

Frequent c-Ki-ras Oncogene Activation in Mucous Cell Hyperplasias of Pancreas Suffering from Chronic Inflammation¹

Akio Yanagisawa,² Keiko Ohtake, Kazuhiko Ohashi, Masaharu Hori, Tomoyuki Kitagawa, Haruo Sugano, and Yo Kato

Departments of Pathology [A. Y., K. O., T. K., H. S., Y. K.], Internal Medicine [K. O.], and Surgery [M. H.], Cancer Institute, 1-37-1 Kami-Ikebukuro, Toshima-ku, Tokyo, 170 Japan

Abstract

In order to scrutinize the possible significance of (nonatypical) mucous cell hyperplasia of the pancreas to neoplasia, we analyzed these lesions in terms of c-Ki-ras activation, which is known to be very frequent in pancreatic carcinomas. A total of 16 such mucous cell hyperplasias were collected from 10 pancreases resected for chronic pancreatitis. Tiny tissue fragments were taken from hematoxylin-stained sections by microdissection, and DNA analysis was carried out by the polymerase chain reaction amplification and oligonucleotide hybridization methods. Activating mutations of c-Ki-ras oncogene at codon 12 were detected in 10 of the 16 lesions (62.5%), a high rate as seen in carcinomas. The results indicated a clonal origin of cells comprising the mucous cell hyperplasia suggesting a neoplastic and/or precancerous nature.

Introduction

Mucous cell hyperplasia or mucous cell hypertrophy are frequently seen in pancreas tissue of patients suffering from chronic inflammation or carcinoma (1-3). Such lesions have further been subdivided into simple (nonpapillary) hyperplasia, papillary hyperplasia, and atypical hyperplasia categories (3). Since atypical hyperplasia is frequently encountered in the vicinity of cancers on the one hand and also demonstrates an incidental association with nonatypical hyperplasias on the other, all these hyperplastic lesions have been suggested to be forerunners of carcinomas. It is, however, still a matter of dispute whether these lesions, especially ones without atypia, are indeed neoplastic or merely develop in response to inflammatory and/or obstructive stimuli (4, 5).

It has recently been shown that the development of human pancreatic carcinoma is associated with c-Ki-ras oncogene activation at high frequencies, ranging from 75 to 100% (6-10), suggesting that this may be an important event in the neoplastic process. We have also studied *ras* activation in a specific type of pancreatic neoplasm, designated as the ductectatic mucinous cystic neoplasm (11, 12), and found *Ki-ras* point mutation in 62.5% (5 of 8) of the cases analyzed (13). Interestingly, when we analyzed cases in which histological variation was noted in one and the same tumor, ranging from mucous cell hyperplasia with no atypia to carcinoma, c-Ki-ras point mutations of the same type, when present, were found in all sites regardless of the histological appearance. This suggests a mucous cell hyperplasia-adenoma-carcinoma sequence in the ductectatic mucinous cystic neoplasm together with a relatively early occurrence of c-Ki-ras activation. The results also prompted us to study the presence or absence of

c-Ki-ras point mutation in mucous cell hyperplasias of the pancreas developing in association with chronic pancreatitis. This paper documents the results.

Materials and Methods

A total of 16 mucous cell hyperplasias and 13 FOM³ collected from 10 pancreas tissues of patients with chronic pancreatitis were used for analysis. All these pancreatic tissues were resected in our Cancer Institute Hospital under strong suspicion of carcinoma on image diagnosis by endoscopic retrograde pancreatography and computed tomography. Mucous cell hyperplasias were further subclassified into 5 FMH, 7 PMH-A, and 4 PMH-B. Representative pictures of FOM, FMH, PMH-A, and PMH-B are presented in Figs. 1-4, respectively. The ducts with the FOM (Fig. 1) are dilated and surrounded by thickened connective tissue. The cells comprising FMH (Fig. 2) and PMH-A (Fig. 3) are similar, with clear high columnar cytoplasm and flattened nuclei, the difference being only the presence or absence of papillary growth pattern. PMH-A is characterized by tall columnar cells with clear cytoplasm, which contain much mucin, whereas PMH-B (Fig. 4) demonstrates columnar epithelium containing small mucin droplets. No nuclear atypism is visible.

DNA extraction from paraffin sections was performed by the following procedure (13). Serial sections 4 and 20 μ m thick were made from paraffin-embedded tissue blocks and attached to slide glasses. The 4- μ m section was stained with hematoxylin and eosin. The 20- μ m section was stained with hematoxylin only after deparaffinization. With comparative microscopical observation of the hematoxylin and eosin-stained section for orientation, tiny fractions of epithelial lesions composed of 100 to 1000 cells were cut out from the 20- μ m section under the stereomicroscope ($\times 40$). These microdissection samples were further deparaffinized with xylene, cleared with ethanol, completely dried, and boiled in distilled water before PCR.

Amplification of DNA corresponding to the 12th codon of c-Ki-ras was performed by the PCR technique essentially according to the method described by Saiki *et al.* (14) and Wright and Manos (15) using a PCR machine and a kit (Parkin Elmer Cetus). The primers used for amplification were TTGTGGATCATATTCGTC and GGCCTGCTGAAAATGACTGA which yielded a 118-base pair amplified DNA fragment around codon 12 c-Ki-ras. Cloned *Ki-ras* sequences with a point mutation at specific sites of codon 12 were used as positive controls.

A 2- μ l sample of each amplified DNA was spotted onto Hybond-N⁺ filters (Amersham) and fixed by the alkaline procedure. Oligonucleotide hybridization was performed according to the method described by Verlaan-de Vries *et al.* (16). A series of specific synthetic 19-mer antisense single-stranded DNA probes, corresponding to the possible point mutations at codon 12, were end-labeled, respectively, by [γ -³²P]ATP (Amersham) and T4 polynucleotide kinase (Toyobo). The final wash was made with 3 M tetramethylammonium chloride, 50 mM Tris-HCl (pH 7.5), 2 mM EDTA, and 0.1% sodium dodecyl sulfate at 59°C.

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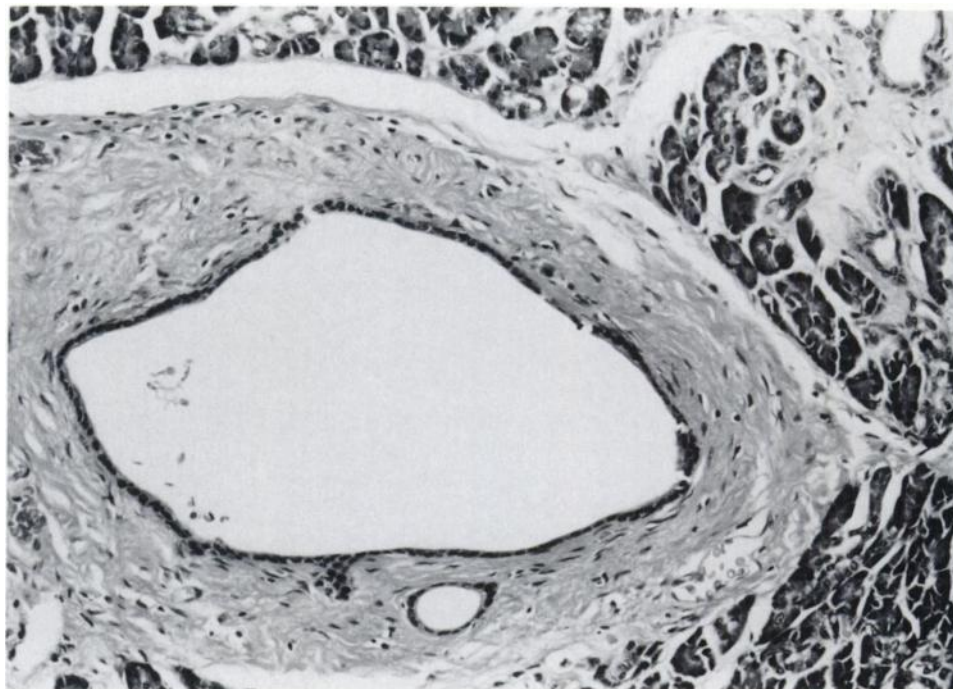
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² To whom requests for reprints should be addressed.

³ The abbreviations used are: FOM, flat ordinary mucosa; FMH, flat mucous cell hyperplasias; PMH-A, papillary mucous cell hyperplasias-type A; PMH-B, papillary mucous cell hyperplasias-type B; PCR, polymerase chain reaction; Gly, glycine; Arg, arginine; Ala, alanine; Ser, serine; Asp, aspartic acid; Cys, cysteine; Val, valine.

Fig. 1. Flat ordinary mucosa.



Results and Discussion

The results of oligonucleotide hybridization are shown in Fig. 5 and Table 1. Samples a-g are positive control samples with *Ki-ras* codon 12 coding for glycine (wild type), arginine, alanine, serine, aspartic acid, cysteine, and valine, respectively. Samples 1-29 are the test samples from the 10 pancreatitis patients, arranged in sets for each case. Upon hybridization, the wild type probe (glycine) gave rise to a positive reaction with all the samples, whereas the arginine, aspartic acid, and valine probes reacted positively with 3, 5, and 2 samples, respectively. Samples 22 and 24, which were obtained from the same pancreatic tissue (patient 8) revealed different *Ki-ras* point mutations, giving arginine and aspartic acid, respectively.

The investigation thus revealed high incidences of *Ki-ras* point mutation at codon 12 in FMH (3 of 5; 60.0%), PMH-A (6 of 7; 85.7%), and to a lesser extent PMH-B (1 of 4; 25.0%) but not FOM (0 of 13; 0%) in cases of pancreatitis with chronic inflammation, the average being 62.5% (Table 2). Putting the data for FMH and PMH-A together, since both lesions comprise similar high columnar clear cells, the incidence is 75.0% (9 of 12). These rates are very similar to those found for ductal carcinomas of the pancreas (6-10). The majority of mucous cell hyperplasias, especially those comprising high columnar cells, may thus be the result of clonal cell proliferation, in line with a possible neoplastic or precancerous nature. The results suggest that, when present, *Ki-ras* activation may be an event occurring at a relatively early stage of multistep carcinogenesis in the

Fig. 2. Flat mucous cell hyperplasia.

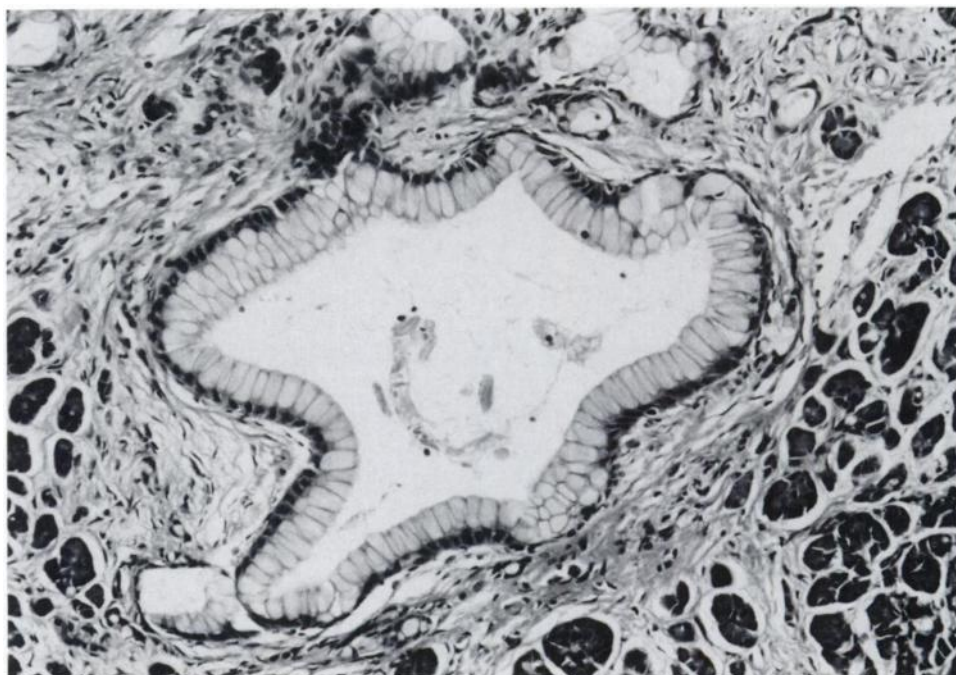
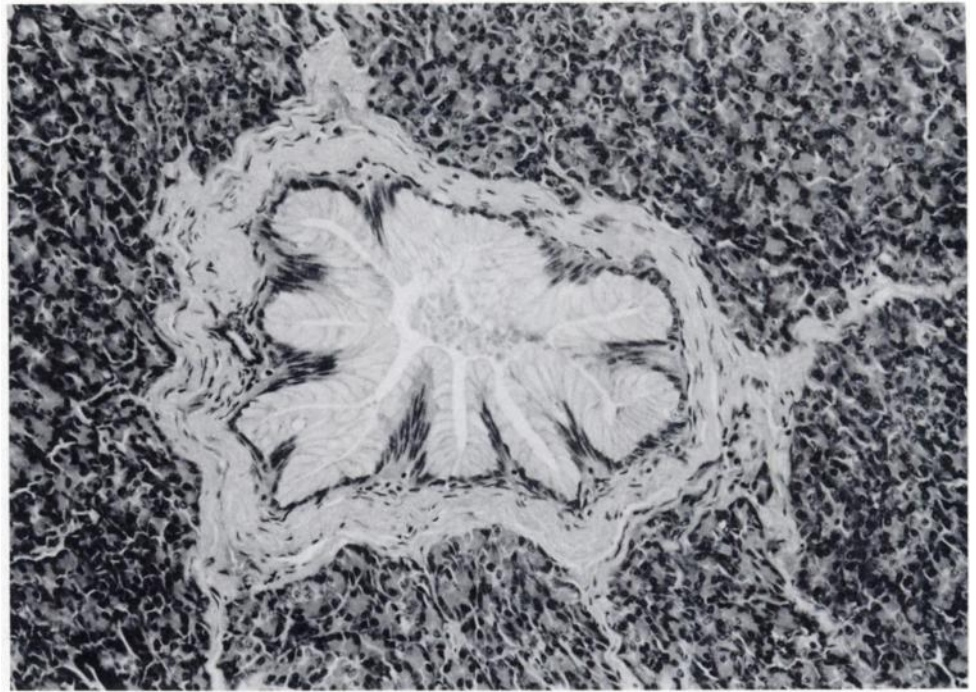


Fig. 3. Papillary mucous cell hyperplasia type A.



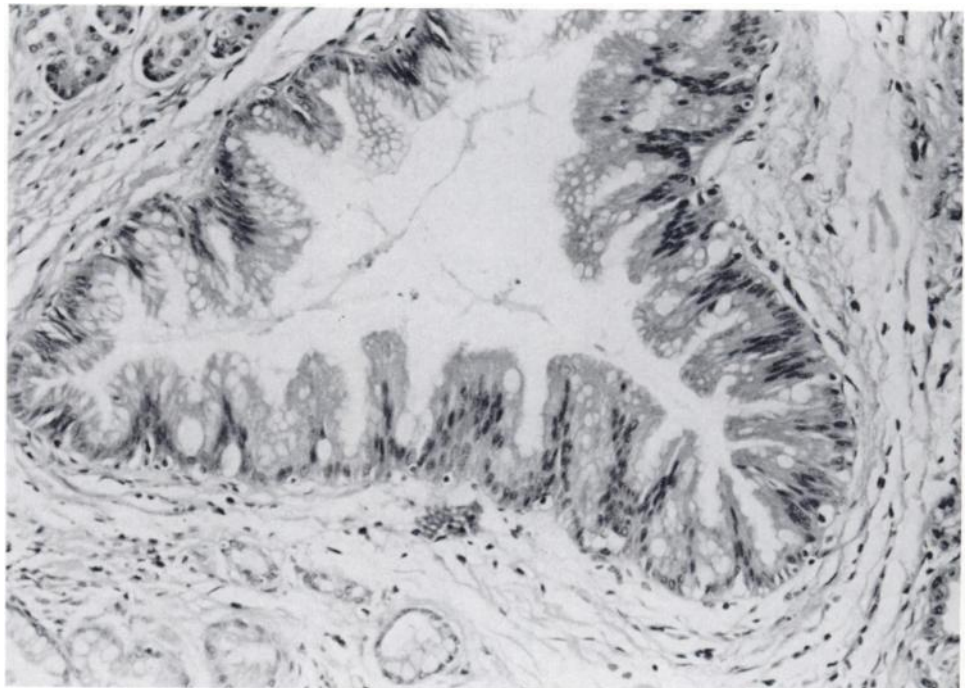
pancreas lesions, in concert with observations on colon lesions(17–19), ductectatic type mucinous carcinomas of the pancreas (13), and myeloid leukemia in humans (20). The conversion rate of the *Ki-ras*-mutated mucous cell hyperplasia to carcinoma, however, awaits future studies. Currently information is lacking even as to the incidence of carcinoma developing in the pancreas with chronic inflammation.

The present results are in contrast with those of Lemoine *et al.* (21) and Tada *et al.* (8), who, respectively, found no *Ki-ras* point mutations in 5 ductal papillary hyperplasias or in 9 nonneoplastic pancreatic tissues demonstrating chronic inflammation. While the reason for this discrepancy is not clear, a major difference in methodology should be pointed out; while we extracted DNA selectively from the epithelial

cells in question using a microdissection method, they collected DNAs from serial paraffin sections of ones showing particular lesions as a whole. Since their DNAs would have been inevitably contaminated with normal tissue DNA, the detection sensitivity for *Ki-ras* point mutation might be expected to be much lower than ours. Recently, Cerney *et al.* (22) reported early occurrence of *Ki-ras* mutation in pancreatic duct carcinogenesis in the Syrian golden hamster. They applied a methodology similar to ours for genetic analysis and found *Ki-ras* point mutation in 26% of hyperplasias and 46% of papillary hyperplasias, quite in concert with the present results in humans.

It has been mentioned that there are differences in the type of *Ki-ras* codon 12 point mutation in pancreatic cancers between Europe and

Fig. 4. Papillary mucous cell hyperplasia type B.



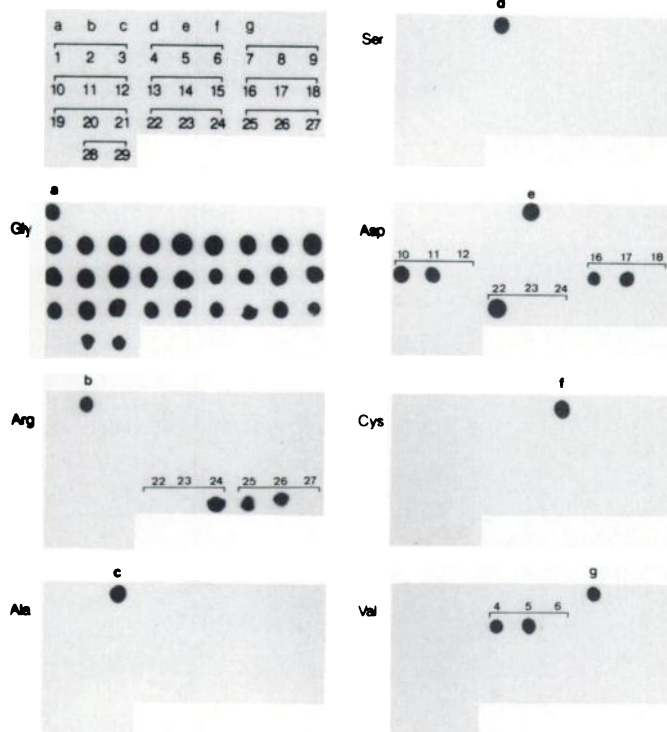


Fig. 5. Results of dot blot hybridization with oligonucleotide probes specific for the normal sequence GGT of codon 12 (a) and mutated sequences CGT (b), GCT (c), AGT (d), GAT (e), TGT (f), and GTT (g).

Table 1 Clinicopathological data of cases and Ki-ras point mutations

Case	Age and sex	Location	Histological classification	No. of samples	Ki-ras mutation	Type of mutation
1	31 M	Body	FOM	3	0	
2	42 M	Body	PMH-A FOM	2	2	Val
3	43 M	Tail	FMH PMH-B FOM	1 1 1	0 0 0	
4	70 F	Tail	PMH-A FMH FOM	1 1 1	1 1 0	Asp Asp
5	71 F	Head	PMH-B FOM	2 1	0 0	
6	47 M	Head	FMH	3	2	Asp
7	47 M	Head	FOM	3	0	
8	57 F	Body	PMH-A	2	2	Asp, Arg
9	72 F	Body	FOM PMH-A PMH-B	1 2 1	0 1 1	Arg Arg
10	57 M	Head	FOM	2	0	

Table 2 Incidence of mutation in Ki-ras codon 12 according to histological classification

Histology	Incidence
Flat ordinary mucosa	0/13 (0) ^d
Flat mucous cell hyperplasia	3/5 (60.0)
Papillary mucous cell hyperplasia	
Type A	6/7 (85.7)
Type B	1/4 (25.5)

^d Numbers in parentheses, percentage.

Japan (10, 23), although the total incidences are similar: in Europe conversions from glycine to aspartic acid, valine, and arginine are common whereas in Japan that from glycine to aspartic acid predominates. The present findings featured relatively high incidences of conversion from glycine to aspartic acid (5 of 10) and glycine to arginine (3 of 10). While the fit is not perfect, these types of mutation are not infrequent in both European and Japanese cases; therefore, the present results for mutation analysis also do not contradict the notion that mucous cell hyperplasias may be precursor lesions for pancreatic cancers.

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