Identification of Genes Showing Differential Expression in Antisense K-ras-transduced Pancreatic Cancer Cells with Suppressed Tumorigenicity

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ABSTRACT

K-ras point mutation occurs in >80% of pancreatic cancer. We reported previously that the transduction of an antisense K-ras RNA expression vector suppressed the growth of pancreatic cancer cells with K-ras point mutations in vitro and in vivo. The RNA differential display method (DD) was used to compare the mRNA expression profile of the pancreatic cancer cell line AsPC-1 and that of the antisense K-ras-transduced, growth-retarded AsPC-1 cells. cDNA fragments were isolated from 20 bands on the DD gel, and their differential expression between the two cell lines was confirmed. A sequence analysis revealed that all of the 11 clones up-regulated in the antisense-transduced were mitochondrial genes. The other nine cDNA clones that were down-regulated in the antisense-transduced AsPC-1 cells included an oncogene PTI-1 (prostate tumor inducing gene-1), matrix metalloproteinase (MMP)-7, the β3 chain of laminin-5, lysosome-associated membrane protein-2, the H chain of apoferritin, ribosomal protein S6, proteasome subunit XAPC7, and two cDNA fragments with no homology to the GenBank database. In addition to the AsPC-1 cells, reverse transcription-PCR analysis on surgical specimens of pancreatic cancer revealed that the PTI-1 and MMP-7 genes were overexpressed in three and four cases, respectively, of five cases examined. This method offers a unique opportunity to identify a set of genes that may be modulated by K-ras activation, at least in a subset of the pancreatic cancer. The information on such genes may facilitate our understanding of the spectrum of the functional genetic changes in pancreatic cancer.

INTRODUCTION

Various genetic abnormalities accumulate during multistage carcinogenesis. The changes include structural aberrations such as point mutation, amplification, and deletion, as well as functional alterations such as overexpression and down-regulation (1, 2). Although the type and incidence of genetic alteration differs according to the type of human cancer, the most striking example may be the mutations of the K-ras gene, the activation of which by point mutation is found at characteristically high frequencies of 70–90% in pancreatic cancer (3–6). That mutation incidence is then followed by 40–50% in colon and thyroid tumors and 20–30% in lung cancers. In other types of cancers such as gastric (4, 7), esophageal, and liver cancers (4), however, the mutation is observed at low frequencies or not at all.

Like most other cancers, pancreatic cancer does not seem to arise from a single genetic change; other than K-ras mutation, structural abnormalities have been reported for the p53 (8–10), p16 (10), DPC4 (11), c-myc (12), APC (13), RB1 (14), BRCA2 (15), and akt2 (16) genes. Abnormal expression has been noted for basic fibroblast growth factor (17), c-met (18), and DCC (9). However, among the constellation of genetic abnormalities that a single pancreatic cancer cell may harbor, the K-ras mutation appears to be essential for expression of the malignant phenotype, because we reported previously that the expression of antisense K-ras RNA alone effectively suppressed the in vitro and in vivo growth of pancreatic cancer cell lines with K-ras mutation and other genetic abnormalities (19, 20). Moreover, the K-ras mutation is considered as an early event in pancreatic carcinogenesis, because it has been found in intraductal pancreatic cancer, ductal hyperplasia, and even chronic pancreatitis (21).

Considering the almost ubiquitous expression of the K-ras gene, the unusual dependency of pancreatic ductal carcinogenesis on K-ras activation is intriguing but may be explained by one of the following: (a) an unidentified carcinogen/mutagen is uniquely present in the pancreatic tissue and specifically causes K-ras point mutation in the duct; (b) the point mutational activation of this housekeeping gene is too toxic to most cells except a few “K-ras-mediated transfection sensitive” tissues, such as pancreatic duct or colorectal epithelium; or (c) the K-ras mutation leads to the activation of a special set of cancer-related genes only in those “K-ras sensitive” tissues. A signaling cascade downstream of the K-ras protein leading to the mitogen-activated protein kinase activation has been well elucidated. However, if this is the only outcome of the K-ras activation, the K-ras point mutation should have been found in many types of cancers, because the K-ras gene is expressed ubiquitously as a housekeeping gene.

The antisense K-ras-transduced AsPC-1 pancreatic cancer cells with a “revertant” phenotype (19, 20) offer a unique opportunity to analyze the gene expression that is dependent on the K-ras activation. We identified 20 cDNA fragments that showed a differential expression between the parental and antisense K-ras-transduced AsPC-1 cells. All of the 11 clones up-regulated in the antisense-transduced cells were mitochondrial genes. By contrast, the remaining nine clones that were down-regulated by antisense K-ras effect included an oncogene that has not been described before in the context of pancreatic carcinogenesis, a matrix metalloproteinase gene, and two unknown genes.

MATERIALS AND METHODS

Cell Lines and Tissue Specimens. Human pancreatic cancer cell lines AsPC-1, MIA PaCa-2, BxPC-3, PSN-1, Panc-1, Hs 700T, and Hs 766T were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, 1 mm NaHCO3, 2 mm l-glutamine, and penicillin-streptomycin in a humidified atmosphere of 95% air-5% CO2 at 37°C. The AsPC-1, MIA PaCa-2, PSN-1, and Panc-1 cell lines have mutations in codon 12 of the K-ras gene, whereas the BxPC-3, Hs 700T, and Hs 766T cell lines contain wild-type K-ras genes. The K-ras mutation in the AsPC-1 cells is GGT to GAT transition at codon 12, the mutation observed in >50% of the pancreatic cancer patients in Japan (5, 6). AsPC-1-AS (4.3), AsPC-1-AS (4.4), and AsPC-1-AS (4.9) are three independent clones of the AsPC-1 cells transduced with the AS-K-ras-LNSX expression vector. The level of the K-ras p21 protein expression and the growth rate is more suppressed in AsPC-1-AS (4.4) and AsPC-1-AS (4.9) than in AsPC-1-AS (4.3) cells (19). Samples of pancreatic cancer tissues and corresponding noncancerous tissues were obtained from five patients at surgery at National Cancer Center Hospital. The cancerous and noncancerous portions were macroscopically identified and excised by an experienced surgeon.
pathologist. All patients had histologically confirmed adenocarcinoma of the pancreas. The nonpancreatic cancer cells and tissues used in this study were gastric cancer cells, TMK-1 (22), KATO III (23), and MKN28 (24); hepatoma, Alexander and HepG2 (American Type Culture Collection); an erythroleukemia, HEL (American Type Culture Collection); HUVEC (Clonetics), and the human muscle (Clontech). Exons 1 and 2 of the K-ras gene were sequenced for all those nonpancreatic samples and found to be wild-type (data not shown).

**Differential Display Analysis.** Total cellular RNA was extracted from parental AsPC-1 cells and from pooled single-cell clones of antisense K-ras-transduced AsPC-1 cells as described (19). Twenty-five μg of total RNAs were treated at 37°C for 30 min with 20 units of DNase I (TaKaRa, Kyoto, Japan) in 50 μl of 1 × PCR buffer containing 2 units of RNase inhibitor (Promega). The reaction was stopped by extraction with phenol:chloroform (1:1, v/v), and the RNA was ethanol precipitated and resuspended in diethylpyrocarbonate-treated H₂O. Single-stranded cDNA was synthesized from 0.5 μg of total RNA by incubation with 200 units of Superscript reverse transcriptase (Life Technologies, Inc.) at 37°C for 60 min in a 50-μl reaction volume containing 10 mM DTT, 250 μM dNTP and 10 μM one-base anchored oligo-dT primers (T11M, where M denotes G, C, or A; Ref. 25, 26). cDNA (0.2 μg) was amplified in a 40-μl reaction volume using 4 mM anchored primer (T11 G, 5'-TGGCGAGACGT-TTTTGTCC-3'; T11 C, 5'-TGGCGAACGC- TTTTTTGTC-3'; or T11 A, 5'-TGGCGAAGCCTTTTTTTTTTTTT-3'). 4 mM arbitrary primer (LH-A1, 5'-TGGCCAGATCGCTACAGC-3'; LH-A2, 5'-TGGCGCAATCGTGCAG-3'; LH-A3, 5'-TGGCGAACGTGGAGC- CTG-3'; LH-A4, 5'-TGGCGCAAGCTTGTGAGG-3'; LH-A5, 5'-TTGC- GAAGCTTGTGAGG-3'; LH-A7, 5'-TGGCGCAAGCTTGTGAGG-3'; or LH-A10, 5'-TGGCGCAAGCTTGTGAGG-3'). Amplified cDNA was treated at 37°C for 30 min with 20 units of DNase I (TaKaRa, Kyoto, Japan) before and after antisense K-ras-transduced, transformation-suppressed cells were the mitochondrial genes (Fig. 1, A5, A10). The list of primers used for differential display is shown in Table 1. Remarkably, all of the 11 clones that were up-regulated in the antisense K-ras-transduced, transformation-suppressed cells were the mitochondrial genes, which have been implicated in PTI-1, EGF, and the human muscle (Clontech). Exons 1 and 2 of the K-ras gene were sequenced for all those nonpancreatic samples and found to be wild-type (data not shown).

**RT-PCR Analysis on Cell Lines.** Amplified cDNA from various sources (AsPC-1, MiAPaCa-2, PSN-1, BxPC-3, Panc-1, HS 700T, HS 766T, KATO III, MKN28, TMK-1, HepG2, Alexander, HUVEC, HEL, and human muscle) were synthesized as described above and subjected to PCR. The primer sequences (and expected sizes of the PCR products) were: PTI-1 (28), 5'-AGGTTGAGCAGCTG-3' and 5'-AACACGCAAGC CAAAAT CAG-3' (252 bp); MMP-7 (29), 5'-TCTTTGGCTTACCTATAACGG-3' and 5'-CTAAGTGCTACCACTCATGTC-3' (420 bp); and β-actin (30), 5'-GAC- TACCTGGAGATCCT-3' and 5'-GGCGATGCTCAGCCTAC-3' (313 bp). PCR amplification for PTI-1 was performed as 30 cycles of 94°C for 45 s, 60°C for 1 min, and 72°C for 1 min. The thermal cycle profile for MMP-7 was 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. Samples were subjected to electrophoresis on 2% agarose gels and stained with ethidium bromide.

**Results**

**RT-PCR Analysis on Surgical Specimens.** One μg of total RNA extracted from the tissues was incubated at 25°C for 10 min, 42°C for 60 min, and 70°C for 15 min with 200 units of Superscript reverse transcriptase (Life Technologies, Inc.) in a 40-μl reaction volume containing 1 μM dNTP. 2 units of RNAse H (Promega), and 10 μM random hexanucleotide primer (TaKaRa, Kyoto, Japan). PTI-1 and MMP-7 expression was determined by semiquantitative RT-PCR based on comparison with the β-actin expression as an internal reference. The 5' ends of the sense primers were labeled with [γ-32P]ATP and T4 polynucleotide kinase, and cDNA from AsPC-1 cells was amplified by PCR for 15–30 cycles with each primer set as described above. The PCR products were separated on 6% polyacrylamide gels, and radioactivity levels of the bands were quantified by Bio Imaging Analyzer (BAS 2000; Fujix, Tokyo, Japan). The range of linear amplification for both the target (PTI-1 or MMP-7) and the reference β-actin genes was determined, and the optimal number of amplification cycles for semiquantitative PCR was found to be 20. Under this condition, a semiquantitative RT-PCR was performed on surgical specimens using the unlabeled primers. For PTI-1 expression analysis, the PCR products were hybridized with the PTI-1-specific Bridge Region probe (Fig. 2A) to further assure the specificity of the analysis. The relative increase in the band intensity was confirmed using the BAS 2000 imaging analyzer.

**Detection of the K-ras Mutations in Surgical Specimens.** The K-ras cDNA fragment was amplified by RT-PCR from total RNAs obtained from surgical specimens of pancreatic cancer as described (19). Mutations at codons 12, 13, and 61 were subsequently determined by direct sequencing using the Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer Corp., Foster City, CA) and the ABI PRISM 310 DNA sequencer (Perkin-Elmer Corp., Foster City, CA).

**RESULTS**

**DD and cDNA Southern Blot Analysis of Antisense K-ras-Transduced AsPC-1 Cells.** The bands that showed significant differences on the DD analysis between the parental and antisense K-ras-transduced AsPC-1 cells were cloned and hybridized to cDNA from the respective cells to confirm the difference in the expression level. The bands marked by an arrow in Fig. 1A were cloned and hybridized to cDNA (Fig. 1, B and C).

In total, 20 DD clones were found to be differentially expressed before and after antisense K-ras transduction, and their identity is listed in Table 1. Remarkably, all of the 11 clones that were up-regulated in the antisense K-ras-transduced, transformation-suppressed cells were the mitochondrial genes (Fig. 1B). On the other hand, the genes down-regulated by antisense K-ras included seven known genes (Fig. 1C) and two clones, DD53 and DD134, which showed no homology to the current database. Cloning of full-length cDNAs for the two unknown clones are under way, and we are analyzing the PTI-1 and MMP-7 genes, which have been implicated in transformation and tissue invasion, respectively.

**References**

1. The abbreviations used are: HUVEC, human umbilical vein endothelial cell; dNTP, deoxynucleotide triphosphate; RT-PCR, reverse transcription-PCR; PTI-1, prostate tumor-inducing gene 1; MMP, matrix metalloproteinase; DD, differential display; UTR, untranslated region; EF, elongation factor.
PTI-1 Expression and cDNA Southern Blot Analysis of PTI-1 and MMP-7. The reported full-length PTI-1 cDNA has a unique structure consisting of 630-bp 5' UTR with significant homology with *Mycoplasma hyopneumoniae* 23S rRNA gene fused to a truncated and mutated EF-1α (31). DD17 corresponds to the 5' UTR of PTI-1, nucleotide positions 431–605, and it appears that the anchored primer T11C annealed to the sequence 5'-AAAAAG-3', at positions 431–436. To confirm that DD17 represents the PTI-1 oncogene and not the contaminating *Mycoplasma*, PTI-1-specific RT-PCR primers were used to amplify the “Bridge Region” (28) of PTI-1, which spans the *Mycoplasma*-homology UTR and truncated EF-1α coding region (nucleotide positions 537–788; Fig. 2A). The product of the expected size (252 bp) was amplified from the RNA of the AsPC-1 cells (Fig. 2B), and its sequence was confirmed to be identical to the sequence of the Bridge Region. The Bridge Region sequence was not detected by RT-PCR in RNAs of the antisense K-ras-transduced clones AsPC-1-AS (4.4) and AsPC-1-AS (4.3) (Fig. 2B). By cDNA Southern blot analysis, however, the Bridge Region probe weakly hybridized to the amplified cDNA of the AsPC-1-AS (4.3) cells, but not to that of the AsPC-1-AS (4.4) and AsPC-1-AS (4.9) cells (Fig. 2C). A similar finding also obtained for the MMP-7 (clone 135 in Table 1) probe (Fig. 2D). We have reported previously that the degree of p21 K-ras protein suppression and hence the growth inhibition was less in the AsPC-1-AS (4.3) cells than the AsPC-1-AS (4.4) and AsPC-1-AS (4.9) cells (19). The Bridge Region sequence of the PTI-1 gene was detected in the genomic DNAs from AsPC-1, MI-APaCa-2, BxPC-3, and PSN-1 cell lines as well as the human peripheral blood lymphocytes by PCR, and the amplified bands hybridized correctly to the Bridge Region probe (data not shown).

Expression of PTI-1 and MMP-7 in Pancreatic Cancer Cell Lines and Various Cell Lines. In contrast to the AsPC-1 cells, the 252-bp Bridge Region fragment of the PTI-1 gene was not detected by RT-PCR in other six pancreatic cancer cell lines examined (MI-
suggesting that PTI-1 is expressed only in a subset of the pancreatic cancers. A very small amount of multiple PCR products, 252 bp, were observed in MIAPaCa-2, PSN-1, and Hs 700T cells (Fig. 3A). Although those bands hybridized to the Bridge Region probe, their significance remains to be shown. In cells other than the pancreatic cancer cells, the PTI-1 expression was detected by RT-PCR in TMK-1 and Alexander cells derived from a gastric cancer and hepatoma, respectively, but it was not detectable in the KATO III and MKN28 (gastric cancers), HepG2 (hepatoma), HEL (erythroleukemia) cell lines, HUVEC, or in human muscle (Fig. 3A). In addition to AsPC-1, MMP-7 was expressed in two other pancreatic cancer cell lines, Panc-1 and BxPC-3, as well as in a gastric cancer cell line KATO III and HUVEC (Fig. 3B). We confirmed that BxPC-3, Hs 700T, Hs

Table 1 List of clones that showed significant differences in the level of expression in the antisense K-ras-transduced AsPC-1 cells

<table>
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<th>Clone ID</th>
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</thead>
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<tr>
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<td>Mitochondrial 16s rRNA gene</td>
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<tr>
<td>DD 8</td>
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<td>DD 9</td>
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</tr>
<tr>
<td>DD 23</td>
<td>273</td>
<td>Mitochondrial cytochrome oxidase subunit 1</td>
</tr>
<tr>
<td>DD 14</td>
<td>271</td>
<td>Mitochondrial NADH dehydrogenase</td>
</tr>
<tr>
<td>DD 26</td>
<td>151</td>
<td>Mitochondrial NADH dehydrogenase subunit 4</td>
</tr>
<tr>
<td>DD 16</td>
<td>190</td>
<td>Mitochondrial ATP synthase</td>
</tr>
</tbody>
</table>

Clones that showed decreased expression in the antisense-transduced cells

<table>
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<th>Identity with human sequences in GenBank</th>
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</thead>
<tbody>
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<td>175</td>
<td>PTI-1</td>
</tr>
<tr>
<td>DD 135</td>
<td>133</td>
<td>MMP-7</td>
</tr>
<tr>
<td>DD 46</td>
<td>169</td>
<td>Laminin-5 β3 chain</td>
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<tr>
<td>DD 28</td>
<td>150</td>
<td>Lysosome-associated membrane protein-2</td>
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<tr>
<td>DD 10</td>
<td>219</td>
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<td>DD 53</td>
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<tr>
<td>DD 134</td>
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Fig. 2. A, structure of PTI-1 cDNA and its relationship with the EF-1α gene and M. hyopneumoniae genome. BR, Bridge Region, is the PTI-1-specific fragment aimed as a target of PCR. B, RT-PCR analysis of Bridge Region to examine PTI-1 expression in parental AsPC-1 cells and the antisense K-ras-transduced AsPC-1 clones. M, 100-bp ladder size marker; Lane 1, parental AsPC-1 cells; Lane 2, AsPC-1-AS (4.4); Lane 3, AsPC-1-AS (4.3). C, cDNA Southern blot analysis of parental AsPC-1 cells and the antisense K-ras-transduced AsPC-1 clones. Probes are: PTI-1 fragment (upper panel) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH; lower panel). Lanes 1–3, same as B; Lane 4, parental AsPC-1 cells; Lane 5, AsPC-1-AS (4.9). D, cDNA Southern blot analysis of parental AsPC-1 cells and the antisense K-ras-transduced AsPC-1 clones. Probes are: MMP-7 fragment (upper panel) or GAPDH (lower panel). Lanes 1–5, same as C.
NADH dehydrogenase genes. One of the hallmarks of cancer cells is risk and progression deficient mitochondrial content (32). In colon cancers, even the cancer demonstrated previously that highly glycolytic tumor cells had a markedly mitochondrial mediated oxidative phosphorylation, and it was dem-

their propensity to depend on glycolytic metabolism and diminution in abnormally low expression of the mitochondrial cytochrome c3 of patients #1 (GGT ras K- gene). GAT), and #4 (no mutation in codon 12, 13, or 61 of the 3 ras carcinogenic transformation. show a similar change in mitochondrial gene expression during their mitochondrial genes, such as the 16S rRNA, cytochrome oxidase, ras DISCUSSION on April 19, 2017. © 1999 American Association for Cancer Research.

Among the genes that were down-regulated in the antisense K-ras-transduced AsPC-1 cells, we focused first on the PTI-1 gene, because it is an oncogene that has not been implicated in pancreatic cancer before. The antisense K-ras-induced suppression of PTI-1 gene expression was observed in three independent antisense-transduced clones, and the suppression of PTI-1 gene expression was greater in the clones AsPC-1-AS (4.4) and (4.9), which showed a higher degree of K-ras p21 protein suppression. Moreover, infection of the AsPC-1 cells with an antisense K-ras RNA expression adenovirus vector induced growth suppression and decreased expression of the PTI-1

766T, and all of the nonpancreatic cells and tissues have a wild-type K-ras gene (data not shown).

Expression of PTI-1 and MMP-7 in Surgical Specimens. To explore the involvement of the PTI-1 and MMP-7 gene activation in human pancreatic cancer in vivo, five surgical specimens were analyzed by semiquantitative RT-PCR. The PTI-1 expression was elevated in cancerous pancreatic tissues as compared with the noncan-
cerous tissues in patients #2 (GGT→GAT mutation in codon 12), #3 (GGT→GAT), and #4 (no mutation in codon 12, 13, or 61 of the K-ras gene). PTI-1 expression was not increased in cancerous tissues of patients #1 (GGT→CGT) and #5 (GGT→CGT) (Fig. 4A). MMP-7 expression in the cancerous tissues were higher than the normal counterparts in all of the cases except patient #4 (Fig. 4B).

DISCUSSION It is noteworthy that all of the 11 DD clones that were up-regulated in the antisense K-ras-transduced AsPC-1 cells turned out to be mitochondrial genes, such as the 16S rRNA, cytochrome oxidase, or NADH dehydrogenase genes. One of the hallmarks of cancer cells is their propensity to depend on glycolytic metabolism and diminution in mitochondrially mediated oxidative phosphorylation, and it was demonstra-

Presented formerly that highly glycolytic tumor cells had a markedly deficient mitochondrial content (32). In colon cancers, even the cancer risk and progression in vivo were found to be associated with an abnormally low expression of the mitochondrial cytochrome c oxidase subunit 3 (33). Although no previous study has reported the down-regulation of mitochondrial genes during the course of pancreatic carcinogenesis, two ultrastructural studies, one on human pancreatic carcinomas (34) and the other on hamster pancreatic carcinomas (35), described a paucity of mitochondria as a feature of the pancreatic duct adenocarcinoma. However, the role of mitochondria appears more complex, and the cancer phenotype, at least a part of it, depends on the multifunctional organelles (36, 37).

More recently, mitochondrion was found to be the reservoir for key components involved in apoptosis (38), probably playing a pivotal role in coordinating cell proliferation and death. Our data suggest that alteration of the mitochondrion is one of the major elements in K-ras-induced transformation in AsPC-1 cells. It remains to be shown whether other pancreatic cancer cells with or without K-ras mutation show a similar change in mitochondrial gene expression during their carcinogenic transformation.

Fig. 3. Expression of PTI-1 (A) and MMP-7 (B) in pancreatic cancer and other cell lines. In A, the PTI-1-specific Bridge Region fragment was detected. β-Actin was analyzed as an internal control for both A and B. M, 100-bp ladder size marker; Lane 1, AsPC-1; Lane 2, MIAPaCa-2; Lane 3, PSN-1; Lane 4, BaPC-3; Lane 5, Panc-1; Lane 6, Hs 700T; Lane 7, Hs 766T; Lane 8, KATO III; Lane 9, MKN28; Lane 10, TMK-1; Lane 11, HepG2; Lane 12, Alexander; Lane 13, HUVEC; Lane 14, HEL; Lane 15, human muscle.

Fig. 4. K-ras point mutation status (A) and expression of PTI-1 (B) and MMP-7 (C) in human pancreatic cancer tissues. In B, the Bridge Region fragments amplified in the exponential phase of PCR were transferred onto a nylon membrane and hybridized with a 32P-labeled Bridge Region probe. wt, wild-type K-ras sequence. For cases 1 to 5, N and T, noncancerous and cancerous tissues, respectively. M, 100-bp ladder size marker.
gene as compared with the parental AsPC-1 cells. Thus, we conclude that the PTI-1 gene expression is indeed functionally modulated by the K-ras activation status in the AsPC-1 cells, and the clonal variation is not the reason for the decreased PTI-1 expression in the three transfected AsPC-1 clones.

PTI-1 was originally isolated as a transforming gene from human prostate carcinoma DNA (39). PTI-1 has a unique fusion structure consisting of the 5' UTR with a high homology to the 23S rRNA gene from Mycoplasma hominis, juxtaposed to a sequence that encodes a truncated and mutated human EF-1α (31). A genomic PCR using primers to amplify the 5'-untranslated, Mycoplasma-homology region of PTI-1 demonstrated that PTI-1 is present in human brain and kidney tissues (28). Moreover, our PCR-Southern blot analysis showed the presence of the Bridge Region, which spans the 5' Mycoplasma region and the 3' EF-1α region of PTI-1 in genomic DNAs obtained from human pancreatic cancer cell lines and peripheral blood mononuclear cells. These genomic analyses indicate that the transforming gene PTI-1 is an authentic human gene.

In addition to prostate cancer, PTI-1 expression has been reported in breast, colon, and lung carcinoma cell lines (28, 31). Our study has added the PTI-1 gene to the list of the possible genes involved in pancreatic carcinogenesis. It seems that the PTI-1 gene is activated only in a subset of pancreatic cancer; three of five surgical specimens of pancreatic cancer showed increased PTI-1 gene expression, and one of the three cases had no K-ras mutation at codons 12, 13, or 61. The elevated expression of the gene should not be the result of the relative enrichment of ductal cells in the carcinous tissues, because six cell lines and two similar surgical specimens of pancreatic ductal carcinoma showed low or undetectable PTI-1 gene expression. The role of PTI-1 in the pancreatic carcinogenesis may be a modulation of the cancer phenotype, the nature of which awaits further studies.

Another apparently cancer-related gene in Table 1 was MMP-7, one of the matrix metalloproteinases implicated in cancer invasion. Recently, a study showed that mRNA expression of MMP-7 was higher in pancreatic carcinoma than in the normal pancreas (40), an observation congruent to our data. As in the case with PTI-1, MMP-7 expression was also observed in the cells without K-ras mutation, and some pancreatic cancer cells with K-ras mutation did not show an increased MMP-7 transcription, suggesting the presence of a molecular pathway other than the K-ras mutation to modulate the expression of these genes.

Laminin-5 is also considered to play a role in tissue invasion of cancer cells. The anchoring filament protein has been shown to be synthesized and deposited by pancreatic cancer cells in the basement membrane in an abnormal manner (41). In contrast to MMP-7 and laminin-5 β3, the other four known genes found to be down-regulated in the antisense-transduced cells have not been described in the context of pancreatic carcinogenesis. However, available information suggests that involvement in a malignant phenotype is also conceivable for the lysosomal membrane-associated glycoproteins 1 and 2 (42–44), apoferritin (45), ribosomal protein S6 (46), and proteasome subunit XAPC7 (47, 48).

In sum, 20 clones have been isolated from the DD gel to compare the parental and growth-suppressed, antisense K-ras-transduced pancreatic cancer cells. Eighteen clones turned out to be known genes, and two of them, those encoding MMP-7 and the laminin-5 β3 chain, have been reported by others to be overexpressed in pancreatic cancers. The other 16 clones corresponded to nine distinct genes, including four mitochondrial genes. A literature survey suggests that all of them could play a role in cancers and/or cellular transformation, although no report except the present study has suggested their involvement in pancreatic cancers.

On the other hand, this analysis also revealed a heterogeneity of the pancreatic cancer cells, because K-ras-induced overexpression of the PTI-1 and MMP-7 genes was not a universal phenomenon among the pancreatic cancers; some pancreatic cancer cells and tissues with K-ras mutation did not overexpress those genes, and some with the wild type K-ras gene appeared to have increased expression. Although it is conceivable that PTI-1 and MMP-7 contribute to the expression of a certain phenotype in a subset of pancreatic cancers, they may not belong to a set of genes that are crucial and indispensable for the process of pancreatic carcinogenesis. The biological significance of those genes in the pancreatic cancer should be determined by further investigation.

The experimental strategy of antisense K-ras transduction to pancreatic cancer cells and mRNA DD has been generating more clones that await characterization, and it may enhance our understanding of the oncogenic mechanisms of K-ras activation and/or pancreatic carcinogenesis.

REFERENCES
GENE EXPRESSION IN ANTISENSE K-ras-TRANSUCED CELLS


Identification of Genes Showing Differential Expression in Antisense K- ras-transduced Pancreatic Cancer Cells with Suppressed Tumorigenicity

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