c-Ki-ras Point Mutations in Ductectatic-type Mucinous Cystic Neoplasms of the Pancreas

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Ductectatic-type mucinous cystic neoplasms of the pancreas constitute a recently recognized new human pancreatic tumor entity. Examination for the presence of point mutations at codon 12 of K-ras by oligonucleotide hybridization in 5 adenomas and 3 carcinomas revealed alteration in 3 and 2, respectively. In 4 of these positive cases, the transition was GGT→GAT (Gly→Asp) with the remaining one, found in a cancer, being GGT→GTT (Gly→Val). In two carcinoma cases, the same point mutation was detected both in the carcinoma area and in a coexisting adenoma component. Thus K-ras point mutation appears to be associated with this particular type of neoplasm in the same manner as observed for typical exocrine pancreas carcinomas. Our study also indicates the possible existence of an adenoma-carcinoma sequence in the evolution of this type of neoplasm and we suggest that K-ras activation may be an important event in the phase of adenoma development.

Key words: K-ras mutation — Pancreas cancer — Mucinous cystic neoplasm

Recent advances in diagnostic techniques including endoscopic retrograde cholangiopancreatography, ultrasonography and computed tomography, have allowed detection of a considerable number of relatively small neoplastic and/or pseudo-neoplastic lesions of the human pancreas, some of them being previously undescribed in the literature.¹⁾ Among such newcomers are ductectatic-type mucinous cystadenomas and cystadenocarcinomas. These lesions were first recognized and characterized in our laboratory and have now found acceptance as a new clinicopathological entity.²⁻⁴⁾ They are characterized by the presence of multilocular cysts, reminiscent of bronchiectasia of the lung, and papillary proliferation of the lining epithelium, being quite different not only from typical ductal pancreatic carcinomas but also from classically described mucinous cystic tumors in terms of morphological features, intrapancreatic location, and patients' age and sex predilection.⁵⁾

In the present investigation, we analyzed K-ras activation in this particular type of pancreatic neoplasm with special attention to point mutation at codon 12, since this has been very frequently found in human pancreas carcinomas of exocrine origin.⁵⁻⁹⁾

Five ductectatic-type cystadenomas and 3 cystadenocarcinomas which were operated in our Cancer Institute Hospital were used for the analysis. DNA extraction from paraffin sections was performed by the procedure described by Shibata *et al.*¹⁰⁾ with some modifications. Serial sections of 4 μ m and 10 μ m thickness were made

from paraffin-embedded tissue blocks and attached to slide glasses. The 4 μ m section was stained with hematoxylin and eosin. The 10 μ m section was stained with hematoxylin only after deparaffinization. Based on comparative microscopical observation of the H-E-stained section, limited areas of epithelial lesions comprising 100 to 1000 cells were cut out from the 10 μ m section under stereo-microscopic observation ($\times 40$). Since the 3 carcinomas were all accompanied with adenoma components, specimens were taken from both types of lesions. At least six separate samples including a normal (non-neoplastic) epithelial portion were taken from one material. One of the carcinoma-adenoma cases is shown in Fig. 1a-c. The tissue samples were deparaffinized with xylene again after dissection, cleared with ethanol, completely dried, digested with Proteinase K and boiled before the polymerase chain reaction (PCR) procedure.

Amplification of DNA corresponding to the 12th codon of c-K-ras was performed by the PCR technique essentially according to the method described by Saiki *et al.*¹¹⁾ and Wright and Manos¹²⁾ using a PCR machine and a kit (Perkin Elmer Cetus). The primers used for amplification were TTGTTGGATCATATTCGTC and GG-CCTGCTGAAAATGACTGA, which yielded a 118 bp amplified DNA fragment around codon 12 of K-ras.

A 2 μ l sample of each amplified DNA was spotted onto Hybond-N⁺ filters (Amersham) and fixed by the alkali procedure. Oligonucleotide hybridization was performed as described by Verlaan-de Vries *et al.*¹³⁾ A series of

Fig. 1. A representative case of ductectatic-type mucinous cyst adenocarcinoma with adenoma component of the pancreas (H-E staining) a. Low magnification view ($\times 2.7$). b. Higher magnification of a site of the adenoma component (single arrow in a. $\times 130$). c. Adenocarcinoma component (double arrow in a. $\times 130$). The areas enclosed by the lines in b and c were cut out and used for PCR.

specific synthetic 19mer anti-sense single-stranded DNA probes, corresponding to the possible point mutations at codon 12, were end-labeled by using [γ - 32 P]ATP

(Amersham) and T4 polynucleotide kinase (Toyobo). The final wash was done with 3 M tetramethylammonium chloride, 50 mM Tris/HCl (pH 7.5), 2 mM EDTA and 0.1% sodium dodecyl sulfate at 59°C.

In 3 out of 5 cystadenoma cases, K-ras activation with a G→A transition, resulting in an alteration of amino acid coding from glycine to aspartic acid, was detected. Additionally, in 2 of 3 carcinoma cases, G→A or G→T (Gly→Val) transition was noted. Samples from different portions of one and the same tumor revealed the same point mutation (Fig. 2). It is interesting to note that the same point mutations were found in the adenoma component coexisting with the carcinoma in the two positive cases. K-ras activation was never detected in samples taken from normal epithelial portions of the material.

The present investigation thus revealed frequent occurrence of K-ras activation at codon 12 in ductectatic neoplasms, comparable with that seen in typical duct-type carcinomas of the pancreas. Such K-ras activation may therefore be a common feature of pancreatic exocrine neoplasms playing an important role in carcinogenesis. As to the types of mutation in codon 12, Smit *et al.*⁶⁾ found mutations leading to cysteine, valine or aspartic acid substitution with essentially equal incidence, whereas Grünwald *et al.*⁷⁾ noticed changes to arginine, valine and aspartic acid almost equally. On the other hand, Mariyama *et al.*⁸⁾ and Nagata *et al.*⁹⁾ independently reported that point mutation resulting in glycine to aspartic acid transition was by far the most frequent in pancreas cancers in Japan. The present results are in concert with these previous Japanese data. Whether such a geographical difference reflects differences in environmental causal agents remains to be seen.

Increasing evidence indicates that carcinogenesis proceeds in multisteps in most instances and that the *ras* gene mutation may occur at any step with the significance being different according to the organ (cell type) and/or species (see Lee *et al.*¹⁴⁾ and Hunter¹⁵⁾). The fact that we found the same K-ras mutation in both the carcinoma and coexisting adenoma component in two cases would suggest the existence of an adenoma-carcinoma sequence also in the genesis of ductectatic-type mucinous cystadenocarcinomas of the pancreas and indicates that *ras* activation may be an important event in the development of adenomas rather than being involved in malignant progression from adenomas to carcinomas.

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Fig. 2. Results of dot blot hybridization with oligonucleotide probes specific for the normal sequence GGT of codon 12 (a), and mutated sequences TGT (b), AGT (c), CGT (d), GTT (e), GAT (f) and GCT (g). Samples 1–8 and 18–24 are from two adenomas with sample 9 being a normal area. Samples 10–17 and 25–36 are from two adenoma-carcinoma cases, respectively, with 10, 11, 13, 15–17, 26–28, 32–36 being adenoma components, 12, 14, 29–31 carcinoma components and 25 a normal area.

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