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Duct changes and K-*ras* mutations in the disease-free pancreas: analysis of type, age relation and spatial distribution

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Abstract Recent molecular studies have suggested that hyperplastic duct lesions of the pancreas are potential precursors of pancreatic ductal carcinoma. This study examines the type, distribution, age-related incidence and K-*ras* codon 12 mutation rate of duct lesions in the normal pancreas. Postmortem pancreases from 140 patients were screened for the presence of mucinous cell hypertrophy (MHT), ductal papillary hyperplasia (DPH), adenomatoid ductal hyperplasia (ADH), and squamous metaplasia (SQM). Microdissected cell samples were analyzed for K-*ras* codon 12 mutations by polymerase chain reaction amplification of exon 1 of the K-*ras* gene, combined with constant denaturing gel electrophoresis, and analyzed by sequencing. Of the 140 specimens 114 showed duct lesions. The lesions were evenly distributed throughout the pancreas. They were more common beyond the age of 40. MHT was present in 68%, DPH in 36%, ADH in 40%, and SQM in 36% of the cases. K-*ras* mutations were found in 19 samples from 15 out of 79 pancreases (18%), including all types of duct lesions and a variant of ADH with dense stroma. 67% of the K-*ras*-positive specimens showed the transition GGT to GAT (8) or GTT (5). Hyperplastic/metaplastic duct changes of the pancreas increase with age, but their distribution pattern in the pancreas differs from that of ductal carcinomas.

Key words Normal pancreas · Duct lesions · K-*ras* mutation

The paper contains parts of the doctoral thesis of Barbara Möllmann.

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Introduction

It has been suggested that hyperplastic changes of the duct epithelium of the pancreas may give rise to ductal adenocarcinoma, which is by far the most frequent tumor type in this gland [19]. This assumption is based on three arguments. (1) A number of early histological studies [3, 4, 6, 9, 20] showed that ductal papillary hyperplasia occurs more commonly in pancreases with a ductal carcinoma than in pancreases lacking any neoplastic changes. (2) Moreover, it has been noted that ductal papillary hyperplasia is similar to carcinoma in situ lesions seen in the vicinity of invasive ductal carcinomas [4, 10]. (3) Finally, it was found that hyperplastic duct lesions may harbor mutations of the K-*ras* gene at codon 12, which are characteristic of ductal adenocarcinoma [11, 13, 15, 23, 24, 27]. However, it is difficult to relate the hyperplastic duct lesions and their concomitant genetic changes to carcinoma development. First, none of the studies available so far has systematically examined the type and incidence of duct lesions in the different parts of the normal pancreas in a sufficient number of cases. Second, there has been no study analyzing the K-*ras* status in relation to age and localization in the pancreas. Both the last topics are of particular interest, because pancreatic ductal carcinoma is characterized by a sharply increasing incidence in subjects older than 50 years and preferential occurrence in the head of the pancreas [19]. If certain duct changes are considered to be carcinoma precursors, they will presumably have age-related, spatial or histological features in common with ductal carcinoma.

In this study we therefore investigated a large series of normal, well-preserved postmortem pancreases from different age classes for the presence, type, localization and age distribution of pancreatic duct lesions. Using a microdissection technique that allowed us to pick up single cells, the lesions identified were analyzed for their

K-*ras* status by an assay combining denaturing gradient gel electrophoresis and direct sequencing on PCR-amplified material. This method had a sensitivity of 5%.

Materials and methods

A total of 140 entire pancreatic specimens were selected from more than 300 postmortem pancreases, which were removed within 4–24 h of death. The selection was based on the histological preservation of the tissue (i.e. the duct epithelium was preserved and did not show any shedding into the duct lumen). The specimens came from five different institutions: the Departments of Pathology of the University of Kiel, Germany, the Free University of Brussels and the Catholic University of Leuven, Belgium, the University of Graz, Kaiser Franz-Josef Hospital, Vienna, Austria, and Semmelweis University, Budapest, Hungary.

The deceased patients were 91 men and 49 women. Their mean age at death was 65.5 years (6–92); 31 had been below the age of 50.

All patients had died of nonpancreatic disease. The most frequent causes of death were cardiovascular disease (*n*=48), various carcinomas (*n*=15), respiratory disease (*n*=15), and hematologic neoplasms (*n*=4). There were 16 suicides/accidents. Six patients, all in the age group 60–80 years, were known to have diabetes mellitus (type II). All pancreases were sectioned perpendicular to the main duct; 110 specimens were fixed in 10% buffered formalin for at least 5 days, and the remaining cases were fixed in Bouin's solution. In order to examine both the duct of Wirsung and the duct of Santorin, two blocks of tissue were taken from the head region, one from the lower part of the head with the main pancreatic duct (region A) including the uncinate process, and one from the upper part of the head with the accessory duct (region B). One block each was taken from the body (region C) and the tail (region D) (Fig. 1). In 50 random cases a second block was obtained from the body and tail region. The fixed tissue was embedded in paraffin. The Bouin-fixed material was excluded from the K-*ras* analysis and only assessed histologically. Two sections, one 2 µm thick and one 10 µm thick, were cut from each block and mounted on glass slides. The 2-µm-thick sections were deparaffinized and stained with H&E. On average a histological slide represented 2 cm^2 of pancreatic tissue. Pancreatic duct lesions were classified according to established criteria (described in [8]) as mucinous cell hypertrophy (MHT), ductal papillary hyperplasia without (DPH–) or with mild atypia (DPH+), adenomatoid ductal hyperplasia (ADH), and squamous metaplasia (SQM), including basal cell hyperplasia (Fig. 2). If any of these duct lesions was present, the number of lesions per slide and case was recorded (Table 1). Per case each type of duct lesion was recorded only once. The incidence of lesions was specified separately for the different anatomical sites.

The adjacent unstained 10-µm-thick sections were used for microdissection and K-*ras* DNA extraction. The sections were overlaid with buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). One to three samples, each containing 3–20 cells (Fig. 3), were microdissected from duct lesions, normal duct epithelium, or normal acini with a stereomicroscopically guided micromanipulator (Narishige, Japan) using aspiration capillaries with a diameter of 10–25 µm. Aspiration of buffer only served as contamination control; cells from definite carcinoma tissue were used as positive control. The aspirated cells/buffer were transferred to 5 µl proteinase K digestion buffer (20 µg proteinase K/ml in 10 mM Tris/HCl, 0.5% Nonidet P40) in PCR reaction vials. Digestion was performed for 10 min at 55°C to demask the DNA, followed by inactivation of the proteinase K (15 min at 96°C).

K-*ras* analysis was performed by a previously described technique [14]. Briefly, to amplify the K-*ras* DNA, PCR buffer was added to a final concentration of 12 mM Tris/HCl, pH 8.0, 50 mM KCl, 0.1% Nonidet P40, 0.2 mM each dNTP and 2 mM $MgCl_2$. In a total volume of 25 µl the reactions contained 50 ng 3' primer (cta ttg ttg gat cat att cg), 100 ng 5' primer (cgccgccgcgccccgcgc ccgtcccgccgcgcccccgcccc ctg aat ata aac ttg tgg), and 0.5 U *Taq*-DNA polymerase (Boehringer Mannheim, Mannheim, Germany). PCR was performed in an MJ-Research PTC-200 thermocycler (Biozym, Hess.-Oldendorf, Germany) using a program of 45 cycles (45 s 95°C, 40 s 58°C, 30 s 70°C). The final extension was for 5 min at 70°C. PCR products were analyzed via constant denaturing gel electrophoresis (CDGE) using a D-Gene chamber (Bio-Rad, Munich, Germany) (Fig. 4). Gels contained 35% denaturant, 1× TAE, 7.5% polyacrylamide (30:1) and were run for 240 min at 200 V, 60°C and silver stained. For sequencing, the deviant bands were cut out of the gel; the DNA was eluted by soaking in TE, reamplified and TA cloned in the pCR2.1 vector (Invitrogen, DeSchelp, The Netherlands). Sequencing was performed with an ABI PRISM Dye Terminator Cycle Sequencing kit (Applied Biosystems, Weiterstadt, Germany) and analyzed on an ABI PRISM 310 automated sequencer (Applied Biosystems, Weiterstadt, Germany). In a preliminary study, we examined this method using various mixtures of a mutant control (Panc1 pancreatic carcinoma cell line; ATCC CRL-1469; K-*ras* codon 12: GGT→GAT) and a normal control (human DNA from peripheral blood monocytes; Boehringer Mannheim, Mannheim, Germany). The results indicated that mutations could be detected if the mutation was present in at least 5% of the DNA.

Statistical calculations were performed with SPSS [1]. The significance of mean differences was evaluated with Pearson's Chi-square test.

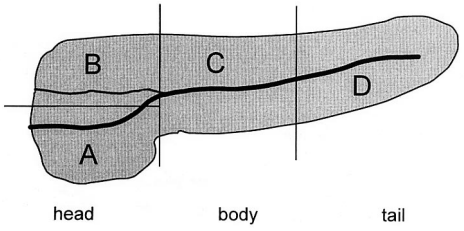

Fig. 1 Diagram of the pancreas with the investigated areas in the anterior head (*A*), superior head (*B*), corpus (*C*), and tail (*D*) indicated

Results

Prevalence and distribution of duct lesions within the pancreas

Of the total of 140 pancreases, 114 (81%) showed duct lesions. MHT, the most frequent type, was found in 96 cases (68%). Of the other types, ADH was detected in 57 (40%), DPH in 51 (36%), 29 of which (20%) revealed mild dysplasia (DPH+), and SQM in 51 (36%) of the cases. There was no sex difference in the incidence of duct lesions. All types of lesions were evenly distributed throughout the four sites that were examined (i.e., upper and lower head [uncinate process], body and tail) (Table 1).

The frequency of duct lesions of the mucinous cell type (MHT, ADH and DPH), in particular MHT, increased beyond the age of 40 (Table 2). Beyond the age of 80 a slight decrease in frequency was observed, with the exception of ADH, which remained constant over the years. The differences were not, however, statistically significant ($P>0.05$ Pearson's Chi-square test). The mean total number of lesions per case also showed only a minimal increase, from 5.00 in the age group below 40 to

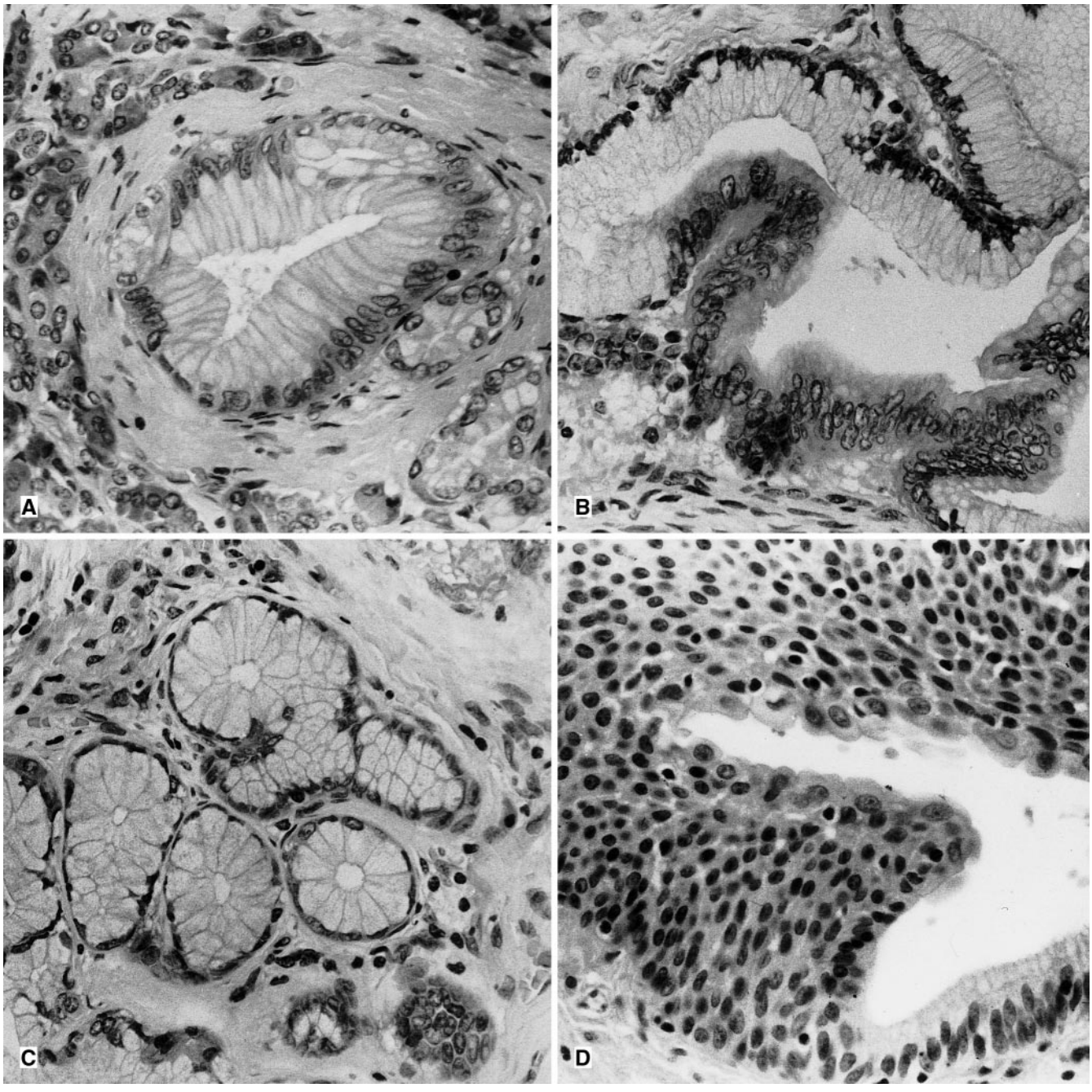

Fig. 2A–D Histological types of lesions from the interlobular ducts: **A** mucinous cell hypertrophy with tall columnar cells; **B** papillary cell hyperplasia with distinct central fibrous core and multilayered cylindrical epithelial cells (*right*) and papillary cell hyperplasia with atypia with slightly enlarged nuclei (*left*); **C** adenomatoid hyperplasia with tubular arrangement of mucinous cylindrical cells surrounded by a loose stroma; **D** squamous metaplasia. H&E, original magnification ×325

5.32 between 40 and 60 and 5.25 between 60 and 80. In the age group over 80 there was even a slight decrease, to 4.38 (Table 2). In 50 cases, an additional second block was investigated from the body and tail. The frequency of lesions did not differ from that of the first series, thus confirming that no lesion showed a preference for any given site. Severe dysplasia was not observed in any case. However, of a total of 126 ADH lesions, 7 (from 6 different cases) were surrounded by a dense fibrous stroma similar to that of carcinomas (Fig. 5). The stromal reaction was not related to pancreatitis or to duct obstruction. Four of these lesions were located in the head of the pancreas, one in the corpus and two in the tail. The mucinous cytoplasm was less well developed, and the tubule formation was less uniform. But the nuclei were regular in shape and size. There were no mitotic figures. Three patients were in the age group 40–60 and four, in the age group 60–80.

Table 1 Distribution of the duct lesions within in the parenchyma of normal pancreas specimens (*n*=140). There were no statistically significant differences in the distribution according to Pearson's Chi-square test. (*MHT* mucinous cell hypertrophy, *DPH all* all cases with ductal papillary hyperplasia, *DPH+* ductal papillary hyperplasia with slight cell atypia, *ADH* adenomatoid hyperplasia, *SQM* squamous metaplasia)

Site	MHT	DPH all	DPH+	ADH	SQM
Head, upper	56 (40%)	25 (17%)	11 (7%)	21 (15%)	26 (18%)
Head, lower	61 (43%)	18 (12%)	8 (5%)	26 (18%)	27 (19%)
Body	62 (44%)	31 (22%)	17 (12%)	29 (20%)	29 (20%)
Tail	73 (52%)	31 (22%)	16 (15%)	34 (24%)	31 (22%)

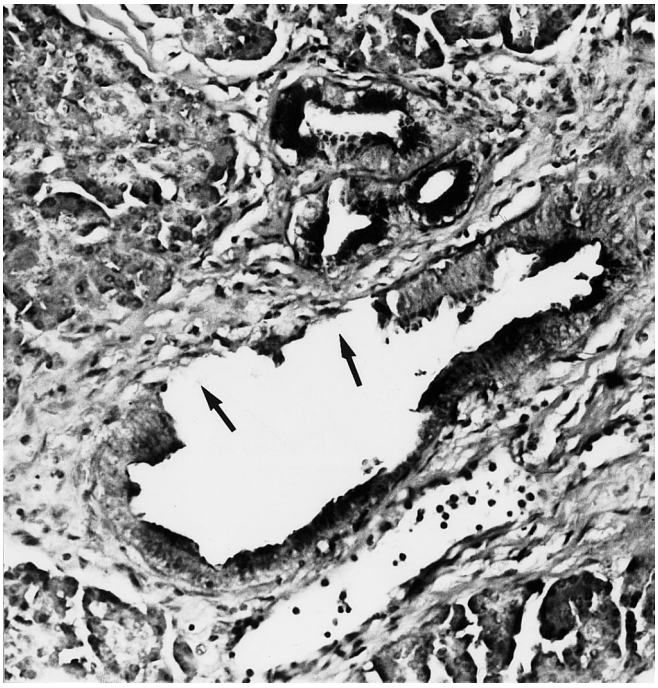

Fig. 3 Duct segment with mucinous cell hypertrophy lacking three cells and five cells at the dissection sites (*arrows*). Original magnification ×250

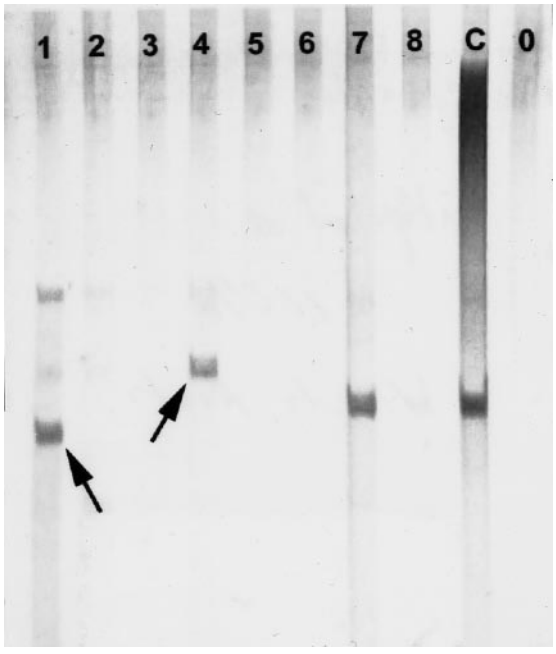

Fig. 4 Gel electrophoresis with two deviant bands revealing a K-*ras* mutation (*arrow*). *Lanes 1–8* samples of the various lesions, *lanes 1* and *4* showing a K-*ras* mutation, *lanes 1* and *7* the wild type, *lanes 2*, *3*, *5*, *6* and *8* not amplifiable material. (*C* positive control, *0* negative control with no DNA)

Frequency of K-*ras* mutations

A total of 1141 samples from 79 well-preserved specimens were microdissected and analyzed for the K-*ras* mutation. The region encompassing codon 12 of the K-*ras* gene was successfully amplified in 924 of the 1141 samples (82%). These included 383 different duct lesions, 268 samples of normal duct epithelium and 278 acinar cells. K-*ras* mutations were identified in 15 of the 79 specimens (19%). In these 15 specimens they were found in 17 duct lesions, i.e., 3.9% of the amplifiable samples. MHT most often harbored K-*ras* mutations. They were found in 10 out of 153 samples (6.5%). In DPH+ they were found in 1 of 26 (3.8%), in ADH in 5 of 100 (5%) and SQM in 1 of 106 (0.9%). K-*ras* mutations also occurred in 2 out of 268 (0.7%) of the amplifiable samples of microscopically normal appearing duct epithelium, whereas acinar cells (0/278) lacked K-*ras* mutations (Table 3).

The age range of patients with mutations was 36–82 years (mean age 67.8±11.7). The sex ratio (f:m) was 1.5:1 (12:8).

K-*ras* mutation patterns

Five different types of K-*ras* mutations were identified in the 16 samples with mutated DNA (Table 4). The most common nucleotide sequences, occurring in 69% of the cases, were GAT (8) and GTT (5). Less common were CGT (2), GCT (3) and TGT (2). K-*ras* mutations were most frequently observed in lesions of the mucinous cell type and occurred in 10 out of 141 MHT lesions (7%), in 5 of 82 ADH lesions (6%) and in 1 of 17 DPH+ lesions (5%). One of 87 SQM lesions (1.1%) harbored a K-*ras* mutation. Of the seven ADH lesions with the stromal reaction, three showed K-*ras* mutations, two with the nucleotide sequence GAT and one with CGT (reproducible in at least one control sample from each lesion).

Table 2 Frequency of duct lesions related to age. The differences between the age groups were not statistically significant according to Pearson's Chi-square test

Age (cases)	Cases with lesions/ relative frequency	Type of duct lesion	No of cases (relative frequency)	Mean number of lesions per case (range; SD)
<40 (*n*=13)	8 (61%)	MHT	6 (46)	5.0 (1–13; 4.1)
		DPH all	5 (38)	
		DPH+	4 (30)	
		ADH	1 (7)	
		SQM	4 (30)	
40–60 (*n*=45)	36 (80%)	MHT	32 (71)	5.32 (1–12; 3.05)
		DPH all	19 (42)	
		DPH+	6 (13)	
		ADH	18 (40)	
		SQM	16 (35)	
61–80 (*n*=65)	57 (87.7%)	MHT	47 (72)	5.25 (1–12; 3.89)
		DPH all	24 (36)	
		DPH+	17 (26)	
		ADH	30 (42)	
		SQM	27 (41)	
>80 (*n*=17)	13 (76.5%)	MHT	11 (64)	4.38 (1–9; 2.59)
		DPH all	3 (17)	
		DPH+	2 (11)	
		ADH	8 (47)	
		SQM	2 (11)	
Total (**n**=140)	114 (81.4%)			

Table 3 Frequency of K-*ras* mutations in microdissected samples from 79 nonneoplastic pancreases [*DPH–*: (ductal) papillary hyperplasia without mild atypia, *DPH+*: (ductal) papillary hyperplasia with mild atypia]

Type of lesion	Number of lesions	Samples	K-*ras* mutation/amplifiable samples	Not amplifiable samples
MHT	141 (37.6%)	193	10/153 (6.5%)	40
DPH–	55 (14.7%)	57	0/47 (0%)	10
DPH+	17 (4.6%)	34	1/26 (3.8%)	8
ADH	82 (21%)	126	5/100 (5%)	26
SQM	87 (23.2%)	134	1/106 (0.9%)	28
Total lesions/samples	383 (100%)	544	17(432) (3.9%)	112 (20%)
Normal duct cells		319	2/268	51
Acinar cells		278	0/224	54
Total samples		1141	19/924 (2.0%)	217 (19%)

Table 4 Cases with K-ras mutations. Localization of the lesions and nucleotide sequences(*inf*. inferior, *ant*. anterior)

Case	Type of lesion	Age	Sex	Localization	Nucleotide sequence
1	ADH	82	F	Body	GGT→GAT
	MHT			Tail	GGT→GTT
2	Normal	76	M	Body	GGT→TGT
3	MHT	76	F	Head, ant.	GGT→GTT
4	MHT	75	F	Tail	GGT→GAT
5	Normal	75	F	Head, inf.	GGT→GTT
	ADH			Head, inf.	GGT→GTT
6	MHT	72	F	Tail	GGT→CGT
7	MHT	70	F	Head, inf.	GGT→ΓAT
	MHT			Head, inf.	GGT→GAT
8	SQM	66	M	Body	GGT→GCT
9	MHT	65	F	Body	GGT→TTT
	MHT			Tail	GGT→TGT
10	DPH+	64	M	Tail	GGT→GTT
11	MHT	56	M	Tail	GGT→GAT
12	MHT	36	F	Body	GGT→GAT
13	ADH	55	M	Tail	GGT→GAT
14	ADH	71	M	Head	GAT→CGT
15	ADH	46	F	Head	GGT→GAT

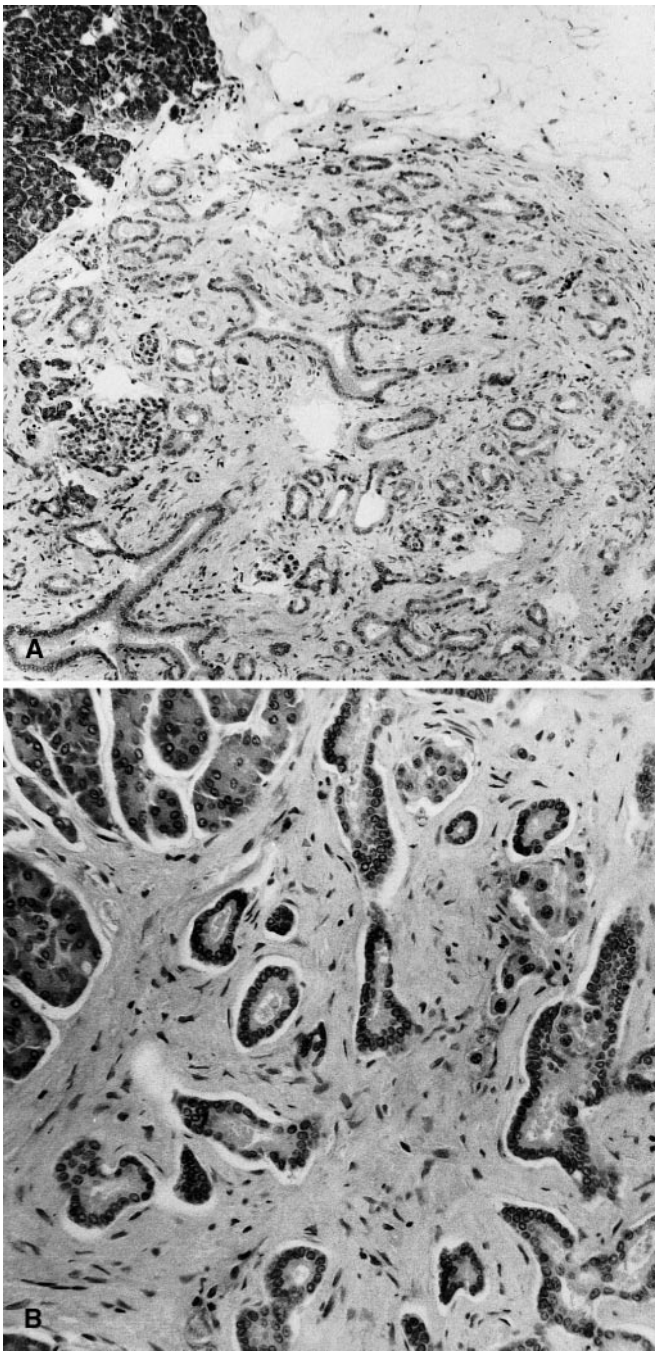

Fig. 5 Variant form of adenomatoid hyperplasia with **A** a dense stroma and **B** irregularly shaped tubules without nuclear atypia. Original magnification **A** ×125, **B** ×325

In 4 of the 79 cases (5%) we detected two mutations in one and the same specimen. Two of the cases showed an identical nucleotide sequence (GAT or GTT) in both sites: one case in two lesions of MHT type in the head region and the other in a lesion of ADH type and in normal duct epithelium). The other two cases had different nucleotide sequences in lesions occurring in the body and the tail. One of these cases showed two MHT lesions, one with TGT and one with GCT, and the other one an ADH lesion with GAT and an MHT lesion with GTT.

The duct lesions that harbored mutations were evenly distributed throughout the pancreas and did not show a preferential localization in the head. No mutation pattern showed a preference for any specific region of the organ.

Discussion

Because most pancreatic carcinomas show a ductal/ductular phenotype it is thought that they originate from ductal/ductular cells [6]. This assumption seems to be supported by the finding of an increased incidence of certain types of duct lesions in association with carcinoma and by the demonstration of K-*ras* mutations in hyperplastic changes of the duct epithelium, which have therefore been held to be tumor precursors [15].

In our study of 140 normal postmortem pancreases we found MHT to be the most frequent hyperplastic duct change. Severe epithelial dysplasia or carcinoma in situ (termed atypical hyperplasia in some studies) [4, 9] was not observed. All duct lesions were evenly distributed throughout the pancreas. There was an age-related increase beyond the age of 40, but no type of lesion showed a statistically significant increase, and nor did any specific site. The average number of lesions was almost equal within the different age groups and regions.

Our finding that duct lesions were diffusely and evenly distributed throughout the pancreas differs from the results of several studies, but confirms others. Since ductal carcinomas have a clear preference for the head region, tumor precursors should develop primarily in this area. The early study of normal autopsy pancreases by Sommers et al. [20] was the first to support this assumption. The subsequent studies by Kozuka et al. [9], Klöppel et al. [6] and Mukada and Yamada [16] largely confirmed Sommers' results. However, there are some inconsistencies between these studies. Neither Sommers' nor Kozuka's groups systematically analyzed the pancreatic tissue. Both groups stated that in some cases only one section per pancreas was available and that in other cases step sectioning had revealed more duct lesions. Mukada and Yamada [16] systematically analyzed the parenchyma in their study, but included lesions in the ampulla and periampullary region. Hence their data are not fully comparable to ours. In an earlier study by one of the authors [6] the exact number of lesions per region was not mentioned. The only study fully comparable to ours is that by Stamm [21], who investigated tissue from each region separately and. also found duct lesions to be evenly distributed throughout the pancreas. In the light of Stamm's and our own findings, the validity of the conclusion that duct changes preferentially occur in the head region needs to be reconsidered.

We found a relationship between the frequency of certain duct lesions and age. Beyond the age of 40 there was an increase in the frequency of MHT, ADH and SQM, which decreased slightly after the age of 80. These data

generally confirm those of other studies [4, 9, 16, 20, 21], although the decrease in lesions in subjects 80 years and older has not yet been described, because this age class has not been separately analyzed. The reason for this decrease is so far not clear. We failed to confirm an increase in DPH with advancing age, as reported by others [6, 9, 16, 21].

We did not detect DPH with severe cellular atypia, also termed severe dysplasia–carcinoma in situ [8, 19] or atypical hyperplasia [4, 9]. Papillary hyperplasia with severe atypia is considered the most likely tumor precursor [6, 15, 24]. This duct lesion is a common feature of carcinoma-associated ducts [3, 7, 10, 15, 22] and most probably represents an intraductal tumor extension of an elsewhere invasive carcinoma. In these cases the lesion is part of the manifest carcinoma and not a precursor. There are only a few reports describing atypical hyperplasia not associated with an invasive carcinoma. In their investigation of 1,174 cases, Kozuka et al. [9] found atypical hyperplasia in 13 cases, 6 of them unrelated to a pancreatic carcinoma. Pour et al. [17] serially sectioned 83 pancreases obtained from consecutive autopsies and found "ductular carcinoma in situ" in 7 cases (3 with cancer in the head of the pancreas) and ductal carcinoma in situ in 1 case. Judging from the illustrations provided by Kozuka and Pour, it is unclear whether all lesions that were labeled as carcinoma in situ or atypical hyperplasia meet the strict criteria recently defined in the WHO classification [8] or elsewhere [5, 19]. These data should therefore be discussed with caution. This is also true of atypical hyperplasia in chronic pancreatitis, which has been reported by one group [22] but not confirmed in other studies [25].

Squamous metaplasia differs in several respects from lesions of the mucinous cell type. It occurs in a lower frequency [4, 6, 21] and seems to be gender related, with a preponderance of males [21]. Squamous metaplasia has been reported more frequently in association with prolonged stenting of the duct system [11] or intraductal inspissated amorphous material [4]. A remarkably high frequency of 45% for SQM was reported by Washington et al. [26] in autopsy cases of young adults receiving high-dose chemotherapy followed by bone marrow transplantation. They conclude that SQM is related to duct injury caused by high dose chemotherapy. In our study, which included 14 patients suffering from different types of organ cancers treated with chemotherapy, we also found SQM rather frequently.

K-*ras* mutations at codon 12 have been detected in histologically nonneoplastic pancreatic duct lesions occurring in pancreases with invasive ductal carcinomas [11, 13, 15, 22] and in many cases of chronic pancreatitis [2, 27], though only in a few selected lesions, mainly of the papillary mucinous type. K-*ras* mutations have also been found in pancreases without any known disease [11, 23]. In this study we microdissected 383 foci from 79 normal postmortem pancreases and found K-*ras* mutations in 20 samples from various regions in 15 pancreases. Mutated K-*ras* was identified in all types of duct lesions (Table 4). In particular, it showed no preference for dysplastic lesions, but was also found in normal duct cells, though never in acinar tissue. The youngest patient with a positive sample was 37 years old, but most of the affected subjects were older than 50. These data extend those of Tada et al. [23], in that they clearly demonstrate that K-*ras* mutations can occur anywhere in the pancreatic duct system, whether it is normal, hyperplastic, metaplastic, mucinous or nonmucinous. It is therefore difficult to ascribe a certain role in pancreatic tumorigenesis to the K-*ras* mutation or any of the affected duct lesions. However, the almost universal presence of mutated K-*ras* in established ductal carcinomas and the selective occurrence of the K-*ras* mutation in duct cells suggest that (a) the pancreatic cell giving rise to a carcinoma is usually a K-*ras* mutated duct cell and (b) if K-*ras* positive carcinomas are preceded by precursor lesions, they most probably develop from those with the highest incidence of K-*ras* mutations.

So far, ADH has been investigated only in two studies examining resection specimens with ductal carcinomas [11, 24]. These lesions were shown to have the highest mutation rate. ADH seems therefore to be the duct change with the greatest potential as a precursor lesion. In this regard it is of particular interest that four of our five ADHs with K-*ras* mutations showed a peculiarly dense stroma, which is also a feature of invasive ductal carcinoma. Moreover, two of the four lesions were found in the head of the pancreas. These peculiar ADHs might therefore represent a distinct lesion whose role in pancreatic tumorigenesis deserves further attention.

Tada et al. [23] emphasized the difference in the prevailing nucleotide sequence pattern of the K-*ras* mutation in normal pancreases from that found in most ductal carcinomas. Fifty-three percent of their foci showed TGT and AGT mutations, which are rare in carcinomas. In contrast, GAT and GTT, the typical sequences in ductal carcinomas, were also most common in our study, while TGT was only observed twice. The high proportion of rare nucleotide sequences in Tada's study is difficult to explain. It might reflect a geographical influence, since considerable differences in the prevailing K-*ras* mutation patterns have been revealed between different countries [12, 18].

In conclusion, duct lesions without severe cellular atypia do not show a preference for the head of the pancreas, which is the most common site of carcinomas. This finding throws doubt on their importance as tumor precursors. Moreover, K-*ras* mutations were not restricted to a certain type of duct lesions and also occurred evenly distributed in the pancreas. K-*ras* mutations therefore seem to have a limited role in the pathogenesis of ductal pancreatic carcinoma. However, as K-*ras* mutations may be an indicator of cells with the potential to undergo malignant transformation, lesions with a high incidence of K-*ras* mutations, such as ADH, are of interest in further studies on pancreatic tumorigenesis.

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