K-ras and p53 in cancer of the pancreas and extrahepatic biliary tract

Sturm, P.D.J.

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Figures
Representative hybridizations of DNA from duodenal fluid and primary tissue are shown. Rows 2-11 represent an ampullary carcinoma, 3 intraductal mucinous papillary neoplasms, and 6 pancreatic adenocarcinomas. Five of the seven possible membranes are shown, each hybridized to 1 sequence specific probe. The first two columns on each membrane contain primary tissue DNA; the last two contain duodenal fluid DNA. The first and third columns on each membrane are non-enriched for K-ras mutations. A mutant specimen should create a weak signal in the non-enriched and a strong signal in the enriched columns, respectively, because enrichment increases the proportion of mutant DNA. Wild-type DNA from nonneoplastic cells was present in all specimens. As examples, Row 3 contains valine (GTT)-mutant DNA in both the primary tumor and duodenal fluid columns. In contrast, Row 11 contains the experiment's only discrepant specimen, with valine (GTT)-mutant duodenal fluid DNA but wild-type primary tissue DNA. The columns of Row 1 contain the following: wild-type control DNA, control DNA corresponding to each membrane, cysteine (TGT)-mutant control DNA, and a negative control (no DNA added). WT: wild-type; Val: valine mutation; Asp: aspartic acid; Arg: arginine; Cys: cysteine.

Example of an autoradiogram of the K-ras mutational analysis. Seven nylon membranes, each hybridized with a different radioactive labeled oligonucleotide, specific for the sequence of the wild-type codon 12 (left) and the 6 possible permutations. Row 1 and 2: Hybridization controls, DNA complementary to the labeled oligonucleotides. On each membrane in the left lane the non-enriched PCR products, and in the right lane the mutant-enriched PCR products are spot-blotted. Row 11: Control for contamination, water. Row 12: control for amplification, placenta DNA. Rows 3 and 4, 5 and 6, 7 and 8: mutation in a carcinoma and corresponding brush cytology. Row 9 and 10: resection specimen without malignancy and corresponding brush cytology, both only showing signals on the membrane hybridized with the wild-type sequence specific oligonucleotide.

Immunohistochemistry for p53. Brush cytology smear with nucleus specific staining of malignant appearing epithelial cells (200x).

Example of an autoradiogram of the K-ras mutational analysis. Seven nylon membranes each hybridized with a different radioactive labeled oligonucleotide specific for the sequence of the wild-type codon 12 (left) and the 6 possible mutations. On each membrane in the left lane the non-enriched PCR products and in the right lane the mutant-enriched PCR products. WT: wild-type = glycine; Cys: cysteine; Ser: serine; Arg: arginine; Val: valine; Asp: aspartic acid; Ala: alanine.

c: Hybridization controls, on each membrane cloned DNA fragments with a known codon 12 sequence complementary to the labeled oligonucleotides used for the hybridization of that membrane. 1 to 8: Brush cytology specimens with the following K-ras codon 12 sequences coding for the following amino acids: aspartic acid, arginine, glycine, glycine, valine, glycine, aspartic acid, aspartic acid, pla: Placental DNA. H2O: Water. 1:100: 1 cell with mutant codon12, coding for the amino acid valine, mixed in 100 cells with wild-type codon 12. 1:1000: 1 cell with mutant codon 12, coding for the amino acid valine, mixed in 1000 cells with wild-type codon 12.

Cytology positive for carcinoma from the 2 patients with a diagnosis of postsurgical stenosis (Giemsa stained, 132X).
FIGURE 6 A and B
Positive p53 immunostaining of endobiliary brush cytology and the corresponding bile duct carcinoma.

FIGURE 7
Hematoxylin and eosin (H&E)-stained section of a bile duct hamartoma (von Meyenburg complex). Note the proliferation of ectatic bile ducts that contain bile. This lesion was wild-type at codon 12 of K-ras. Magnification, X65.

FIGURE 8
Representative hybridizations of DNA derived from the primary carcinoma (left lane of each membrane) and the liver lesions (right lane of each membrane). These seven membranes, which contained identical PCR products, were probed for wild-type (WT) and mutant (Cys, Ser, Arg, Val, Asp, Ala) K-ras as labeled. Rows 1 and 2 are the controls. In row 1, the mutant cysteine was loaded into the left side of all membranes, whereas wild-type DNA was loaded into the right side of all membranes. In row 2, the left side was loaded with the appropriate positive control for that membrane, whereas the right side was loaded with a negative control (water). Rows 3 to 13 are 11 cases. Note that in cases 8 and 12 the primary carcinoma and the patients' liver lesions both harbor valine mutations. In case 5, 6, 9, and 10, mutations were found only in the primary carcinomas. In the remaining five cases, both the primary carcinomas and the liver lesions were wild-type.

FIGURE 9
H&E-stained sections of a bile duct adenoma (A) and an infiltrating primary adenocarcinoma of the pancreas (B) obtained from the same patient. In the adenoma, note the proliferation of bile ductules lined by cuboidal epithelial cells that lack significant nuclear atypia. In contrast, the infiltrating carcinoma (B) is characterized by poorly formed glands, individual infiltrating tumor cells, and marked nuclear pleomorphism with bizarre mitotic figures. Both lesions harbored the same mutation in codon 12 of K-ras. Magnification, X160.

FIGURE 10
An example of automated DNA sequencing for the K-ras oncogene. The figure shows the fluorescence spectroscopy curve and sequence of the PCR product from a liver metastasis with a mutation in codon 12 at the first base: GGT (wild-type) to CGT (arginine).
FIGURE 11

FIGURE 12
Detection of p53 mutations by DNA sequence analysis. Example of a carcinoma (patient 7, Table 3) with a point mutation in exon 8 at position 273 (lanes 3) and the same region for two carcinomas with the wild-type exon sequence (lanes 1 and 2).

FIGURE 13
An example of an autoradiogram of the K-ras analysis. Seven nylon membranes, each hybridized with a different radioactive-labeled oligonucleotide specific for the wild-type sequence and the 6 mutant sequences of codon 12 (W, GGT i.e. wild-type; A, TGT; B, AGT; C, CGT; D, GTT; E, GAT; F, GGT). Row 1, hybridization controls, i.e. PCR products with known codon 12 sequence. Row 2-9, cholangiocarcinomas. Row 4, GGT to GTT mutation; row 5, GGT to AGT mutation; row 6 and 7, GGT to GAT mutation. The remaining cholangiocarcinomas, row 2, 3, 8, and 9, are wild-type at codon 12.

FIGURE 14
Microdissection of an individual osteoclast-like giant cell. The osteoclast-like giant cells were first manually separated from the tumor, and then removed for subsequence analysis.

FIGURE 15
Phagocytosis of the mononuclear tumor cells by the osteoclast-like giant cells (KP1 immunostain).

FIGURE 16
Osteoclast-like cell tumor of the liver (patient 2). The cut surface of the liver shows a lobulated hemorrhagic mass with central necrosis.

FIGURE 17
Osteoclast-like giant cell tumor of the liver (patient 2). (A) The tumor is comprised of mononuclear cells admixed with evenly spaced multinucleated giant cells. (B) Severe epithelial dysplasia is present in the surrounding intrahepatic bile ducts.
FIGURE 18
Osteoclast-like giant cell tumor of the pancreas arising in a mucinous cystic neoplasm (case 3). Osteoclast-like giant cells and infiltrating mononuclear cells are intimately associated with the mucinous duct epithelium.

FIGURE 19
Immunohistochemical staining of an osteoclast-like giant cell tumor of the pancreas arising in association with a mucinous cystic neoplasm (case 3). Only the lining epithelium is immunoreactive for AE1: AE3 (A), whereas the osteoclast-like giant cells and scattered mononuclear cells are immunoreactive for KP1 (B).

FIGURE 20
Allele-specific oligonucleotide hybridization of all five cases. Hybridization with cloned polymerase chain reaction products harboring the wild-type (WT) sequence and four different codon 12 mutations (Cys, cysteine; Ser, serine; Arg, arginine; Val, valine) are shown in the top row. Normal tissues and water serve as controls for contamination and nonspecific hybridization. In patient 4, a sample without DNA template served as an additional negative control (c). Each lane has two dots showing hybridization with the amplification products before (left) and after (right) digestion of the wild-type sequence. Patient 1: a, normal; b, intraductal lesion (IDL); c, infiltrating mononuclear cells (IMC); d, adenocarcinoma (AC); e, osteoclast-like giant cells (OGC). Patient 2: a, OGC; b, normal; c, IDL; d, IMC. Patient 3: a, OGC; b, normal; c, IDL; d, IDL; e, IMC. Patient 4: a, IMC; b, normal; c, no DNA; d, OGC; e, OGC; f, IDL. Patient 5: a, normal; b, IMC; c, OGC; d, IDL. In patient 1 (case 1), arginine mutations are present in the intraductal lesion, the adenocarcinoma, the infiltrating mononuclear, and the osteoclast-like giant cells. For patients (cases) 2, 3 and 4, valine mutations are present in the IDLs, the IMCs, and the OGCs. In patient (case) 5, only the wild-type sequence was noted.
Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17 A and B