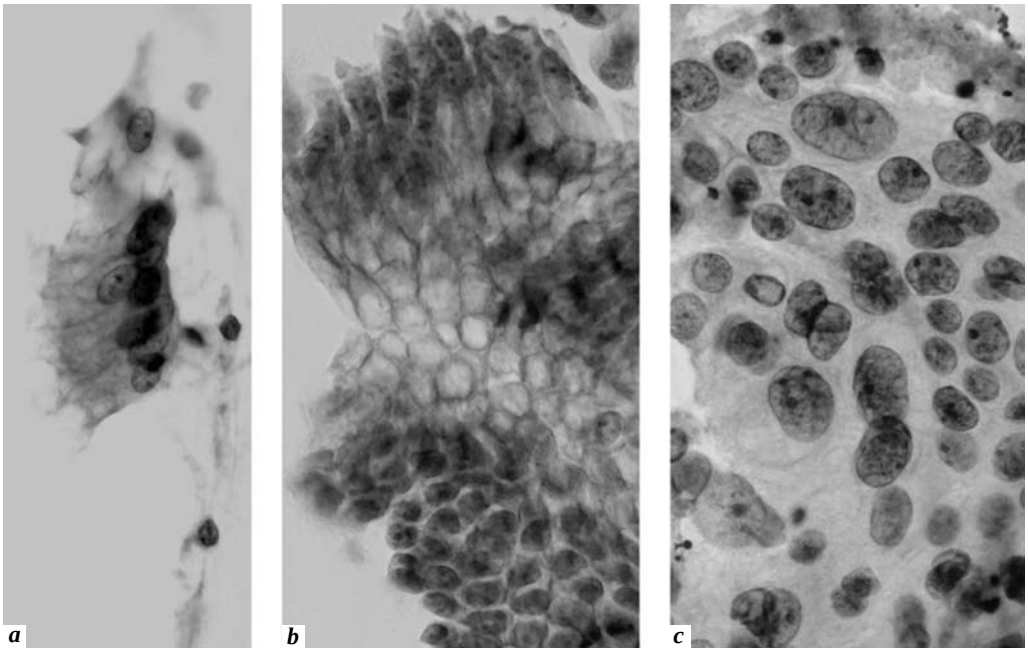

Fig. 3. Cytoplasmic border of benign IPMN (hyperplasia), noninvasive IPMC and IDA. ***a*** Benign IPMN (vinyl tube aspiration of the main duct of the resected pancreas, Papanicolaou stain, ×400) and ***b*** noninvasive IPMC (scrap smear, Papanicolaou stain, ×400) have columnar cytoplasm and a clearly defined cytoplasmic border. ***c*** IDAP are amorphous with a poorly defined cytoplasmic border, and have prominent anisocytosis. Scrap smear, Papanicolaou stain. ×400.

Noninvasive IPMC with a Special Type of Goblet Cell

In IPMC, there is a noninvasive IPMC with a special type of goblet cell that has a clinically very good prognosis and suggests only a noninvasive carcinoma, ruling out benign lesion (normal epithelia, mucinous metaplasia, papillary hyperplasia, atypical hyperplasia) and invasive carcinoma [28]. In carbohydrate histochemistry, these goblet cells were shown to be differentiated goblet cells of the large intestine (staining with dye 8-O-acetylated N-acetylneuraminic acid that is a special marker of goblet cells of the large intestine: periodic acid-sodium borohydride-potassium hydroxide-periodic acid Schiff stain [29, 30] is positive). In our hospital, this staining has been performed for 10 IPMC cases, but we have found only one positive case (fig. 5). This case was operated on, and the patient survived with a stump-positive carcinoma for two years from first surgery. Then, a re-operation was performed. The histology has

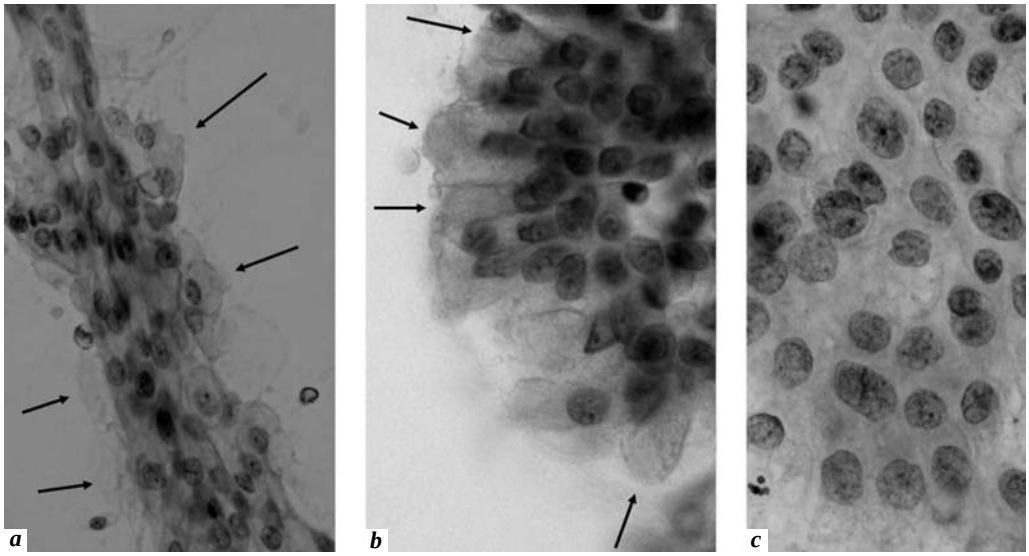

Fig. 4. Clonality of benign IPMN (hyperplasia), noninvasive IPMC and IDA. ***a*** Benign IPMN (arrows exhibit goblet cells, including orange mucin). Vinyl tube aspiration of the main duct of the resected pancreas, Papanicolaou stain. ×400 and ***b*** noninvasive IPMC (arrows show goblet cells, including translucent mucin). ERCP, Papanicolaou stain. ×400 show a polyclonal pattern. ***c*** IDA are monoclonal-like. Scrap smear, Papanicolaou stain. ×400. Mixture of goblet cells that is often observed in benign IPMN (***a***) and noninvasive IPMC (***b***) shows polyclonality. In contrast, a mixture of goblet cells is hardly found in IDA cases (***c***).

remained noninvasive IPMC, the same as the initial histologic diagnosis. There has been no recurrence for eight years after the re-operation. The goblet cells of the large intestine were characteristic of clearly defined cytoplasmic boundaries and lucent cytoplasmic contents with lucent brush borders;it may therefore be safely said that they were classic goblet cells (fig. 6).

Invasive IPMC with Two Cell Types (Noninvasive and Invasive Components)

Invasive IPMC with a Mucinous Noncystic Carcinoma Invasive Component

Among invasive IPMC, noninvasive component cells have papillary-cohesive clusters, suspicious of malignancy, and polyclonal-like cytoplasm (fig. 7a), while invasive component cells (mucinous noncystic carcinoma) have

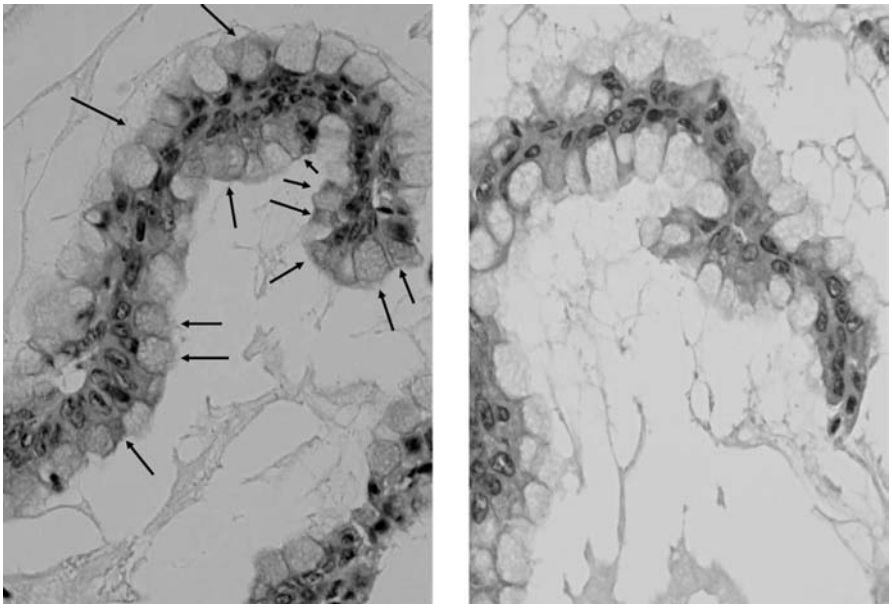

Fig. 5. Noninvasive IPMC with special type goblet cells. ***a*** Periodic acid-sodium borohydride-potassium hydroxide-periodic acid Schiff (a special marker of goblet cells of the large intestine) stains pink (arrows). Periodic acid-sodium borohydride-potassium hydroxide-periodic acid Schiff stain. ×200. ***b*** The cells that differentiated the goblet cells of the large intestine are classic goblet cells. This patient survived with carcinoma for two years after first surgery, and was then re-operated on. The histology at re-operation remained noninvasive IPMC, the same as the initial histology. There has been no recurrence for eight years after the re-operation HE. ×200.

sheet-solid loose clusters with monoclonal-like cytoplasm and are conclusive for malignancy (fig. 7b). The histology is shown in figure 8 (at left: noninvasive IPMC, at right: mucinous noncystic carcinoma).

Invasive IPMC with an IDA Invasive Component

Among invasive IPMC, noninvasive component cells are small nuclei (about 10 μm in short diameter), with euchromatin, strongly suspicious of malignancy, clearly defined cytoplasmic borders, sheet-papillary-cohesive clusters, and a monoclonal-like appearance (upper part of fig. 9). Invasive component (IDA) cells are coarsely granular chromatin, are in a sheet-like arrangement, have large nuclei (>15 μm at the shortest diameter), are conclusive for malignancy, and are monoclonal (lower portion of fig. 9). The histology is shown in fig. 10 (at right: noninvasive IPMC, at left: IDAP).

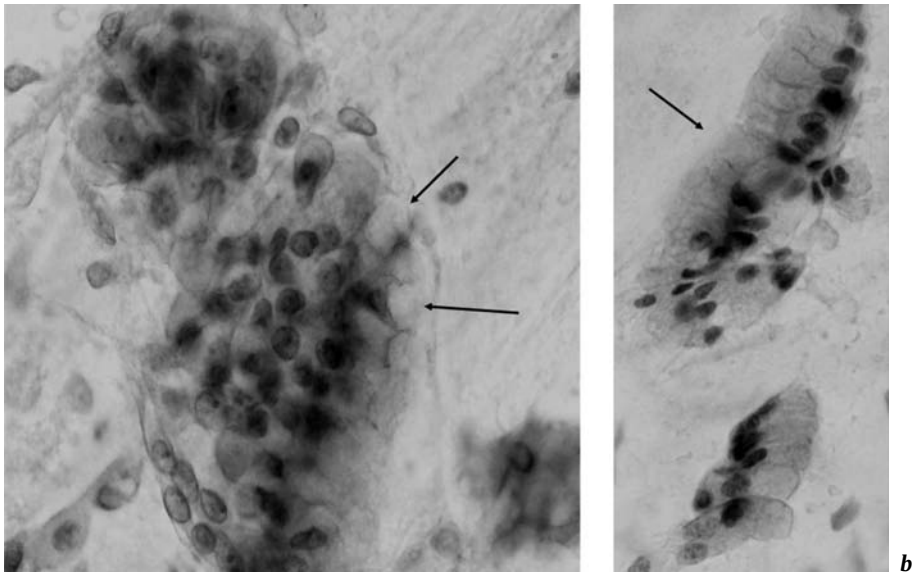

Fig. 6. Noninvasive IPMC with special type goblet cells. Cytology of the case in figure 5. ***a***, ***b*** The cells differentiating to goblet cells of the large intestine appear to have characteristic clearly defined cytoplasmic boundaries, lucent cytoplasmic contents and lucent brush borders (arrows). It is safe to say that they were indeed classic goblet cells. ERCP, Papanicolaou stain. ×400.

PanIN-3 (SD/CIS)

In the spread of IDAP, there are two types of intraductal spread [5, 31], noninvasive and ordinary invasive spread [1, 7, 23, 31, 32], and PanIN-3[1] is detected as noninvasive intraductal spread [5, 31]. PanIN-3 is commonly found in association with IDAP [33–35], and the lesions are present in 30–50% of pancreata with IDAP [33, 36–39]. This association alone suggests that at least the higher grades of PanIN can be precursors of invasive carcinoma [40]. PanIN-3 was established as a term for high grade pancreatic intraepithelial neoplasia in substitution for SD/CIS by the World Health Organization (WHO) [1], because it is very difficult, if not impossible, to draw a clear distinction between SD and CIS [1]. IDAP with intraductal spread is frequently of a well-differentiated type [23, 31] and shows a tendency, although not significant, to be associated with longer survival than those without intraductal spread [31]. Accordingly, if PanIN-3 is found in IDAP samples, it may be expected that the pancreatic cancer will have a better prognosis than IDAP without PanIN-3,

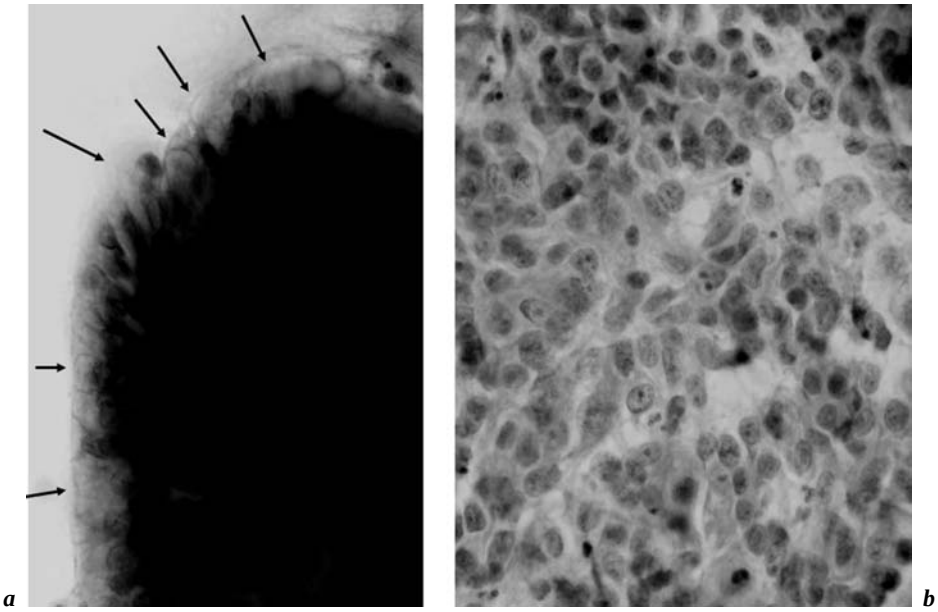

Fig. 7. Invasive IPMC (Invasive components mucinous noncystic carcinoma). ***a*** Noninvasive IPMC has a relatively clearly defined cytoplasmic border and a mixture of goblet cells, and is polyclonal-like (arrow cells stain pale red, the remaining cells stain green). Scrap smear, Papanicolaou stain. ×400. ***b*** Invasive components are a mucinous noncystic carcinoma, with a poorly defined cytoplasmic border and are monoclonal-like appearance. ***a*** and ***b*** are the same magnification. Scrap smear, Papanicolaou stain. ×400.

provided that the tumor size, site and stage are similar. However, there are no reports on a differential diagnosis between PanIN-3 and IDAP except for one report showing that 'there was no cytologic difference between CIS and invasive carcinoma' [41]. PanINs and IPMNs histologically share many fundamental features [27, 40, 42, 43]. In fact, a given focus of intraductal neoplasia may be almost impossible to classify by morphology alone [40]. This is because both are equally inherently intraductal, both are composed predominantly of columnar, mucin-producing cells that may grow in a flat configuration or produce papillae, both exhibit a range of cytologic and architectural atypia, both are recognized as precursors to invasive adenocarcinoma, and both sequentially accumulate similar genetic alterations with increasing cytoarchitectural atypia [44, 45]. These features confirm that cytologic features of PanIN-3 are similar to those of IPMC.

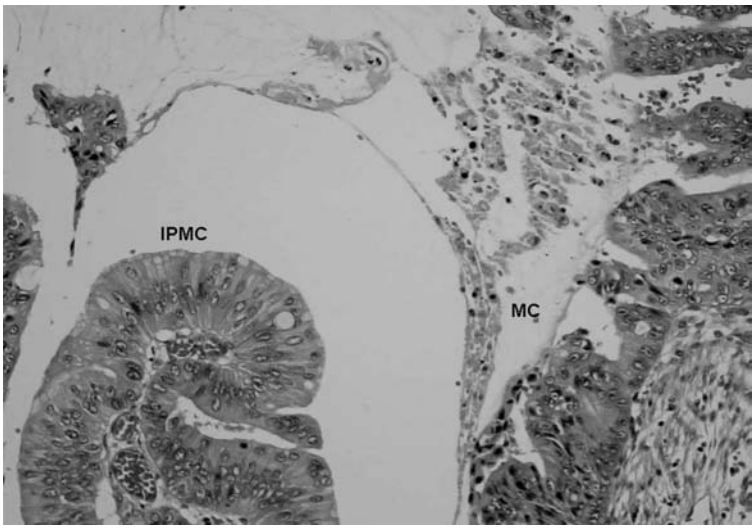

Fig. 8. Invasive IPMC (Invasive components mucinous noncystic carcinoma). Histology corresponds to figure 7. The left side corresponds to noninvasive IPMC, and the right side to the invasive component (mucinous noncystic carcinoma) HE. ×200.

Cytological Differential Diagnosis of PanIN-3 Type Cells from IDA-Type Cells

Arrangement

Small papillary-cohesive and compact clusters are the most consistent features of PanIN-3 (upper portion of figs. 11a, b and 13a, b) [5, 41, 46–49]. Small papillary-cohesive clusters appeared to result from excessive proliferation and strong cohesion of well-preserved epithelial cells within strong uninfiltrated ducts. These reflect low papillary projections/palisades that are a histologic characteristic of PanIN-3 (right portion of fig. 12 and upper portion of fig. 14) [1, 5, 7, 23, 31, 32, 41, 46–49]. IDA-type cells are loose sheet-solid clusters (lower portions of figs. 11a, c and 13a, c) reflecting histologically well/poorly differentiated tubular formation (left portion of fig. 12 and lower portion of fig. 14). These observations seem to support the concept that as a result of the weakening of intercellular cohesion, cell clusters become loose [50]. Nuclear crowding and/or overlapping and nuclear contour irregularities have been cytologically reported as indexes of malignancy [20], but these two features were common in the cytologic profiles of both PanIN-3 [41, 46–49, 51] and IDA [20, 21, 52]. For these reasons, it seems to have been reported that CIS is indistinguishable from IDA. However, peer reviewing nuclear crowding and/or

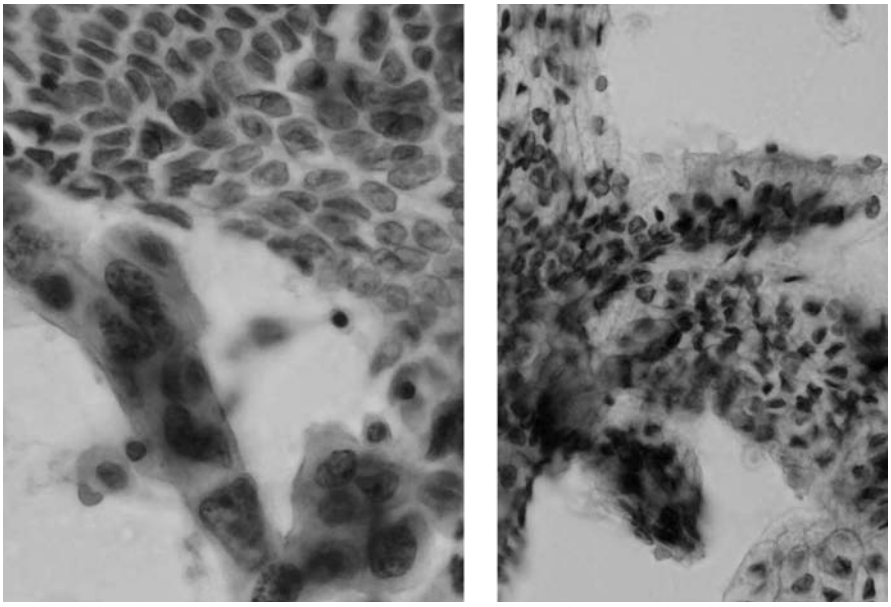

Fig. 9. Invasive IPMC (Invasive components IDA). ***a*** and ***b*** are the same sample. ***a*** The upper part (showing sheet clusters) shows noninvasive IPMC components, and the lower part is the invasive component that exhibits IDAP. The noninvasive IPMC has euchromatin, while the invasive component has coarsely granular chromatin. ***b*** The left part (showing sheet clusters), the right central part (showing palisading clusters) and the right lower part (showing papillary clusters) are noninvasive IPMC components and with clearly defined cytoplasmic borders. Noninvasive IPMC and the invasive component are both monoclonal-like. Scrap smear, Papanicolaou stain. ×400.

overlapping, in the invasive component-type cells, the cytoplasm between nuclei was poorly preserved, and the nuclei tended to adhere to each other [5]. In contrast, in PanIN-3, the cytoplasm between the nuclei tended to be well preserved, the nuclei tended to be separate from each other when focusing the microscope up and down, and the cytoplasmic borders between nuclei tended to be clearly defined. These findings appear to be one of the differences between PanIN-3 and IDAP type cells [5].

Nuclei

Small nuclei without anisonucleosis (upper portions of figs. 11a, b and 13a, b) are characteristic of PanIN-3 [5, 41, 46–49, 51]. In order to differentiate between PanIN-3 and IDA type cells, however, it is more useful to focus on the paired hyperchromatin and large nuclei [5]. The combination of large nuclei

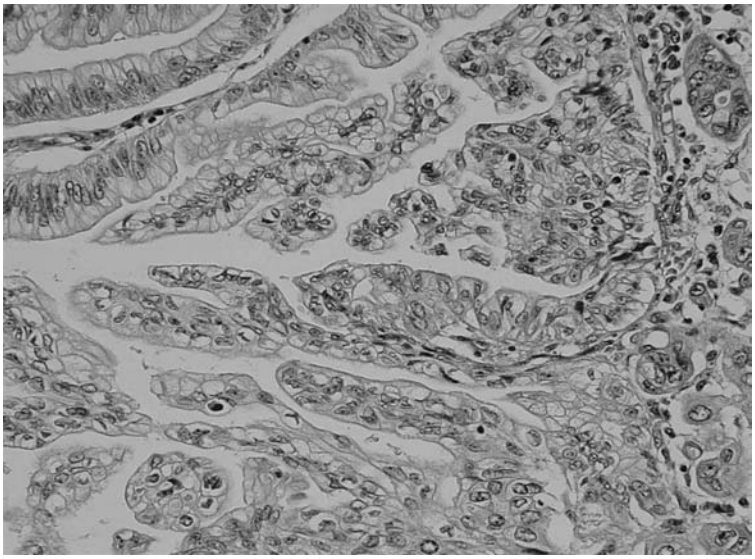

Fig. 10. Invasive IPMC (Invasive components IDA). Histology corresponds to figure 9. The major portion corresponds to noninvasive IPMC, and the right end corresponds to the invasive component (IDAP) HE. ×200.

(>15 μm at the shortest diameter) and hyperchromatin is never observed in PanIN-3, but often observed in IDAP (the lower portion of figs. 11a, c and 13a, c) [5]. IDAP is conclusive for malignancy, while PanIN-3 is mostly strongly suspicious of malignancy [5]. Hyperplasia, adenoma, and moderate dysplasia are all benign [5]. Although cytologic materials of CIS [41, 46–49]. were previously reported to be all pancreatic juice, the assessment was cancer cells (the detection rate was 6 of 11 patients: 6/11 [41], 2/2 [49], 2/3 [48] and 1/1 [46]), suspicious for malignancy (1/1 [47–48]), atypia (3/11 [41]) and no abnormality (2/11 [41]). Accordingly, small monotonous nuclei, no combination of large nuclei and hyperchromatin, and being strongly suspicious of malignancy are characteristic of PanIN-3.

Cytoplasm

Clearly defined cytoplasmic borders are observed in cytologic views of SD [51]/CIS [41, 46–49] and PanIN-3 [5, 20]. According to Monzat et al. [53], gap junctions play a crucial role in proliferation, differentiation and secretion processes, and during the growth of human pancreatic duct cells in vitro and in vivo, gap junctions develop progressively. Hence, clearly defined cytoplasmic borders seem to result from the double factors of strong intercellular cohesion

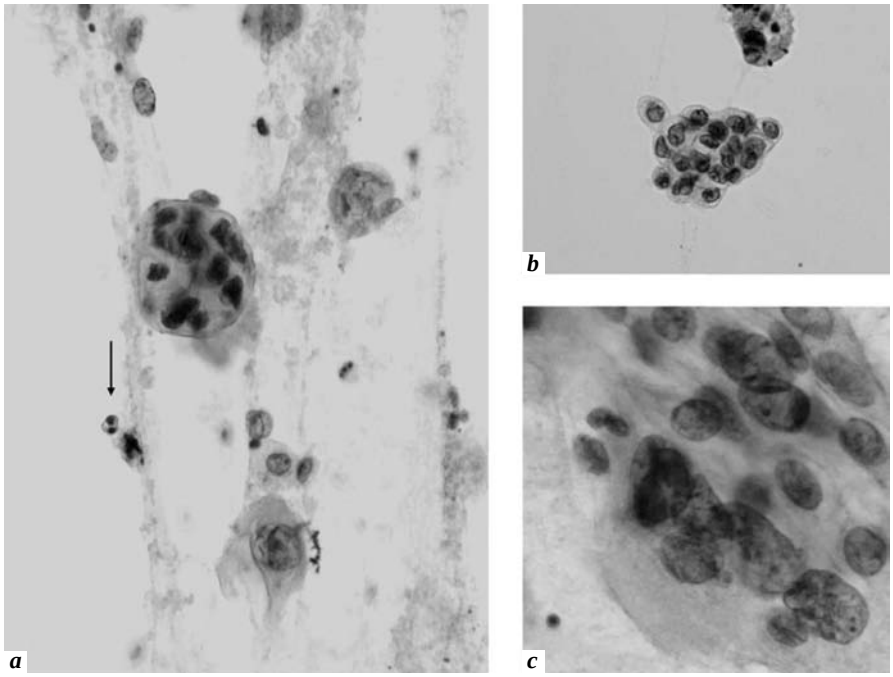

Fig. 11. IDAP with PanIN-3. ***a*** PanIN-3 (the upper portion) and IDA (the lower portion) type cells. ↓ = Neutrocyte. ***b*** PanIN-3 type cells. ***c*** IDA type cells (***a***–***c***: scrap smear, Papanicolaou stain. ×400). ***b*** and ***c*** are the same magnification of the same preparation. PanIN-3 type cells have small papillary-cohesive and compact clusters, and the cytoplasm is dense and well preserved, but meager, without prominent anisocytosis and with no cytoplasm >21 μm in the short diameter. The nuclei tended to be separated from each other (as seen when focusing the microscope up and down), and were often centrally located. In contrast, IDAP type cells were composed of loose sheet-solid clusters, the cytoplasm was poorly preserved, and the nuclei tended to adhere to each other. A combination of large nuclei (short diameter >15 μm) and hyperchromatin is observed in IDAP (the lower portion of ***a*** and ***c***). Commonly, IDAP is conclusive for malignancy, while PanIN-3 is strongly suspicious for malignancy. However, this PanIN-3 case is conclusive for malignancy.

by highly developed gap junctions and pressure on the cytoplasmic border due to excessive proliferation. The cytoplasm of SD [51]/CIS [47] and PanIN-3 [5] has individually well enveloped and often centrally located nuclei, tending to be well preserved. In invasive component cells, there is a tendency for nuclei to protrude from the cytoplasm, and preservation of the cytoplasm is lost [5]. Whether the nucleus is included within, or protrudes from, the cytoplasm appeared to be due to the degree of preservation of the cytoplasm and its

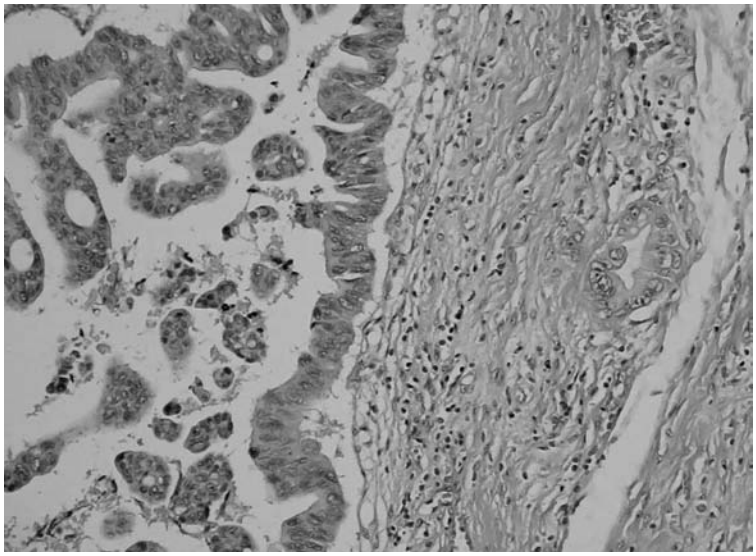

Fig. 12. IDAP with PanIN-3. Histology corresponds to IDAP of figure 11. The left side is PanIN-3 with small papillary-compactly packed patterns with small regular nuclei and dense cytoplasm, and the right side is the invasive component (IDAP) with a tubular pattern with large nuclei HE. ×200.

membrane. Consequently, a nucleus individually enveloped in well-preserved cytoplasm and centrally located nuclei may be also characteristic of PanIN-3. Small and dense cytoplasm without prominent anisocytosis seemed to be characteristic of the cytologic profile of SD [51]/CIS [41, 46–49] and the description of PanIN-3[5], but in order to differentiate between PanIN-3 and IDAP-type cells, it is more useful to focus on abundant cytoplasm rather than small dense cytoplasm, because abundant cytoplasm ($>$21 μm at the shortest diameter) is never observed in SD [51]/CIS [41, 46–49] and PanIN-3 [5], but is observed in most IDAP [5, 20] The nuclear arrangement of PanIN-3-type cells is regular compared with that of IDA-type cells. The irregularity of the nuclear arrangement generally appeared to be due to prominent anisocytosis. In IDA-type cells, the nuclei in large amounts of cytoplasm appeared to contain a greater quantity of total chromatin than the nuclei in a small amount of cytoplasm. Namely, cytoplasmic size appeared to be proportional to the total quantity of chromatin. Consequently, a large amount of cytoplasm itself could be used to make a diagnosis of IDAP. Accordingly, cytoplasm that is dense and meager (without prominent anisocytosis and abundant cytoplasm $>$21 μm in diameter) may be characteristic of PanIN-3.

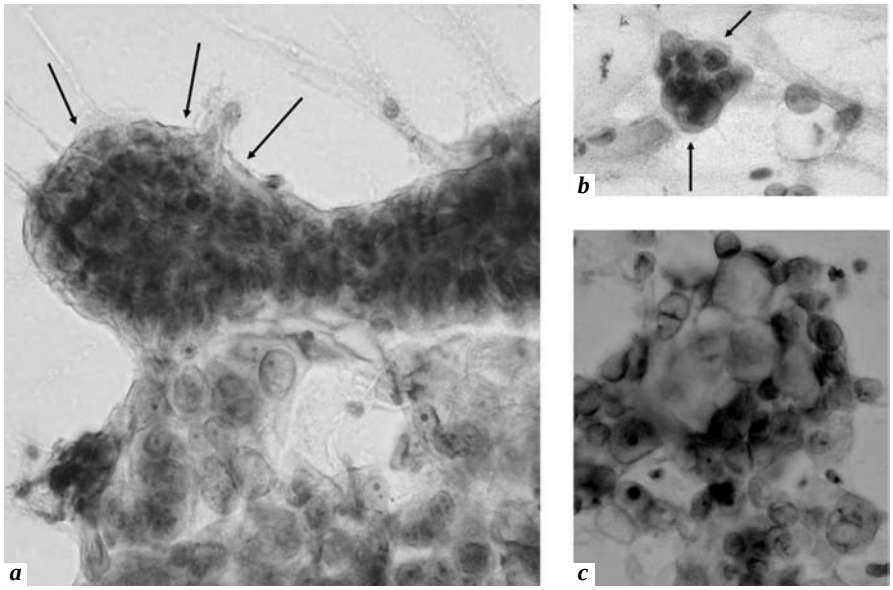

Fig. 13. IDAP with PanIN-3. PanIN-3 type cells (the upper portion of ***a*** and ***b***) and IDAP type cells (the lower portion of ***a*** and ***c***) (***a***–***c***: scrap smear, Papanicolaou stain. ×400). (***b***) and (***c***) are the same magnification of the same preparation. PanIN-3 type cells form compactly packed clusters with small regular nuclei and are polyclonal-like (arrow cells are yellow, the other cells are green). In contrast, IDAP type cells form loose tubular clusters with anisonucleosis with large nuclei and are monoclonal-like.

Analysis and Differences Between PanIN-3 and Noninvasive IPMC

Analysis of PanIN-3 and IPMC (with Dense Cytoplasms and Scarce Goblet Cells)

Both PanIN-3 and IPMC tumors are similar in terms of small papillary-cohesive clusters, small nuclei (about 10 μm at the shortest diameter) without prominent anisonucleosis (fig. 15a–e). As for nuclear crowding/overlapping, the nuclei of both tumors tend to exist in the well-preserved cytoplasm and to be separated from each other; the cytoplasmic borders between the nuclei can be clearly defined by focusing the microscope up and down (fig. 15a–f).

Differences between PanIN-3 and IPMC

Compactly packed clusters are often observed in PanIN-3 (fig. 15a, b), but are rare in IPMC. The cytoplasm is relatively abundant in most IPMC (fig. 15c, f),

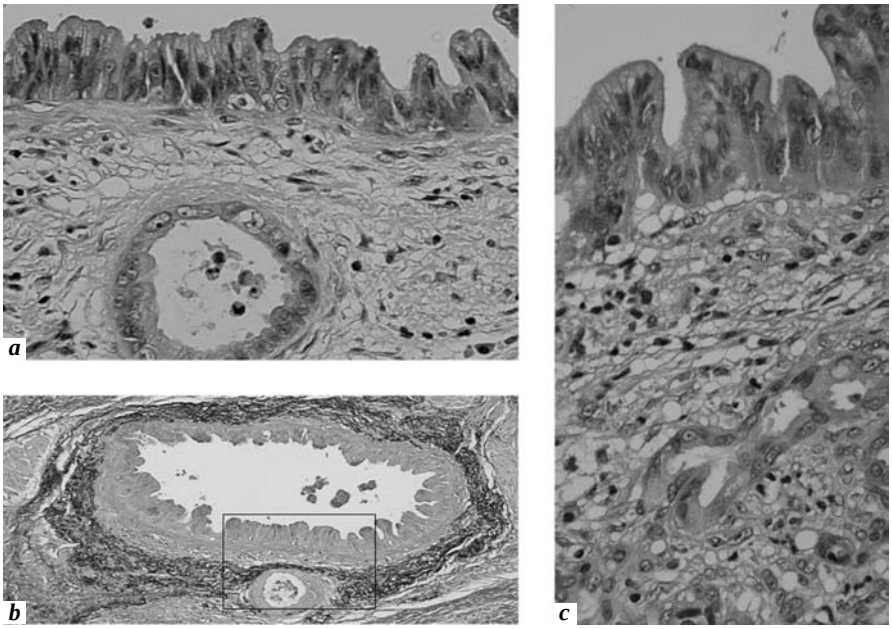

Fig. 14. IDAP with PanIN-3. Histology corresponds to figure 13. The upper half of (***a***–***c***) is PanIN-3. The epithelium has a different quality of cytoplasm to polyclonality and is similar to the upper portion of figure 13. The lower half of (***a***–***c***) shows a tubular pattern, composed of invasive components, and the quality of the cytoplasm is regular and monoclonal-like. HE. ×400. ***b*** is an elastica van Gieson stain corresponding with the hematoxylin-eosin stain in (***a***) and shows that PanIN-3 is surrounded by elastic fibers, while the invasive component is not. Elastica van Gieson stain. ×200.

but is small in most PanIN-3 (fig. 15a). The cytoplasm, including apparently abundant mucin, is large in IPMC (fig. 15c, e and f), but small in PanIN-3 (fig. 15a). Euchromatin is largest in IPMC (fig. 15c–f), but is small in PanIN-3. IPMC cells have mostly polyclonality (figs. 15d–f and 16d–f). Most cases with polyclonality progress to mucinous noncystic carcinoma later (figs. 15d, e and 16d, e). However, there are cases in which most cells have monoclonality (figs. 15c and 16c) and these progress to IDA later. PanIN-3 cells are mostly monoclonal (figs. 15a and 16a), but there was a rare case that showed polyclonality (figs. 15b and 16b) (mixture of dense cytoplasm and mucin-producing cytoplasm) but the tumor size was small (1.8 cm in diameter), and the prognosis was very good (there has been no recurrence for nine years since the operation). Consequently, the good prognosis is considered to be due to tumor size and/or polyclonality.

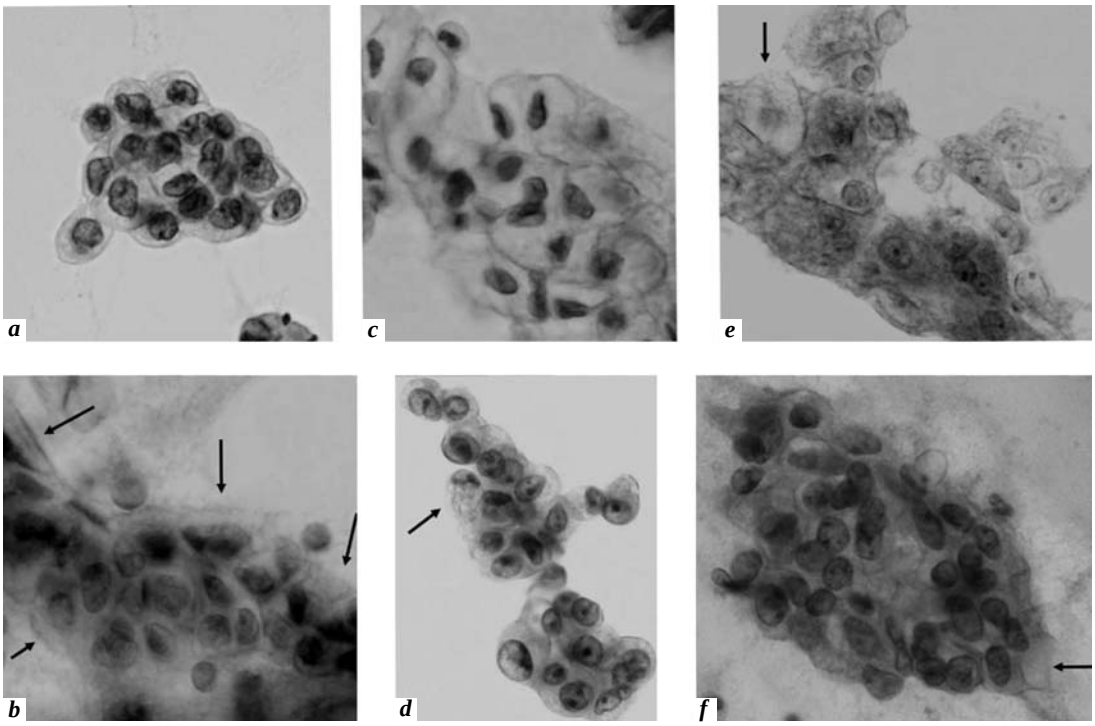

Fig. 15. PanIN-3 and noninvasive IPMC. ***a***, ***b*** are PanIN-3-type cells in IDAP cases. ***c***–***f*** are noninvasive IPMC-type cells. ***a***–***c***, ***e***: scrap smear, ***d***: vinyl tube aspiration of the main duct of the resected pancreas, ***f***: ERCP, ***a***–***f***: Papanicolaou stain. ×400. ***a*** is an ordinary type of PanIN-3 that has a bad prognosis. ***b*** is a rare case of PanIN-3 that has a good prognosis. ***c*** is noninvasive IPMC that is simultaneously accompanied by IDAP. ***d*** and ***e*** were only noninvasive IPMC at first surgery, but histology at re-operation after several years showed features of mucinous noncystic carcinoma. ***f*** Although the carcinoma was neglected for two years from the first surgery, the histology of re-operation remained noninvasive IPMC. ***a*** and ***c*** are monoclonal-like. ***b***, ***d***–***f*** are polyclonal-like (arrows show translucent/yellow mucin).

Clonality of Cytoplasm

As mentioned above, in IDAP cases, there is a rare case with a very good prognosis. In such IDAP cases, the columnar cells of PanIN-3 are peculiar in that the morphology of each cell is different (figs. 15b and 16b). These make the tendency towards polyclonality more prominent like the mixture of goblet-like cells in IPMC. The findings that the papillary epithelium of IPMNs is typically composed of various cytoplasms of tall columnar cells with a slightly basophilic dense cytoplasm, tall columnar cells with clear cytoplasm, or round-to-cuboidal cells with eosinophilic dense cytoplasm [12, 25] suggest polyclonality

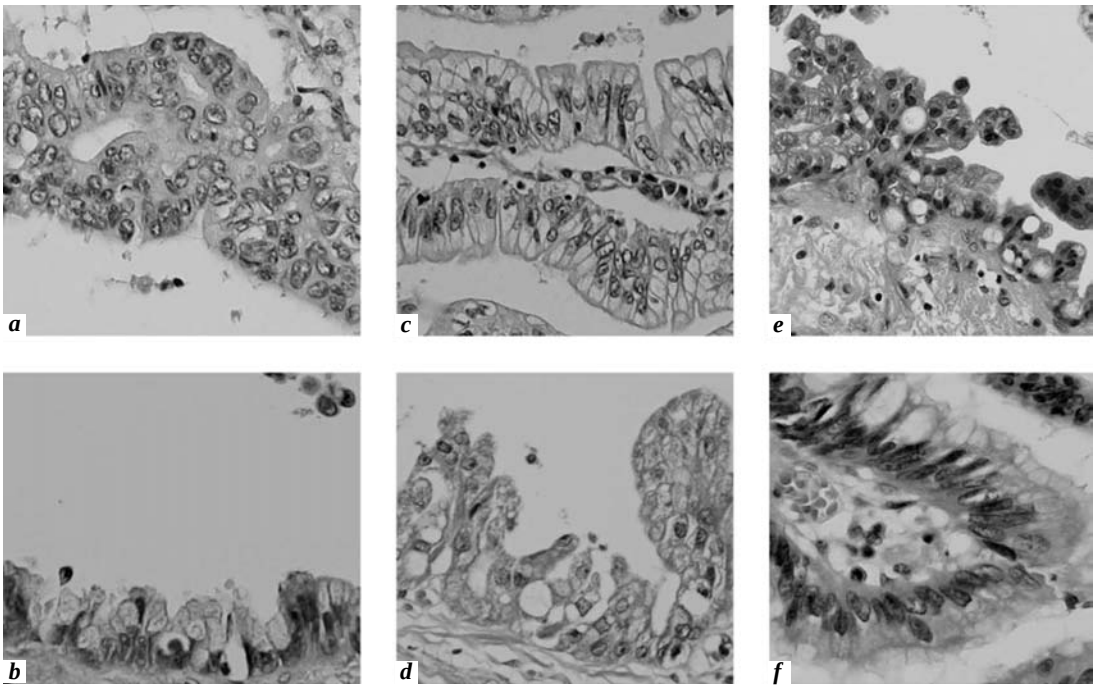

Fig. 16. PanIN-3 and noninvasive IPMC. Histology corresponds to each cytologic feature of noninvasive carcinoma in figure 15. HE ×200.

(figs. 15d–f and 16d–f). One of the important clinicopathologic and molecular features of IPMT is that multifocal occurrence of IPMTs has been observed in the same pancreas (9.8–32%) [54–56], there was genetic heterogeneity in an individual IPMN focus [57], and a hyperplasia-adenoma-carcinoma sequence in the evolution of IPMT has also been recognized [58–61]. Histomorphologically, IPMNs may have a variety of different cytoarchitectural features, even in different regions of a single neoplasm [40]. Comparative genetic studies of different regions of IPMN suggest that multiple clones may evolve independently [57, 62]. Namely, IPMN comes to have substantial allelic heterogeneity [57]. This marked heterogeneity may, in part, be due to the slow growth rate of these neoplasms [57]. In clonality analysis of IPMN [62], clonality of a single focus of the normal pancreatic duct/acinar epithelium is characteristic of the polyclonal pattern. In contrast, the clonality of a single focus of IDA cells is a monoclonal pattern. Although a single focus of IPMN (including IPMC) had both a polyclonal and monoclonal pattern, the monoclonal pattern was more pronounced than the polyclonal pattern. However, comparing different foci of a single neoplasm, the types of clonality are different. Consequently, on the

whole IPMN (including IPMC) becomes polyclonal [62]. Reviewing a table of clonality of a single IPMC focus, there is a single focus with polyclonal clonality [62]. On IPMC, that single focus has a polyclonal pattern suggesting that the focus retains the nature of normal epithelial cytoplasm. Consequently, despite malignancy, cases showing a polyclonal pattern may be determined to be IPMC. The cytoplasm of IDAP is different in size and shape, but assumes a monoclonal pattern, giving the impression that the product is produced homogeneously, artificially and uniformly (figs. 1c, 2c, 3c, 4c, 11c and 13c). However, the cytoplasm of PanIN-3 has a short diameter and is relatively clear at the border, but assumes a monoclonal aspect (upper portion of figs. 11a, 15a and 16a). In a rare case of PanIN-3, the size, height, shape and quality of the cytoplasms were slightly different. The cells seemed independent, although they gave a faint impression on high magnification. They assumed a polyclonal aspect impression, similar to plants that independently and separately result from the same kind of seeds (upper portion of figs. 13a, b, 15b and 16b).

Differences between PanIN-3 de novo and PanIN-3 in IDAP

PanIN-3 exists in the pancreatic ducts, while the invasive component exists in the stroma. Studies on p53 protein overexpression were done on both the infiltrating and intraductal carcinoma components [31, 63, 64]. These revealed that IDA has a tendency to spread intraductally [31]. However, studies with Ki67 [31], cell proliferative activity [65–67] and Dpc4 [68] suggest that the biologic behavior of intraductal spread carcinoma is suited to PanIN-3 de novo [31, 65–68]. Consequently, PanIN-3 in IDAP was the same as the intraductal spread of infiltrating carcinoma, but the biologic behavior suggested PanIN-3 de novo.

Significance of Identification of PanIN-3 Type Cells in IDAP

IDAP can spread through the ducts beyond the tumor mass, and such intraductal extension has been cited as a particular problem in determining the appropriate surgical resection margin [69]. A study on the extent of intraductal spread shows that has limited to 2.0 cm [31]. Consequently, detection of PanIN-3 type cells in IDAP decides the appropriate surgical resection margin. Prognostic factors include grade [1, 23, 31, 70], diameter (survival time is longer in patients with <3 cm) [1, 23, 71], site (carcinomas of the body or tail are more advanced) [1, 23, 72, 73], and stage (lymph node metastasis is worse) [1, 23, 71, 72]. Accordingly, if the size, site, stage and other factors are similar, IDAP cases with PanIN-3 will have a good outcome compared with IDAP cases without PanIN-3. This means that IDAP cases with PanIN-3 are useful when choosing treatment and determining prognosis.

Clinical Application at Diagnosis of PanIN-3

Invasion was found when group IV (corresponding to PanIN-3) epithelia spread 5–8 mm (rarely infiltrated when it spread <4 mm) [2]. Molecular studies revealed that PanIN-2 and PanIN-3 lesions represent a distinct step toward invasive carcinoma [74]. Thus, invasion begins at the occult stage nearby. However, the survival rate after complete resection at the PanIN-3 stage is very good [3]. Consequently, in order to improve prognosis in pancreatic carcinoma, diagnosis and resection at the PanIN-3 stage is necessary. Practically speaking, in PanIN-3, no pancreatic mass could be detected by traditional radiography, ultrasonography (US), endoscopic ultrasonography (EUS) or computed tomography (CT), nor was any ductal stenosis or obstruction detected by endoscopic retrograde pancreatography (ERP) [48]. Furthermore, the original, occult site cannot be visualized even during laparotomy [75]. Total pancreatectomy requires that patients manage diabetes for the rest of their lives [75]. With regard to searching for the original site of such an occult cancer, cytologic examination is very useful [49, 75–79]. To avoid blind total resection and leave a much greater volume and endocrine function, one institution precisely locates the original site by means of a Whipple procedure with the aid of intraoperative cytodiagnosis and then decides on the appropriate extent of pancreatectomy [49, 75–79]. Thus, concerning determining the stage of PanIN-3 and locating the original site, the present cytologic findings are useful.

Conclusion

PanIN-3-type cells form small papillary-cohesive and compactly packed clusters, have small nuclei and small to moderate cytoplasm, and clearly defined cytoplasmic borders, while invasive carcinoma component-type cells are loose and uncohesive and display a combination of hyperchromatin, large nuclei and abundant cytoplasm.

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Genetic Alterations in Pancreatic Cancer

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Abstract

Recently, a classification system for pancreatic intraepithelial neoplasia (PanIN) developed in 1999 was revised at a meeting of international experts on precursors of conventional pancreatic cancer. Many genetic alterations are shared by PanIN, invasive ductal carcinoma of the pancreas, and intraductal papillary-mucinous neoplasm including K-ras, p16, p53, and Smad4/DPC4 mutations. In this paper, genetic alterations in these tumors are discussed.

Pancreatic cancer (PC) is a mostly fatal disease that is now thought to be a genetic disease. Therefore, various research on PC and its precursors has been done. Recently, intraductal lesions associated with PC were characterized at not only the histological but also the molecular level [1, 2]. Klimstra DS and Longnecker DS proposed to group them under the genetic term pancreatic intraductal neoplasia [3]. A classification system for PanIN developed in 1999 was recently revised at a meeting of international experts on precursors of PC [4, 5]. It has been established that the most likely precursor of conventional PC is the high-grade PanIN (PanIN-3). PanIN-3 lesions are found in 30–59% of pancreata harboring invasive ductal carcinomas (IDCs) of the pancreas [4–7], and many genetic alterations are shared by PanIN and IDS including K-ras, p16, p53, and Smad4/DPC4 mutations [5–9]. Moreover, these genetic alterations are also shared by intraductal papillary-mucinous neoplasms (IPMNs) [10]. In this section, genetic alterations in IPMN, PanIN, and IDC are discussed (tables 1, 2).

Genetic Alteration in PanIN and IDC

In recent years, it has been established that the most likely precursor of ordinary IDC of the pancreas is the high-grade PanIN (PanIN-3) [11]. Some

Table 1. Genetic alterations in pancreatic carcinoma

Gene	Locus	Function	Frequency
Caretaker gene			
BRCA2	13q	DNA repair	7%
FANCC, FANCG	9q, 9p	DNA repair	<5%
Oncogenes			
k-ras	12	signal transduction	85%~95%
CCNE	19q	G1/S cell cycle transition	NA
Tumor-suppressor genes			
p16	9p	G1/S cell cycle arrest	98%
p53	17p	Cell cycle arrest, apoptosis	50%~75%
DPC4	18q	TGF-β/activin pathway	55%
LKB1/STK11	19p	Serine/threonine kinase	5%
TGF-β/activin receptor		TGF-β/activin pathway	5%
BAX	19q	Apoptosis	<5%
FBXW7	4q	G1 cell cycle transition	<5%

Table 2. Frequency of mutations in several of the major pancreatic cancer-associated genes

Type of cancer	K-ras (%)	Cyclin D1 (%)	p16 (%)	p53 (%)
Pancreas	~95	~95	~80	~40
Colon	~60	~60	~20	~60
Esophagus	~60	~40	~95	~60
Liver	~60	~20	~80	~60
Gallbladder	~60	~60	~80	~80

papers indicated that residual PanIN-3 after resection progresses to IDC some years later [12–14]. This evidence suggests that PanIN-3 may be one of the precursor lesions of conventional PC.

The best material for investigating true precursor lesions is an intraductal carcinoma with minimal invasion [15, 16]. However, we rarely encounter such carcinomas. Therefore, most immunohistochemical and molecular analyses on the carcinogenesis and histogenesis of IDC were done by investigating the tumor itself and/or intraductal lesions adjacent to IDC.

Yamano et al. used molecular biology to investigate both intraductal lesions adjacent to IDC and IDC with an excellent microdissection technique [17]. Their

loss of heterozygosity (LOH) analysis revealed that IDCs of the pancreas develop from hyperplasia (corresponding to PanIN-1) through severe ductal dysplasia (corresponding to PanIN-3) to invasive carcinoma by a progressive and divergent accumulation of genetic changes. The above reflect the spectrum of intraductal morphological alterations around invasive foci. LOH of 17p and 9p is observed at a high frequency in both invasive and severe ductal dysplastic foci, and a 9p loss may be the earliest event in the transition from hyperplasia to early dysplasia. Other chromosomal alterations including 18q and 6p follow later in the intraductal dysplastic and invasive stages and confer subclones with a further growth advantage.

Genetic Alteration in IPMN

IPMNs of the pancreas constitute a unique clinicopathological entity with an overall incidence of associated invasive malignancy of 20%. Loss of p16 and DPC4/Smad4 expression occur more frequently in intraductal papillary-mucinous carcinoma, or with associated invasive carcinoma, compared with adenoma/borderline IPMN. Aberrant protein expression of cell cycle regulatory genes p16, p21, p27, cycline D1, pRb, and p53 of IPMN in their development is similar to that of PanIN in the current progression model of PC, and may also represent the subsequent risk of invasive carcinoma [10]. In Japan, invasive carcinoma derived from IPMN was classified in the 5th edition of the general rules for the study of PC (in Japanese) and the first English edition of the classification of PC by the Japan Pancreas Society [18, 19]. Invasive carcinoma derived from IPMN with extrapancreatic expansion should be treated as conventional IDC because of its aggressive biological behavior. Some IPMNs are thought to progress to invasive carcinoma through different pathways from that of IDC [20]. The prognosis of invasive carcinoma derived from IPMN is still controversial, i.e. the prognosis in patients with invasive carcinoma derived from IPMN is thought to be as poor as in patients with conventional IDC [21], although it is also thought to be more favorable than in patients with conventional IDC [22].

Molecular Pathology

K-ras Oncogene

Oncogenes can be activated through various mechanisms, including a point mutation within a gene and amplification of the gene itself. Activating point mutations in codon 12 of the K-ras oncogene are among the most common genetic alterations identified to date in IDC [23]. These mutations can be found in 80–95% of PCs [24]. Activating point mutations in the K-ras gene occur early in

the development of a pancreatic neoplasia before an invasive cancer develops. Therefore, detection of a point mutation in the K-ras gene has the potential to detect curable, non-invasive pancreatic neoplasia before it progresses to incurable invasive cancer [25]. However, K-ras mutations are so common in normal, hyperplastic, metaplastic, and neoplastic ductal cells that this mutation may not cause, but only promote, mucinous differentiation. The prevalence of a certain mutation pattern in non-neoplastic and neoplastic ductal cells in an individual pancreas suggests the dominance of one of the carcinogenic factors [26].

Tumor-Suppressor Genes

In sporadic cancers, tumor-suppressor genes can be inactivated by (1) an intragenic mutation in one allele coupled with loss of the second allele, (2) deletion of both alleles (homozygous deletion), or (3) hypermethylation of the promoter of the gene associated with silencing of gene expression. In familiar cancers with germline mutations of one allele, the tumor suppressor gene can be inactivated by loss of the second allele.

The p16 gene on chromosome 9p is inactivated in 40% of pancreatic cancers by homozygous deletion, in another 40% by an intragenic mutation in one allele coupled with the second allele, and in 15% by silencing associated with hypermethylation of the gene's promoter [27]. The p16 functions are to regulate the cell cycle through the 16/Rb pathway. The inactivation of the p16 gene in almost all PCs means that a critical regulator of the cell cycle is lost.

The p53 tumor-suppressor gene on chromosome 17p is inactivated in 55–75% of PCs, almost always by an intragenic mutation in one allele coupled with loss of the second allele [28]. The p53 protein regulates the G1/S cell cycle checkpoint, maintenance of G2/M arrest, and the induction of apoptosis. The loss of p53 means loss of cell division and cell death in the majority of PCs.

The Smad4 (DPC4) gene on chromosome 18q is inactivated in 55% of PCs; in 35% by homozygous deletion and in 20% by an intragenic mutation coupled with loss of the remaining allele [29]. Smad4 plays a critical role in signaling through the transforming growth factor type B (TGF-β) pathway. The TGF-β pathway is activated by the TGF-β protein's binding to specific surface receptors. This triggers an intracellular cascade and results in the nuclear location of Smad4. Once in the nucleus, Smad4 has growth-controlling effects by regulating the expression of specific target genes.

Mismatch Repair Genes

Genome-maintenance genes repair damage to DNA. When a genome-maintenance gene is inactivated, DNA damage is not repaired efficiently and DNA mutations accumulate. Mutations occurring in cancer-associated genes can contribute to tumorigenesis.

The DNA mismatch repair genes MLH1 and MSH2 are examples of genome-maintenance genes targeted in pancreatic cancer [30, 31]. When one of these genes is inactivated, either by mutation or promoter hypermethylation, mutations accumulate in these repetitive tracts, producing DNA changes called microsatellite instability. Approximately 4% of pancreatic cancers have microsatellite instability and these cancers show a specific histology as poorly differentiated medullary carcinomas [30, 31].

The BRCA2 gene on chromosome 13q is also a genome-maintenance gene and it is targeted in a small percentage of PCs (<10%) [32]. However, germline (inherited) mutation in BRCA2 can cause the familial aggregation of PC, and carriers of this mutation have a 10-fold increased risk of developing PC, as well as an increased risk of developing breast, prostate, and ovarian cancers [33].

Telomere Shortening

Telomeres are caps at the ends of chromosomes that normally function to protect the terminal sequences and prevent the ends of chromosomes from joining aberrantly [34]. Telomeres are composed of short repeated DNA sequences and associated proteins. It appears that telomeres become abnormally short very early in the development of pancreatic neoplasia, in the non-invasive PanIN stage [35]. These shortened telomeres can presumably lead to the abnormal fusion of chromosome ends and ultimately to chromosome instability, promoting further neoplastic progression in these cells. When cells with critically short telomeres divide, the abnormally fused chromosomes will break, resulting in the gain of genetic material by some daughter cells and the loss of genetic material in other daughter cells. This process, called a breakage-fusion-bridge cycle, has been observed in PCs and is believed to be one of the major causes for the loss of tumor-suppressor genes and the gain of oncogenes.

Conclusion

In this section, genetic alterations in IPMN, PanIN, and IDC were discussed. PC is a mostly fatal disease that is now thought to be a genetic disease. Novel discoveries of genetic alterations in PC may play an important role in the prevention and early detection of small PC.

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Islets of Langerhans in Various States of Glucose Intolerance

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Abstract

Hyalinization of islets of Langerhans is generally a characteristic finding in non-insulin dependent diabetes mellitus. However, hyaline material/amyloid is sometimes found in elderly nondiabetic patients and also in patients with endstage renal failure on dialysis treatment and in patients after gastrectomy, which are known as states of impaired glucose tolerance. Glucose intolerance also occurs in patients with cirrhosis of the liver, showing enlarged/swollen islets of Langerhans accompanied with B cells, and decrease in insulin-secreting cells, and in patients with hemochromatosis which consists of hemosiderin deposition in B cells.

The islets of Langerhans of the pancreas are a representative component of the endocrine pancreas, related to glucose metabolism. Abnormal glucose metabolism, or impaired glucose tolerance, occur in various diseases.

Hyalinization of the islets of Langerhans is generally a characteristic feature of patients with type 2 (non-insulin dependent) diabetes mellitus. Prevalence of islet amyloid in type 2 diabetic patients ranges up to 87%, while in elderly non-diabetic subjects it ranges between 0 and 17% [1–4]. These hyalinized islets demonstrate amyloid with nonbranching, randomly distributed 100- to 120-Å thick fibrils and are immunostained positive for anti-amylin (or islet amyloid polypeptide), which is a major component of hyaline replacement [6, 7]. According to the studies in a group of spontaneously diabetic monkeys, amyloid development precedes glucose intolerance [5].

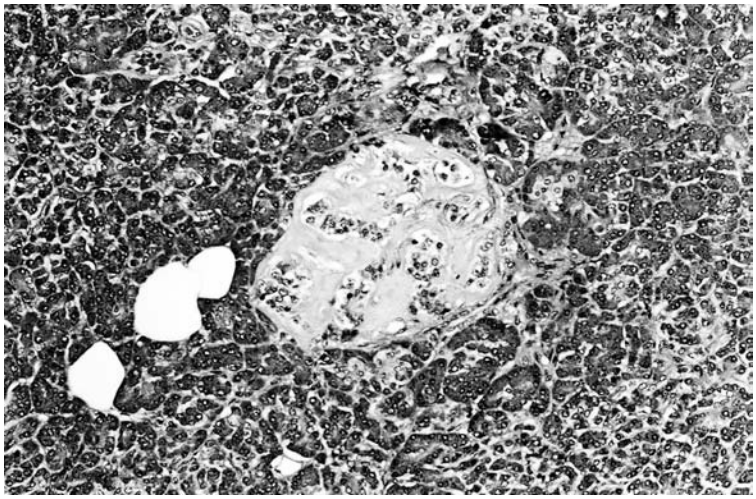


Fig. 1. Hyaline replacement in an islet of Langerhans in an autopsied endstage renal failure patient who had received dialysis treatment for 5 years. HE. ×200.

In this chapter, various diseases related to impaired glucose tolerance are described.

Hyaline Replacement/Amyloid of Islets of Langerhans

Hyaline material/amyloid of the islets of Langerhans is also found in patients with endstage renal failure (ESRF) on dialysis treatment [8, 9] and in patients after gastrectomy [10, 11].

In Patients with Endstage Renal Failure on Dialysis Treatment

With the advent of periodic dialysis, patients with chronic renal failure can be maintained in relatively good health over long periods of time. It is well known that these patients often develop infectious disease, pathological fractures of the bone, renal neoplasm, disturbance of lipid metabolism and entrapment neuropathy, as complications.

It is known that patients with renal failure are unable to adequately handle a glucose load [12] and are unable to augment insulin secretion sufficiently to overcome peripheral antagonism to insulin [13]. In patients receiving dialysis therapy, abnormal glucose tolerance is observed.

According to Suda and Ariwa [8], hyaline replacement of the islets of Langerhans is found in 6 ESRF patients who have received chronic dialysis for more than 2 years and 10 months (fig. 1), as shown in table 1. The percentage of

Table 1. Histopathologic findings of the islets of Langerhans in patients with endstage renal failure (ESRF) on dialysis treatment (10 cases) (8)

Case no.	Age years	Sex	Clinical diagnosis	Periods of hemodialysis	Islets of Langerhans*	Blood glucose level mg/dl	Urine glucose level
1	72	F	chronic nephritis	1 year, 1 month	degenerative	164	<0.25 g/dl
2	45	M	chronic nephritis	1 year, 6 months	degenerative		
3	70	F	chronic renal failure	2 years			
4	51	F	SLE	2 years, 2 months		160	
5	36	F	chronic nephritis	2 years, 10 months	hyaline replaced (44.2)	435	
6	62	M	gout	3 years, 4 months	hyaline replaced (3.4)		negative (2 years ago)
7	49	M	ESRF	5 years	hyaline replaced (14.6)		negative (5 years ago)
8	58	F	chronic nephritis	7 years	hyaline replaced (8.3)		negative (6 years ago) positive (2 years ago)
9	73	M	chronic nephritis	7 years	hyaline replaced (42.1)	186	
10	62	M	chronic nephritis, liver cirrhosis and hepatocellular carcinoma	10 years, 10 months	hyaline-replaced (11.4)	95	

*Figures in parentheses represent the percentage of involved islets.

islets of Langerhans involved by hyalinosis ranged between 3.4 and 44.2%. Clinically, although urine glucose is only positive in 1 of 6 patients with hyaline islets, the blood glucose level is moderately elevated in 2 other cases, and that finding is related to the hyaline replacement of the islets of Langerhans.

The hyaline-replaced islets were stained positively with Congo red and showed green fluorescence under polarized light. Electron microscopy revealed a fine fibrillar structure, 10–12 μm wide, with nonbranching fibrils running in different directions. Hyaline replacement of the islets of Langerhans stained positive for anti-amyline (or islet amyloid polypeptide).

The hyaline replacement of islets of Langerhans in patients with ESRF receiving chronic dialysis seems to be resulting from dialysis therapy. Avram [14] has an explanation for pancreatic abnormality in patients receiving dialysis therapy. Three reasons seem to be related to the change in the islets: (1) Based

on thickened arterioles in the pancreas as part of systolic blood vessel disease; (2) repeated volume contraction occurs during maintenance dialysis. In cases using a glucose-containing dialysate, there will usually be a net gain of glucose during dialysis, depending upon the relative glucose concentrations in serum and dialysate [15]; (3) with increased patient survival, islet changes may be a manifestation of the natural progression of ESRF. According to de Koning et al. [9], amyloid deposits were found in 8 of 23 non-diabetic patients with ESRF (35%) on chronic dialysis treatment, which was higher than the prevalence in non-diabetic control subjects (3%) ($p < 0.01$). There was no relationship was found between the duration of dialysis treatment and the presence of islet amyloid deposits derived from insulin amyloid polypeptide (IAPP), a 37-amino-acid peptide, which is the main proteinaceous component of islet amyloid and is localized in B cell secretory granules [16, 17]. This suggests that long-term exposure to high plasma concentrations of IAPP is not the only factor for islet amyloidosis in patients with ESRF: insulin resistance, the associated B cell hypersecretion of insulin and IAPP are likely to play a more important role in amyloid deposition. Several authors [18, 19] report a high prevalence of amyloid deposition in osteoarticular tissues in patients with ESRF on chronic dialysis treatment. Patients with ESRF on dialysis treatment also have increased concentrations (up to 20 times) of the amyloidogenic protein beta 2-microglobulin [20], which are the major constituent fibrils in dialysis-related amyloidosis. However, plasma concentrations of IAPP have been shown to be elevated in patients with ESRF compared with subjects without renal disease [21, 22]. Impaired renal clearance is an important determining factor for high concentrations of circulating IAPP found in patients with end-stage renal disease.

Therefore, the hyalinosis of the islets of Langerhans seems to be either a complication of dialysis itself or probably the result of long-term endstage renal failure.

In Patients after Gastrectomy

After gastrectomy, patients often experience such complications as the dumping syndrome, gallstones, and malabsorption. These patients cannot adequately process a glucose load [23]; indeed, gastric surgery is a well-recognized cause of impaired glucose tolerance [24]. Alterations in glucose metabolism after partial gastrectomy, with a rapid increase and high peak concentration of glucose, are due in large measure to an increased rate of gastric emptying and, consequently, to an increased rate of intestinal absorption [25].

According to Suda et al. [10], in all but two patients in the Billroth (B) groups shown in tables 2 and 3, fewer than 10% of the islets show hyalinization (fig. 2). Such a small reduction in the percentage of functioning islets may seem to bear no relation to the significant impairment of insulin production. However,

Table 2. Histopathologic findings of islets of Langerhans in post-gastrectomy patients who underwent Billroth's I anastomosis (10)

Case	Age years	Sex	Periods after gastrectomy	Islets of Langerhans*	Blood glucose level mg/dl
1	88	M	2 years, 6 months	Hyaline replaced (0.9%)	115
2	74	F	10 years	NA	72–121
3	78	M	10 years	Hyaline replaced (1.2%)	122–279
4	81	F	12 years	Hyaline replaced (1.2%)	139
5	87	M	12 years	Hyaline replaced (2.2%)	95–123
6	79	M	15 years	NA	88–106
7	58	M	15 years	Hyaline replaced (0.5%)	83–143
8	54	M	15 years	Hyaline replaced (2%)	62
9	77	M	18 years	NA	–
10	78	M	19 years	Hyaline replaced (5.7%)	102
11	87	M	20 years	Hyaline replaced (6.8%)	–
12	77	F	20 years	NA	–
13	81	F	24 years	NA	98
14	85	M	28 years	NA	103–160
15	69	M	34 years	Hyaline replaced (14.3%)	160

*Figures in parenthesis represent the percentage of involved islets.
NA = Not applicable.

blood glucose levels were mildly elevated in three patients with B-I who showed hyalinization in the islets of Langerhans, and one patient with B-II showed an elevated glucose level. Thus such a small reduction in the percentage of functioning islets may reflect mild, but not significant, impairment of insulin production.

Although reasons for the occurrence of hyaline replacement of the islets of Langerhans in these persons after gastrectomy remain obscure and data on carbohydrate metabolism in these persons remain scanty, hyalinization in the islets of Langerhans is obvious [10, 11].

In animals, the vagus nerve is involved in insulin secretion [26, 27]; because vagotomy is performed in most gastric operations, this may contribute to postprandial hyperglycemia. Vagotomy may alter the release of gastrointestinal peptides from the mucosa of the small intestine, which, in turn, affects absorption and glucose tolerance [24].

After total or partial gastrectomy, dumping syndrome followed by secondary hypoglycemia may occur. Early dumping syndrome is due to the rapid passage of ingested food, which causes distension of the jejunum. The rapid absorption of carbohydrate produces marked hyperglycemia, an excessive stimulus [28] that causes the islets to became hyperactive, and this, finally, results in their hyalinization.

Table 3. Histopathologic findings of islets of Langerhans in post-gastrectomy patients who underwent Billroth's II anastomosis (10)

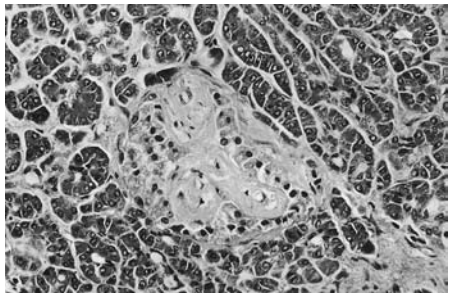
Case	Age years	Sex	Period after gastrectomy	Islets of Langerhans*	Blood glucose level mg/dl
1	77	M	3 years, 8 months	Hyaline replaced (11.6%)	100
2	63	M	8 years, 6 months	Hyaline replaced (0.2%)	–
3	84	M	10 years	NA	–
4	71	F	16 years	NA	–
5	61	M	18 years	NA	87–99
6	76	M	20 years	Hyaline replaced (6.3%)	–
7	52	M	21 years	Hyaline replaced (1%)	102
8	71	M	21 years	Hyaline replaced (0.8%)	90
9	83	F	25 years	Hyaline replaced (2.5%)	131–182
10	56	M	26 years	NA	89
11	69	M	29 years	NA	–
12	58	M	29 years	Hyaline replaced (7.1%)	112
13	80	M	30 years	Hyaline replaced (3.6%)	–
14	54	M	30 years	NA	–
15	61	M	30 years	NA	144
16	78	F	40 years	NA	–

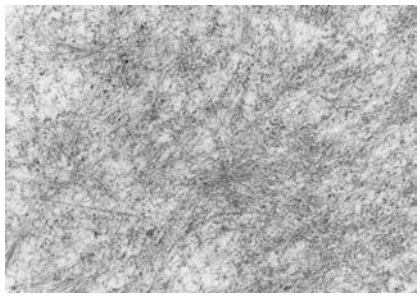
*Figures in parenthesis represent the percentage of involved islets.
NA = Not applicable.

Hence, hyperplasia of the islets of Langerhans and increased numbers of endocrine cells were seen during the early period after gastrectomy. In contrast, the islets of Langerhans showed atrophy and decreased numbers of endocrine cells more than 5 years after gastrectomy. These changes suggested that B cells in the islets of Langerhans subsequently became wasted and atrophic after hypersecretion of insulin [11].

Enlargement of Islets of Langerhans in Patients with Cirrhosis of the Liver

In patients with cirrhosis of the liver (LC), it is known that glucose intolerance [29, 30] and pancreatic change related to portal hypertension [31] occur clinically. Pathologically, there are pancreatic fibrosis [32] and enlargements of islets [33, 34] (fig. 3). However, the relationship between glucose intolerance and the enlarged islets is not known. In 17 LC patients including any types/causes, an area of the islets was statistically enlarged in 14, compared with that of controls as shown in table 4. Such findings were previously





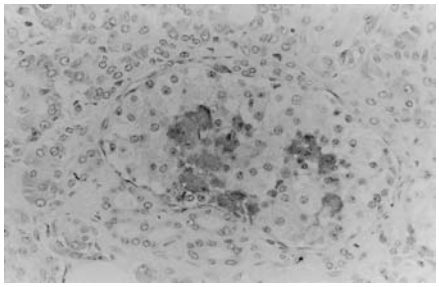
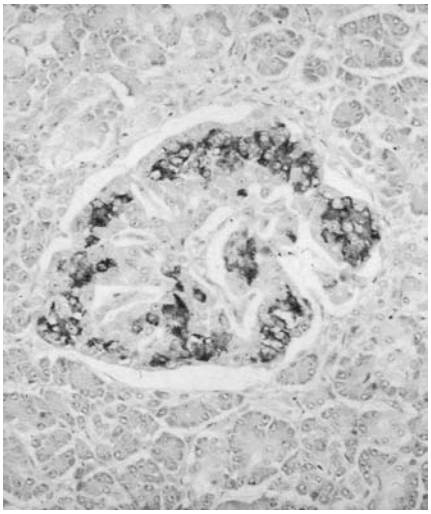


Fig. 2. Islet changes in post-gastrectomy patients. ***a*** Hyaline replacement in an islet of Langerhans. HE. ×200 (From [10] with permission). ***b*** Amyloid with nonbranching, randomly distributed 100- to 120-Å thick fibrils in the islets of Langerhans. Uranyl acetate-lead nitrate. ×72,000. ***c*** Amorphous amyloid deposits were strongly immunostained with anti-amylin. ×320.



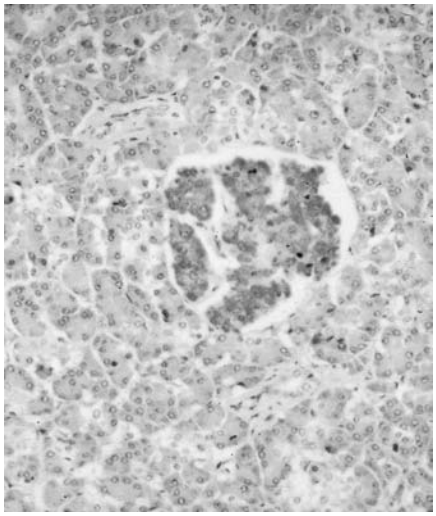


Fig. 3. The islet of Langerhans in a patient with cirrhosis of the liver. The islet was swollen and the number of B cells were decreased (***a***), compared to those in the control (***b***). Anti-insulin immunostain. ×200.

Table 4. Areas of islets, B cells and acinar cells in patients with cirrhosis of liver (LC)

Case*	Blood glucose mg/dl	Islets (mean ± SD) μm^2	B cells (mean ± SD) %	Acinar cells (mean ± SD), μm^2
Control**		1,5701.0 ± 11,396.7	41.8 ± 11.3	743.8 ± 904.4
1	92	27,998.5 ± 14,821.4[a]	54.6 ± 7.5[a]	1,267.1 ± 1,257.1[c]
2	95	28,186.8 ± 11,451.8[a]	42.2 ± 6.2	894.0 ± 748.4
3	86	22,661.6 ± 14,337.0[a]	40.0 ± 8.8	1,466.8 ± 1,137.0[c]
4	90	38,585.5 ± 18,384.8[a]	39.7 ± 10.8	2,290.1 ± 2,124.1[c]
5	101	33,253.0 ± 29,000.4[a]	37.2 ± 10.3[b]	1,212.4 ± 1,622.2[c]
6	235	29,763.7 ± 15,304.9[a]	38.0 ± 8.7[b]	1,058.1 ± 1,031.3[c]
7	…	22,236.5 ± 23,544.6[a]	33.8 ± 9.1[b]	1,753.6 ± 1,466.8[c]
8	100	54,058.0 ± 34,343.2[a]	33.3 ± 6.9[b]	1,589.4 ± 1,157.9[c]
9	97	24,238.5 ± 10,688.3[a]	32.0 ± 9.5[b]	1,960.6 ± 1,703.6[c]
10	…	32,877.1 ± 13,508.4[a]	28.6 ± 7.3[b]	1,005.9 ± 1,121.0[c]
11	228	15,750.6 ± 12,111.7	26.5 ± 7.9[b]	1,667.4 ± 1,978.9[c]
12	215	55,683.6 ± 34,922.4	26.1 ± 13.5[b]	1,272.2 ± 1,438.4[c]
13	325	47,425.9 ± 30,621.3[a]	10.0 ± 5.7[b]	1,273.2 ± 1,747.1[c]
14	…	36,529.4 ± 17,233.2[a]	11.2 ± 4.3[b]	1,625.7 ± 1,252.6[c]
15	91	30,756.1 ± 17,301.3[a]	34.0 ± 16.2[b]	1,849.8 ± 1,555.0[c]
16	…	15,402.6 ± 6,391.2	23.7 ± 6.7[b]	1,876.0 ± 1,953.0[c]
17	…	20,569.0 ± 10,241.0[a]	19.6 ± 5.3[b]	1,389.1 ± 1,239.8[c]

*Posthepatitic LC (cases 1–13); postnecrotic LC (case 14); primary biliary cirrhosis (case 15); Alcoholic LC (cases 16 and 17).
**Mean of 4 cases [a]significantly increased [b]significantly decreased [c]significantly increased.

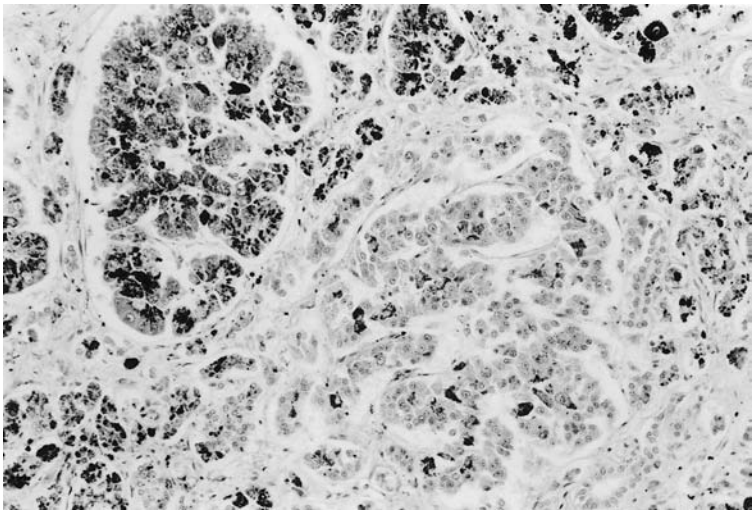


Fig. 4. Pancreatic tissue in a patient with hemochromatosis. Marked hemosiderin deposition in the acinar tissue as well as in B cells of islets. Berlin blue stain. ×100.

described in some chapters [33, 34]. However, the B cell area ratio was decreased in 13 and increased in one. Hence, enlarged islets with B cell decrease may play an additional role in the pathogenesis of glucose intolerance.

Hemosiderin Deposition in the Islets of Langerhans

Hemosiderin deposition has been well investigated by many authors, such as in cases of diabetes mellitus, which is usually related to a deposition of hemosiderin in the B cells of the islets of Langerhans [35]. Hemosiderin deposition occurs predominantly in the acinar tissue in cases of primary hemochromatosis (fig. 4) and in the periinsular acinar tissue in patients who receive a large volume of blood through transfusions [36, 37]. In the latter, the distribution and amount of hemosiderin increase in accordance with the volume of blood given. According to Rahier et al. [36], the histological appearance of the islets was normal as HE staining, their shape and size being unchanged; amyloid deposits were absent, as were atrophic islets. A severe reduction in the number of immunoreactive B cells was noted in the four diabetic patients. Under the electron microscope, iron deposits were restricted to B cells and associated with progressive loss of endocrine granules.

Hence, glucose intolerance is related to selective deposition of hemosiderin in B cells of the pancreas.

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Solid Pseudopapillary Tumor of the Pancreas: Report of Four Cases

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Abstract

Solid pseudopapillary tumor of the pancreas (SPTP) is a rare neoplasia of uncertain histogenesis, which usually occur in young females and is regarded as a tumor of low malignancy potential. We describe the histopathological features of four cases of this tumor and review the literature. All patients were female with ages ranging from 18 to 60 years (mean 35.2 years). Grossly, the tumors appeared as a solid cystic mass, frequently with hemorrhagic and necrotic areas; however, with a predominantly cystic formation in 1 case and of non-cystic type in 1 case. The histological findings of the tumor cells were relatively uniform, showing solid and pseudopapillary or pseudoglandular proliferation and having few mitotic figures or nuclear pleomorphism. On immunohistochemical examination, all cases were similar to those previously reported as SPTP. In addition, all tumors showed low a Ki-67 index (<1.0% of all tumor cells). One of four cases proved to be metastatic to lymph nodes. Despite metastasis, the proliferating ability of the tumor cells was relatively low and this patient is alive with no evidence of recurrence since surgery. SPTPs have distinctive morphologic and biologic features; therefore, it may be difficult to evaluate the malignancy potential by histological and immunohistochemical examination.

Solid pseudopapillary tumor of the pancreas (SPTP) is a rare form of pancreatic neoplasm, usually occurring in young females. With improvements of ultrasonography (US) and computed tomography (CT), the frequency of detection of this tumor is nowadays increasing. However, its etiology and pathogenesis are still unknown. Although SPTP shows relatively low aggressive behavior and a good prognosis, local recurrences and even metastases have been reported in a few cases. Therefore, this tumor is regarded as a neoplasia of low malignancy

potential. We present here four cases of SPTP, including a case with lymph node metastasis, and review previously reported cases in order to clarify the pathological concept of this tumor.

Case Reports

Case 1

A 49-year-old female presented to her family doctor having suffered from lumbar pain. She had an abnormal shadow by abdominal X-ray, but had left it unattended. She was admitted to our hospital because of the tumor shadow being found again by a routine medical check. Abdominal US and CT scan revealed a ping-pong-ball sized tumor in the pancreatic tail. A distal pancreatectomy was performed. The resected tumor measured $6 \times 6 \times 3.7$ cm and the cut surface showed a yellowish-white solid tumor forming focally cystic changes with marked calcification and ossification (fig. 1). The tumor invaded into the capsule in the anterior region of the pancreas, but not into the surrounding pancreatic tissue. Histologically, the tumor was composed of small round cells with pseudopapillary structures and a microcystic pattern with mucinous material.

Case 2

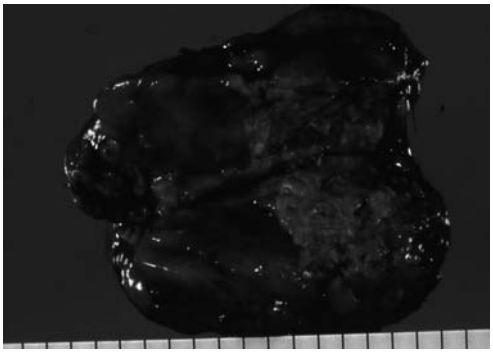
A 12-year-old girl was admitted to a hospital with a chief complaint of abdominal pain and nausea. Abdominal X-ray and US of the abdomen revealed a tumor of the pancreatic tail. A distal pancreatectomy and splenectomy was performed. The resected tumor measuring $5 \times 4 \times 4$ cm was elastically hard and had a thick fibrous capsule. The cut surface showed a solid white tumor with hemorrhagic cyst. Histologically, the tumor was composed of solid sheets of round-to-ovoid cells with solid and pseudopapillary structures, and focally pseudorosettes. Focally, a few mitotic figures were observed. In the solid areas, some tumor cells had PAS-positive globules, and these also were found in the imprint cytological specimens of the tumor (fig. 2). Cholesterol granuloma and calcification were seen in the cystic area. The tumor extended directly into the surrounding pancreatic parenchyma with capsular penetration. Moreover, metastases to the parapancreatic and omental lymph nodes were found (fig. 3).

Case 3

A 20-year-old female presented to her family doctor complaining of abdominal pain. Abdominal US and a CT scan confirmed the finding of a cystic lesion in the pancreatic head, suggestive of cystadenoma. An enucleation of the tumor was performed. The tumor measuring $7 \times 7 \times 3$ cm revealed a well-circumscribed unilocular cyst with a thin fibrous capsule, and the cyst contained dark red material. The inner surface of the cyst wall was covered by papillary tumor cells. Histologically, on the cystic lumen, the oval-shaped tumor cells were arranged around a fibrovascular stalk with a pseudopapillary pattern (fig. 4).

Case 4

A 62-year-old female with no significant previous medical history was admitted to hospital because of abdominal discomfort and mass. Abdominal US and a CT scan revealed a protruded solid tumor arising from the pancreatic body. Complete excision of the tumor was performed. The



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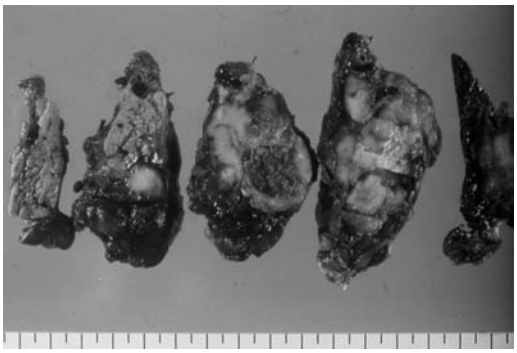

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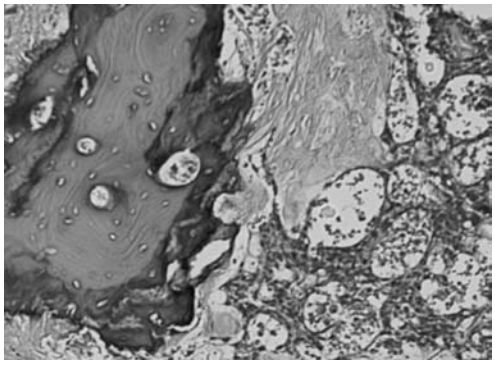

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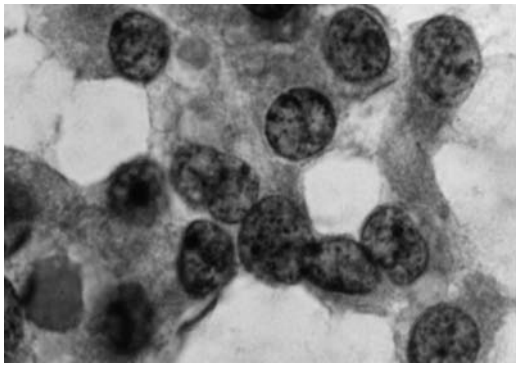

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Fig. 1. Case 1. ***a*** Cut surface of the tumor reveals solid, cystic, and hemonecrotic areas. ***b*** Histological feature of the tumor is composed of solid nests with marked calcification and ossification. HE. ×10.

Fig. 2. Case 2. ***a*** Cut surface shows solid and cystic areas with necrosis. ***b*** In imprint cytological finding, the tumor cells have round nuclei with hyaline globules. Papanicolaou stain. ×1,000.

tumor was well-encapsulated and measured 8 × 7 × 5 cm. The cut surface was solid without cystic formation. Histologically, the tumor was composed of ovoid cells with mild nuclear atypia and a few multinucleated giant cells. However, mitotic figures were very rare (fig. 5).

The postoperative course of all patients was uneventful and no recurrence was observed.

Pathological Features

Grossly, the SPTPs were relatively well-circumscribed tumors and the cut surface had mainly solid and cystic degenerative areas filled with hemonecrotic material, but the tumor in case 3 showed a predominance of cystic formation and in case 4 a solid appearance.

Histologically, the tumors were composed of three architectural patterns in various proportions, namely solid, pseudopapillary and cystic. The tumor cells of the solid area were uniformly medium-sized, and round to polygonal in shape, arranged in sheets with microcystic formation. The cytoplasm was clear or slightly eosinophilic, and occasionally contained small vacuoles. PAS-positive hyaline globules were present within and between the tumor cells of cases 1 and 2. A few mitotic figures were detected in case 2 and mild nuclear pleomorphism was seen in case 4. The stroma between the tumor nests had hyaline fibrous bands with scattered calcification (cases 1–4) and ossification (case 1). In the necrotic and hemorrhagic areas, cholesterol clefts surrounded by foreign body cells were seen. In case 2, the tumor invaded into the adjacent pancreatic parenchyma and lymph node metastasis was detected.

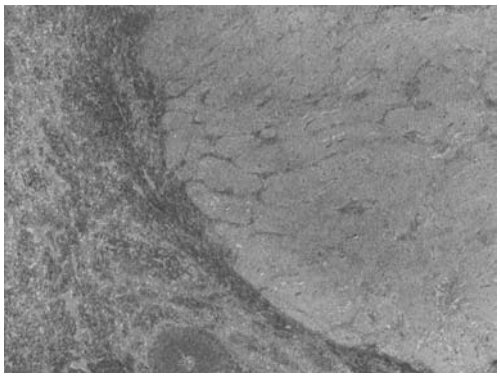
Immunohistochemically, the tumor cells were diffusely positive for vimentin, and in case 1 focally positive for α-1-antitrypsin (AAT) and neuron-specific enolase (NSE). They were positive for vimentin, AAT, and NSE in cases 2 and 3 (fig. 6). In case 4, the tumor cells were diffusely positive for AAT and NSE, and focally positive for vimentin. In addition, the tumor cells were reactive for cytokeratin (AE1/AE3) in cases 3 and 4, and synaptophysin in cases 2–4. CD10 was positive for some tumor cells in cases 2 and 3. Progesterone receptors(PgR) was positive for several tumor cells in cases 1 and 2.

Ultrastructural studies were performed in cases 1, 2 and 4. The tumor cells were connected by junctional complexes and had focally irregular microvilli on the plasma membrane. The nuclei were slightly irregular and had small nucleoli. The cytoplasm contained relatively abundant rough endoplasmic reticulum and mitochondria. In some tumor cells of cases 2 and 4, the cytoplasm contained zymogen-like granules of various sizes (range 200–2,000 nm) and annulate lamellae (fig. 7). Also, there were a few neurosecretory granules in case 2. In case 1, zymogen-like granules could not be identified, but annulate lamellae were seen.

Flow cytometric analysis revealed hyperdiploid with a DNA index of 1.13 in case 3 without metastasis, but case 2 with lymph node metastasis was diploid. Immunohistochemical study using an antibody of Ki-67, a marker of cellular proliferation, showed a low index of tumor cells (<1.0%) for all cases, even in case 2 with lymph node metastasis.

Discussion

In 1959, Frantz [1] first designated this tumor as ‘papillary tumor of the pancreas-benign or malignant?’. In 1981, Klöppel et al. [2] described it as solid cystic acinar cell tumor because of its pathological features, and it had become



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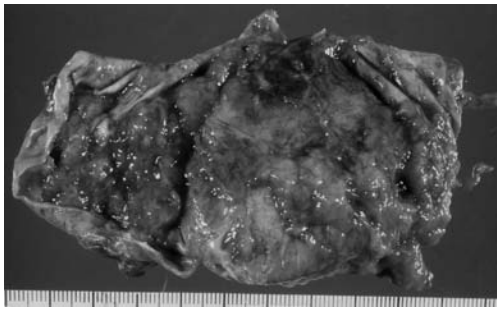

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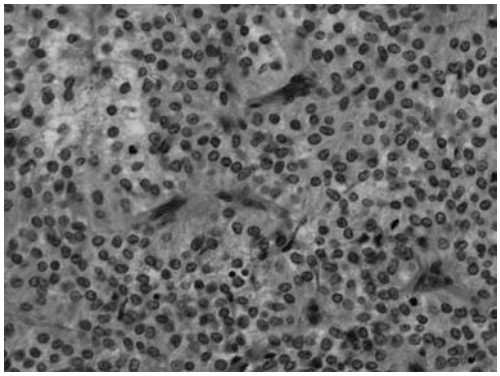

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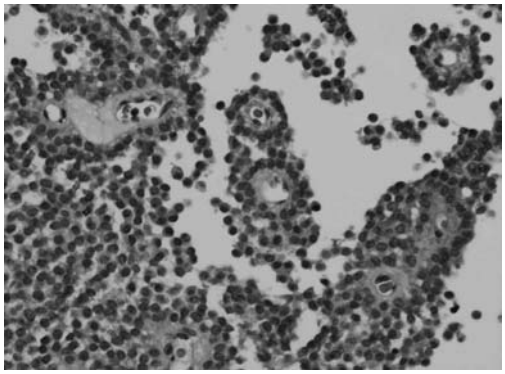

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Fig. 3. Case 2. ***a*** A metastatic tumor is seen in the parapancreatic lymph node. HE. ×1.25. ***b*** The metastatic tumor cells have the same histology as those in the primary tumor, but have a few mitotic figures. HE. ×10

Fig. 4. Case 3. ***a*** The tumor reveals prominent cystic formation with low papillary lesions on the inner surface. ***b*** The tumor displays a pseudopapillary structure with fibrovascular cores. HE. ×10.

a well-known tumor. Since then, the same tumor has been given a variety of names, including solid and cystic tumor, papillary-cystic tumor, and solid and papillary epithelial neoplasm. In 1996, the World Health Organization (WHO) reclassified pancreatic tumors, and named this tumor solid-psuedopapillary tumor [3].

SPTP is a relatively rare tumor that has been reported to account for approximately 1–2% of all exocrine pancreatic tumors [4], but recently the rate has increased. To date, more than 700 cases have been reported in English-language medical literature [5], whereas almost 300 cases have been reviewed in Japanese literature. Kosmahl et al. [6] reported that of 1454 pancreatic

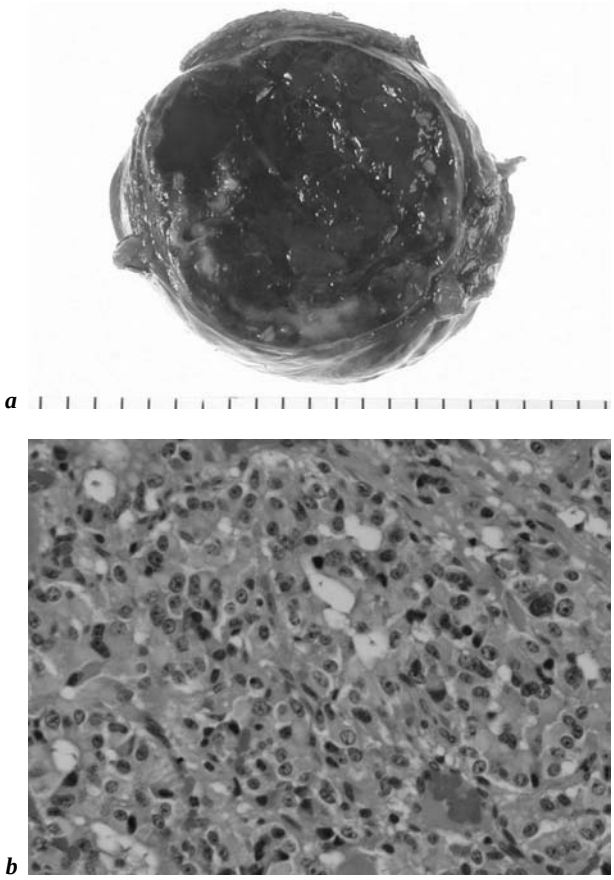

Fig. 5. Case 4. ***a*** Cut surface shows grayish-white solid tumor with hemorrhage. ***b*** The tumor is composed of ovoid cells with mild nuclear pleomorphism. HE. ×10.

tumorous lesions they examined, 418 cases (29%) showed cystic formation, and the most numerous tumors among these cases were SPTPs (89 cases; 21.9%). The mean age of the patients was 30 years (range 11–73) and 87% were females. According to Yoshioka et al. [7], a series of 302 cases of SPTP reported during a 21-year period from 1979 to 1999 in Japan included 262 females (87%) and 40 males (13%) with a median age of 30 years (range 7–79). Based on 292 cases in a cumulative review of the world literature, Mao et al. [8] reported that 90% of patients were females with a mean age of 23.9 years. As mentioned above, most patients are young females, and development of this tumor thus suggests that hormonal [9] and genetic factors may play an important role.

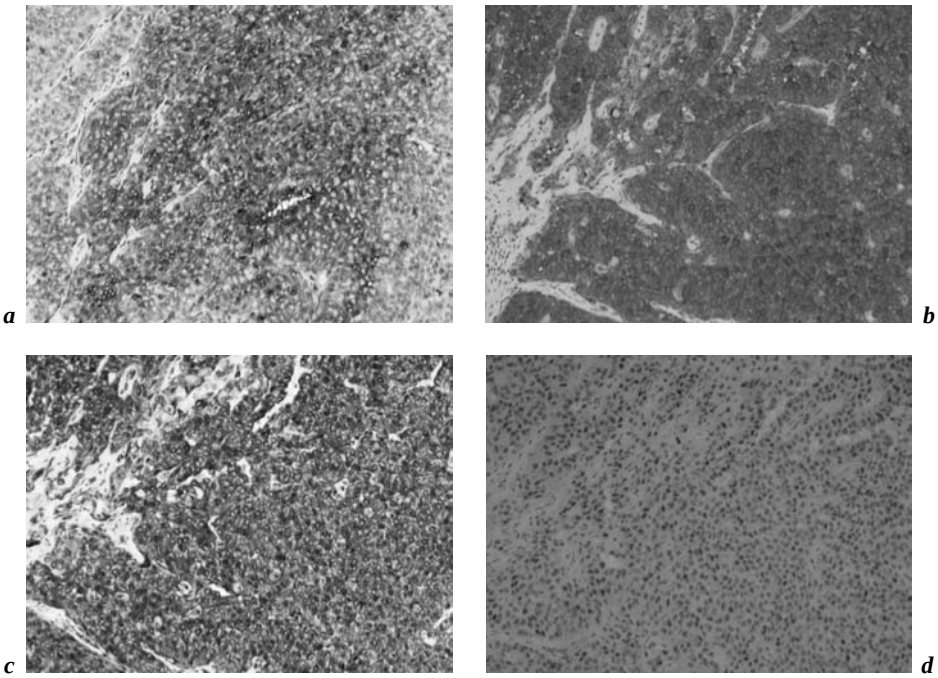

Fig. 6. Case 2. Immunohistochemical findings are positive for (***a***) AAT, (***b***) NSE, (***c***) vimentin, and (***d***) PgR. ×10.

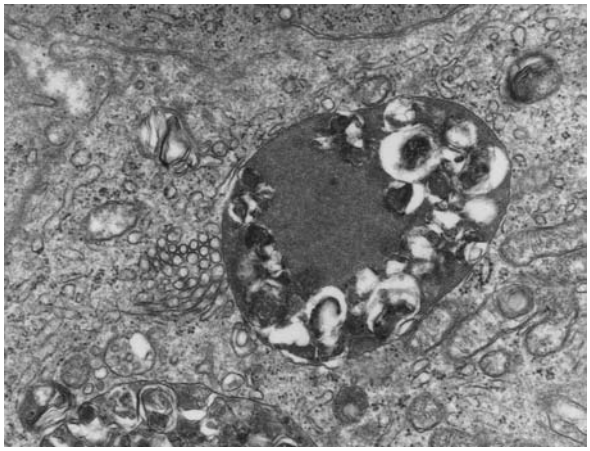

Fig. 7. Ultrastructurally, the tumor cells contain zymogen-like secretory granules and annulate lamellae (×20,000).

In general, the gross appearance of this tumor shows cystic change with a solid component. However, of 173 cases of classic SPTPs reported in Japan, 22 cases were noncystic tumors, and reports of this type of tumor have been recently increasing [10]. Histologically, SPTP is characterized by solid and pseudopapillary patterns which are composed of relatively small uniform tumor cells with clear cytoplasm. The tumor cells have little or no nuclear pleomorphism and mitotic figures are usually rare. Immunohistochemically, this tumor has a wide spectrum, showing different findings for epithelial, mesenchymal and endocrine markers [4]. According to a detailed Immunohistochemical study of 59 SPTPs by Kosmahl et al. [11], immunoreactivity for vimentin, AAT, NSE, and the progesterone receptor is each found in more than 90% of the tumors, whereas cytokeratin is found in almost 70%, and synaptophysin in 22%. Ultrastructural characteristic findings in SPTP are the presence of zymogen-like granules of various diameters or resembling endocrine granules, and annulate lamellae in the cytoplasm [2, 4]. As shown above, SPTP has immunohistochemical and ultrastructural features of both acinar-ductal and endocrine cells. These distinctive findings may be useful for the diagnosis of SPTP.

Despite many studies with immunohistochemistry and electron microscopy, the origin of tumor cells in SPTP is still undetermined. In the general rules for surgical and pathological studies on cancer of the pancreas in Japan [12], this tumor is categorized as an epithelial tumor of uncertain differentiation. Formerly, the origin of SPTP was considered to be acinar cells, because of positivity for AAT in immunohistochemistry and zymogen-like granules in electron microscopy [2]. Moreover, a neuroendocrine cell origin has been suggested by positivity for NSE and synaptophysin. Notohara et al. [13] reported that the tumor cells indicate, at least focally, neuroendocrine differentiation by CD10 and CD56 expression. However, it has now been suggested that SPTP derive from a totipotential stem cell which might be able to develop into all types of ductal, acinar or neuroendocrine cells, because results of immunohistochemical studies are not uniform. In addition, annulate lamellae, which are often ultrastructurally observed in this tumor, are considered to be a marker of immature cells because of their common appearance in germ cells. Therefore, some investigators support this hypothesis [8, 14]. As another unique hypothesis, Kosmahl et al. [11] recently proposed that this tumor might derive from ridge/ovarian anlage-related cells which were incorporated into the pancreas during early embryogenesis.

In general, patients with SPTP show a good prognosis after complete resection. However, approximately 10% of tumors are clinically malignant [6] and metastases have been reported in a few cases; tumor extension into surrounding organs, vessel invasion, local recurrence, and distant metastases have been documented [5–7, 13, 14]. The most common sites of distant metastasis

are the liver, lymph nodes and peritoneum. According to Papavramidis et al. [5], of 497 PSTPs reported in the English-language literature, there were 97 cases of metastatic or invasive diseases, including liver (27 cases), portal vein (26 cases), spleen (17 cases) and other organs (duodenum, omentum, colon, etc.). Cappellari et al. [15] noted that the metastatic tumor cells showed bizarre giant cells and more increased nuclear pleomorphism and mitotic rate, although the primary tumor had the typical histology of SPTP. Moreover, Nishihara et al. [16] reported that venous invasion, nuclear grade, and prominent necrobiotic nests were important parameters of the malignancy potential of this tumor. However, in our case 2, there was histologically no difference between the primary tumor and the metastatic tumor in lymph node. Therefore, in many cases, it may be difficult to evaluate the malignancy potential of this tumor. According to several studies differentiating benign from malignant tumors by using DNA flow cytometry [15, 16], a few cases of PSTP with metastases revealed patterns of aneudiploidy. In addition, the proliferative fraction determined by Ki-67 immunostaining usually shows low indexes in both benign and malignant cases. However, Tang et al. [17] recently reported that in two rapidly fatal cases of SPTP with marked nuclear atypia and high mitotic rate, the Ki-67 labeling indexes were 30 and 40%, although the mean Ki-67 labeling in 34 conventional cases was $<1\%$.

To the surgical pathologist, SPTPs might be still considered as an enigma. Further studies will be required to elucidate their nature and biological behavior.

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Author Index

Subject Index