Expression of Pancreatitis-Associated Protein (PAP) mRNA in Gastrointestinal Cancers

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Summary

Conclusion. Pancreatitis-associated protein (PAP) mRNA is expressed in some cases of gastric and colorectal cancers resulting from an ectopic expression in dedifferentiated cancer cells.

Background. The PAP gene is identical to the hepatoma-intestine-pancreas (HIP) gene, which is expressed in hepatoma. Expression in cancer might be another characteristic of PAP.

Methods. Fresh surgical specimens of 100 gastrointestinal cancers, 14 benign digestive diseases, and six normal organs were studied with nonisotopic in situ hybridization (ISH) using biotin-labeled cDNA probe.

Results. PAP mRNA was detected in 10% (6/60) of gastric cancers, 21.4% (6/28) of colorectal cancers, 20.0% (1/5) of pancreatic cancers and 0% of biliary tract (three), esophageal (one), and hepatocellular cancers (three). Reverse transcription-polymerase chain reaction (RT-PCR) detected PAP mRNA in these ISH-positive cases. PAP mRNA was not detected in noncancerous portions, benign disease tissues, or normal organs except for the small intestine. There was no relationship between PAP mRNA expression and any clinicopathological factors.

Key Words: Pancreatitis-associated protein (PAP); ectopic expression; gastrointestinal cancers; in situ hybridization (ISH); dedifferentiation.

Introduction

Pancreatitis-associated protein (PAP) was first isolated from pancreatic juice of rats with experimental acute pancreatitis (1). PAP is barely expressed in normal pancreas, but is overexpressed in acute pancreatitis (2). Human PAP has been detected after inflammation induced by pancreatic transplantation (3). Cloning of rat PAP was reported by Iovanna et al. (2) and that of human (4) and mouse (5) homologs has been reported. Rat and human PAP genes were further analyzed by Dusetti et al. (6, 7).

PAP can be used for neonatal screening of cystic fibrosis (8), which involves not only the lung but also the pancreas. PAP is an acute phase protein of the pancreas, and PAP is a better serum marker of pancreatic alteration than exocrine enzymes are. Serum PAP levels are elevated before the onset of cystic fibrosis (8).

On the other hand, hepatoma-intestine-pancreas (HIP) gene was reported to be expressed in hepatocellular carcinoma (9). Surprisingly enough, this HIP
gene is identical to PAP gene (10). There seems to be no relationship between hepatoma and pancreatitis. Expression in cancer might be another characteristic of PAP. Because we preliminarily identified the expression of PAP mRNA in some cancer tissue (11), we attempted to examine more cases of cancers for PAP expression and to evaluate its correlation with various clinicopathological factors.

PAP mRNA has been examined mainly by Northern blot analysis, and there has been no report on analysis of human PAP mRNA expression with in situ hybridization (ISH). Only recently, rat PAP mRNA was demonstrated by ISH in a report on pituitary peptide 23 (12), which is identical to rat PAP based on nucleotide sequence analysis (12). In this study, we utilized the technique of nonisotopic ISH, and reverse transcription-polymerase chain reaction (RT-PCR) was used in some cases in order to confirm the ISH findings. Furthermore, subtypes of PAP mRNA were screened using subtype-specific primers (11).

Materials and Methods

Tissues

One hundred cases of cancers of digestive organs (stomach, 60; colon and rectum, 28; pancreas, 5; biliary tract, 3; liver, 3; esophagus, 1), 14 cases of benign digestive diseases (various liver diseases, 10; chronic pancreatitis, 4), and normal tissues (stomach, 2; small intestine, 2; pancreas, 2) were enrolled in this study. All of the pancreatic cancers were pancreatic ductal adenocarcinomas. Fresh surgical tissues were fixed with 4% paraformaldehyde overnight at 4°C and embedded in paraffin.

In Situ Hybridization

Sections of 4 μm were subjected to ISH. Nonisotopic ISH (13) was performed according to the instruction manual of British Biotechnology Products, Ltd. (Oxon, UK) using a biotin-labeled PAP cDNA probe (275 bp, produced at the Shionogi Research Laboratories, Osaka, Japan). The probe concentration was 200 ng/mL. Hybridization was done at 37°C overnight in a humid chamber (14,15). For the detection of mRNA, an ISH detection kit (DAKO, Carpinteria, CA) was used that included streptavidin and biotinylated alkaline phosphatase with color produced by 5-bromo-4-chloro-3-indolylphosphate in the presence of nitroblue tetrazolium (NBT/BCIP), which yielded a blue precipitate. Two investigators checked the slides independently. If more than 25% of all cancer cells were stained, the specimen was classified as positive; otherwise, it was considered negative (16). Negative controls were examined by substitution of the PAP cDNA probe with biotin-labeled pBR328 probe (Boeringer Mannheim, Mannheim, Germany), RNase A (100 μg/mL) treatment at 37°C for 1 h before hybridization, or the use of the hybridization buffer without the PAP cDNA probe.

RT-PCR

Fresh surgical specimens were taken from both cancerous and noncancerous portions of 17 gastrointestinal cancers (colon, 6; stomach, 8; and pancreas, 3). Total RNA was extracted using an RNA isolation kit (Ultraspec-II®, Biotechx Laboratories, Inc., Houston, TX). RT-PCR was performed using an RT-PCR kit (StrataScript RT-PCR kit®, Stratagene Cloning Systems, La Jolla, CA). In order to reduce nonspecific amplification, a DNA polymerase enhancer (Perfect Match®, Stratagene) was used, following the manufacturer's instructions. Human PAP mRNA was specifically amplified according to Dusetti et al. (7) with sense (5'-GAGACTTCGAGGAAAAATACCC-3') and antisense (5'-ACCTGTAAATTTGCAGACGTAGGG-3') primers, in positions 138–161 and 561–584 of the mRNA, respectively. β-actin was defined by the 5'-CGGAACCGCTCATTGCC-3' and 5'-ACCCACACTCTGCCCATCTA-3' primer sets. Predicted sizes of the mRNA for human PAP and β-actin were 448 and 289 bp, respectively.

Detection of PAP mRNA Subtypes

Three different subtypes of PAP mRNA were screened using subtype-specific primers (11). RT-PCR using four primers (p1, p2, p3, and p4) was performed as reported previously (11). Briefly, oligodeoxynucleotides used as p1, p2, and p3 were forward primers corresponding to the nucleotides –47 to –30, –57 to –39, and –23 to –7 within the 5'-untranslated region of PAP transcript 1, 2, and 3, respectively. The primer p4 was a reverse primer corresponding to nucleotides 289–308 within the coding region (5). The expected sizes of the three subtypes were 351, 358, and 324 bp, respectively.