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# Human Pancreatic Ribonuclease 1

## *Expression and Distribution in Pancreatic Adenocarcinoma*

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**BACKGROUND.** Human pancreatic ribonuclease (RNase 1) is a pancreatic enzyme that is present at high levels in the serum of most patients with pancreatic adenocarcinoma. For this reason, the authors studied its patterns of expression at the single-cell level in pancreatic adenocarcinoma tissues by immunohistochemical analysis and in situ hybridization (ISH).

**METHODS.** Immunohistochemical analysis with polyclonal antibodies against RNase 1 and by ISH with digoxigenin-labeled RNase 1 probe were used to detect RNase 1 in the neoplastic cells of ductal type pancreatic adenocarcinomas.

**RESULTS.** Fifteen of 18 carcinoma samples were positive for RNase 1, demonstrating that the expression of ribonuclease that the authors observed previously in human pancreatic adenocarcinoma cell lines was not an artifact of cell culture. The authors also found RNase 1 in some of the metaplastic ducts and atrophic islets in 4 of 6 chronic pancreatitis samples, and they observed RNase 1 immunostaining in hyperplastic ducts adjacent to one of the well-differentiated adenocarcinomas.

**CONCLUSIONS.** The expression levels of RNase 1 by tumor cells from pancreatic adenocarcinomas are consistent with the high RNase 1 levels found in the serum of most patients with pancreatic adenocarcinoma. This expression of RNase 1, which is an acinar protein, demonstrates that the patterns of gene expression in pancreatic adenocarcinoma are distinct from those of normal pancreatic duct cells. Conversely, RNase 1 expression levels in altered ductal cells from some chronic pancreatitis tissues and hyperplastic ducts from carcinoma tissues suggest that abnormal expression levels may be an early event in pancreatic tumorigenesis.
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**KEYWORDS: ribonuclease, pancreatic carcinoma, tumor markers, protein expression.**

Pancreatic adenocarcinoma is the fourth to fifth leading cause of cancer-related mortality in Western countries.[1,2] Patients usually die within 1 year of diagnosis because of the disease's aggressiveness and resistance to chemotherapy and radiotherapy.[3] Identifying sensitive and specific tumor markers for the early detection of pancreatic adenocarcinoma and also developing new therapeutic strategies for the treatment of these patients would reduce mortality.

Pancreatic-type ribonuclease, or ribonuclease 1 (RNase 1), is a digestive enzyme that is produced and secreted by the acinar cells of the pancreas. Its expression also has been found in other human tissues[4] although in a minor quantity. High levels of RNase 1 have been detected in the sera of most patients with pancreatic adenocarcinoma; however, they do not seem to be specific to this type of neoplasm.[5,6] Moreover, the origin of these elevations is still unknown. It has been described, however, that RNase 1 levels, together with other tumor markers, are of value in cancer diagnosis.[7] Our interest

has been to determine whether these high RNase 1 levels could originate from pancreatic adenocarcinoma cells. Previously, we demonstrated that RNase 1 is expressed by pancreatic adenocarcinoma cell lines.[8–10] In this paper, we extend those studies to sections from patients with pancreatic adenocarcinoma and from normal pancreas.

We describe the distribution and expression of RNase 1 in ductal type pancreatic adenocarcinoma tissues and in normal pancreas. The studies were performed using immunohistochemical analysis and in situ hybridization (ISH). The findings presented in this paper describe that RNase 1 is expressed in most pancreatic adenocarcinomas of a variety of histologic types, including well-differentiated, moderately differentiated, and poorly differentiated carcinomas, and in some metastatic tissues. We also studied the distribution of RNase 1 in samples from patients with chronic pancreatitis, because it has been associated with an increased risk for developing pancreatic carcinoma.[11,12]

## MATERIALS AND METHODS

### Tissue Specimens

Twenty-four pancreatic carcinoma tissue specimens and six chronic pancreatitis tissue specimens were obtained from patients who underwent surgical resection at the M. D. Anderson Cancer Center. The median age of the patients with pancreatic carcinoma was 57 years (range, 41–76 years). These patients included 17 males and 7 females with exocrine adenocarcinomas of the duct cell type (18 primary tumors and 6 metastases). Histologically, there were six well-differentiated tumors, six moderately differentiated tumors, and six poorly differentiated tumors. The six chronic pancreatitis tissue samples were from 6 patients (4 males and 2 females; median age, 54 years; range, 42–65 years) who were without any pancreatic malignancy. The median follow-up for the pancreatitis patients was 5 years. During this period, none of the patients developed pancreatic carcinoma. A normal pancreas was obtained from the resection margin of one pancreatic neoplasm. All specimens were fixed in 10% formalin, embedded in paraffin, and cut into 5-$\mu$m serial sections.

### Antibodies

Recombinant human pancreatic ribonuclease (rRNase 1) was a generous gift from Dr. M. Seno.[13] Antibodies to this protein were raised as follows: 0.5 mg of the recombinant protein diluted in 1 mL of phosphate-buffered saline (PBS) was mixed with 1 mL of Freund complete adjuvant and injected subcutaneously into New Zealand White rabbits in five sites. Additional immunizations with 0.5 and 1 mg of the protein with Freund incomplete adjuvant were given after 7 days and 32 days, respectively. Serum was isolated 1 week after the last injection. Antibodies were purified by protein A chromatography. The working dilution was 1:2000 (1.5 mg/mL). The antibody's specificity was tested by Western blot analysis and enzyme-linked immunosorbent assay; the antibody did not cross react with bovine RNase, with human RNases 2–5, or with other proteins (lysozyme, albumin; data not shown).

Monoclonal antibody LK2H10 ($IgG_1$, K) against human chromogranin-A was obtained from Biogenex (San Ramon, CA). The working dilution was 1:1000. Guinea pig antibody against porcine pancreatic insulin, which cross reacts with human insulin, was obtained from Dako (Carpinteria, CA). The working dilution was 1:2500.

### Staining Methods

Immunohistochemical staining was performed by using the avidin-biotin-peroxidase complex technique (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA). All incubations were performed at room temperature in a humid chamber.

After deparaffinization and rehydration, the sections were immersed in 3% $H_2O_2$ in absolute methanol for 10 minutes to block endogenous peroxidase activity and then washed three times with distilled $H_2O$. The slides were placed in 10 mM sodium citrate, pH 6, and microwaved on high for 10 minutes. Next, they were washed 3 times with PBS, pH 7.4, and nonserum protein block (Dako) was applied for 10 minutes and then removed. After washing 3 times with PBS, normal goat serum (when staining with polyclonal antibodies) or normal horse serum (when staining with monoclonal antibodies) was applied for 20 minutes and removed by blotting. The sections then were incubated with primary antibody diluted in normal goat/horse serum for 120 minutes at room temperature, washed three times in buffer, incubated with the biotinylated secondary antibody goat antirabbit/guinea pig (for polyclonal antibodies) or horse antimouse (for monoclonal antibodies) for 30 minutes, washed with PBS, incubated for 45 minutes with avidin-peroxidase conjugate, and washed repeatedly. Finally, the sections were reacted with diaminobenzidine and hydrogen peroxide (stable DAB) (Research Genetics, Huntsville, AL) for 3 minutes, washed, counterstained with hematoxylin, rinsed in tap water, and mounted. For a negative control, preimmune serum instead of a primary antibody was used.

### ISH

The DNA probe for human RNase 1 was generated by polymerase chain reaction (PCR). The primers used were based on the previously reported sequence for RNase 1 cDNA[14] and were 5′-GGGTCCAGCCTTCCCT-GGG-3′ (primer 1) and 5′-ACACAGTAGCAT-CAAAGTGG-3′ (primer 2).

PCR was performed with human blood DNA as template at 94 °C for 3 minutes followed by 35 cycles of denaturation at 94 °C for 90 seconds, annealing at 53 °C for 90 seconds, and elongation at 72 °C for 1 minute. Total blood DNA (0.5 $\mu$g) and 20 ng of each primer were used in the PCR reaction. The PCR products were purified by agarose gel electrophoresis.

For ISH, the DNA probe was cloned into the *Srf*I site of pCR-Script™ (Stratagene, La Jolla, CA), which has flanking T7 and T3 RNA polymerase promoters. The resulting plasmid was purified, linearized with *Not*I and *BamH*I, and used to generate antisense and sense riboprobes with T7 and T3 RNA polymerases, respectively.

Digoxigenin (DIG)-labeled antisense and sense (negative control) probes were transcribed in vitro with T7 and T3 RNA polymerase. The probes were synthesized in the presence of digoxigenin-UTP at 37 °C for 2 hours and digested with pancreatic RNase free DNase at 37 °C for 15 minutes to remove DNA templates. The probes were purified with a Sephadex G-50 RNase free spin column (Roche Diagnostics, Basel, Switzerland) and quantified using DIG-RNA control (Roche Diagnostics).

ISH was performed as described elsewhere.[15] Briefly, sections were deparaffinized in xylene and then rehydrated in decreasing concentrations of alcohol. Fixation was performed with 4% of buffered formaldehyde for 20 minutes, followed by Proteinase K digestion (0.04 mg/mL in 50 mM Tris-HCl and 5 mM ethylenediamine tetraacetic acid [EDTA], pH 8.0) for 7.5 minutes. After a postfixation step with 4% buffered formaldehyde for 5 minutes, sections were treated with 0.1 M triethanolamine, pH 8, and 0.25% acetic anhydride for 10 minutes and dehydrated in increasing concentrations of alcohol. Hybridization was performed at 60 °C for 18 hours in a hybridization buffer (50% formamide; 300 mM NaCl; 10 mM Tris-HCl, pH 8.0; 5 mM EDTA, pH 8.0; 1 × Denhardt solution; 10% dextrane sulphate; 0.2 mg/mL sperm DNA; and 10 mM dithiothreitol) containing the labeled riboprobe (2–3 ng/$\mu$L) in a humid chamber. Posthybridization washes were with 50% formamide, 2 × standard saline citrate (SSC) at 55 °C; digestion of any single-stranded RNA probe with bovine RNase A (0.02 mg/mL) and T1 RNase (20000 U) at 37 °C; and final washings with 2 × SSC and 0.1 × SSC at 50 °C. Slides were blocked with 1% blocking reagent (Roche Diagnostics) for 30 minutes, followed by incubation of alkaline phosphatase antidigoxigenin antibody (Roche Diagnostics) with a 1:100 dilution in 0.5% blocking reagent for 2 hours. After washings, sections were developed in 4-nitro blue tetrazolium chloride (NBT)/5-bromo-4-chloro-3-indolyl-phosphate 4-toluidine salt (BCIP) for 2–16 hours in the dark, washed and counterstained with 0.2% nuclear fast red, and mounted. Sense riboprobes and negative samples were used as controls for ISH.

## RESULTS

Immunohistochemical staining of the normal pancreatic tissue with anti-RNase 1 polyclonal antibodies showed that the acinar cells from normal pancreatic tissues were positive for this protein. The amount of enzyme was variable among the acinar cells, appearing to be a little more abundant in the periinsular acini (Fig. 1, top). Ribonuclease immunoreactivity also was observed in the lumen of the ducts, which is to be expected if ribonuclease is secreted into the duodenum through the ducts (Fig. 1, top). Virtually no duct cells were positive. We observed a small amount of positively stained material within the islet cells. To corroborate these results, ISH was performed with an RNase 1 probe. Abundant expression of RNase 1 mRNA was detected in the acinar cells as well as a little expression in some islet cells. No expression was observed in ductal cells (Fig. 1, bottom).

Eighty-three percent of the pancreatic adenocarcinomas were immunoreactive with antibodies against RNase 1. This protein was detected in the cytoplasm of neoplastic cells in 5 of 6 poorly differentiated adenocarcinomas, in 5 of 6 moderately differentiated ductal carcinomas, and in 5 of 6 well-differentiated ductal carcinomas (Fig. 2, top). The ISH confirmed the immunohistochemical results: The expression of RNase 1 mRNA was detected in the ductal type cells of pancreatic carcinomas (Fig. 2, bottom).

We also studied the expression of this enzyme in metastases, because RNase 1 is expressed in Capan-1,[8,10] which is a human pancreatic adenocarcinoma cell line that was established from a liver metastasis of a well differentiated pancreatic carcinoma. However, of six metastases (five from the liver and one lymph metastasis), only one, a liver metastasis, was positive for RNase 1 immunoreactivity (Fig. 3, top). The expression of RNase 1 in the tumor tissues was detected not only in the tumor cells. RNase 1 also was observed in the cytoplasm of normal islet cells that were adjacent to the tumoral mass and that were surrounded by fibrotic tissue (Fig. 3, bottom). These islet cells were positive when stained for chromogranin or insulin

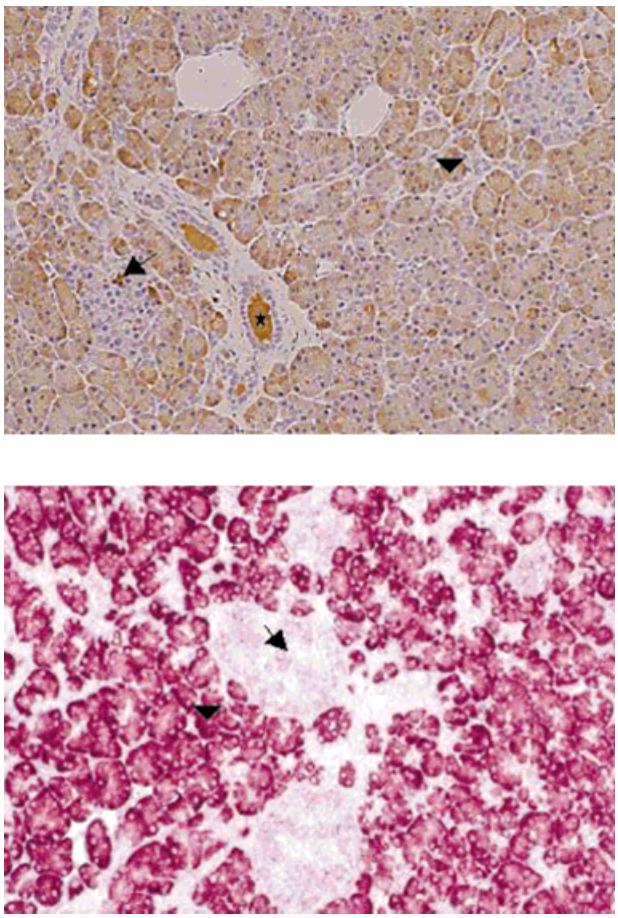

**FIGURE 1.** Ribonuclease 1 (RNase 1) expression in normal pancreas. Representative examples of RNase 1 immunostaining (top) and in situ hybridization (bottom) in the normal pancreas. RNase 1 immunoreactivity was clear in the acinar cells (arrowheads) and in the lumen of the ducts (star, top). Faint staining was observed in some islet cells (arrows) (original magnification, ×500).

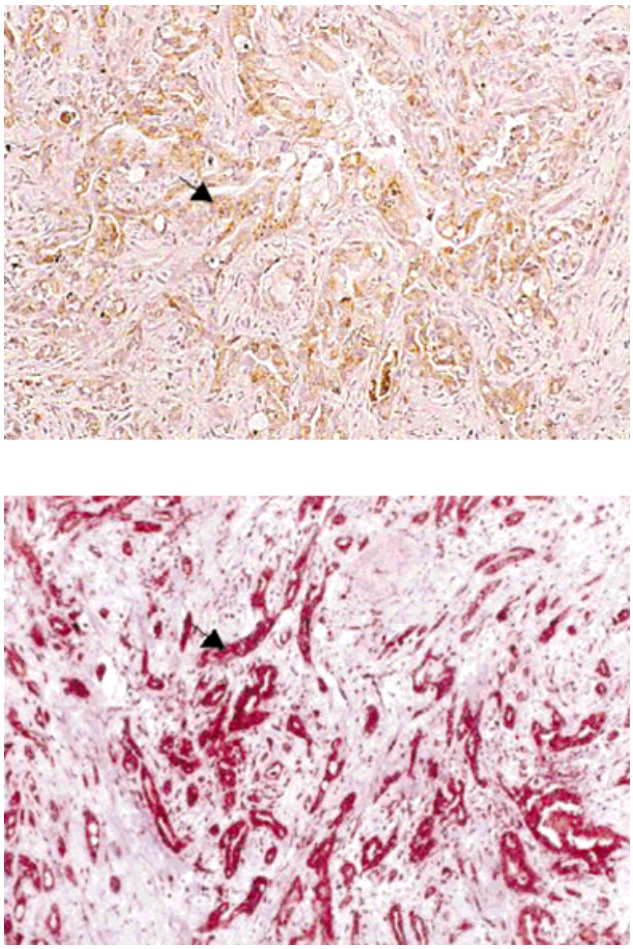

**FIGURE 2.** Ribonuclease 1 (RNase 1) expression in pancreatic adenocarcinoma. (Top) RNase 1 immunostaining was present in tumor cells from a patient with pancreatic adenocarcinomas. (Bottom) RNase 1 expression in tumor cells from a moderately differentiated adenocarcinoma by in situ hybridization. Arrows indicate tumor cells (original magnification, ×500 on the top, ×250 on the bottom).

(not shown), confirming their endocrine character. In addition, RNase 1 also was detected weakly in hyperplastic ducts adjacent to a well differentiated carcinoma (Fig. 4, top).

Several chronic pancreatitis tissues also were analyzed. Four of six samples from these tissues showed RNase 1 staining. The protein was located in some ducts with squamous metaplasia, in dilated ducts (Fig. 4, bottom), and in some isolated atrophic islets. Pancreatitis tissues that were negative for RNase 1 had less fibrosis.

## DISCUSSION

The general expression of RNase 1 in pancreatic adenocarcinoma tumors of different histologic types is described. About 80% of the tumors were positive for this enzyme. RNase 1 also is present in the serum from many pancreatic adenocarcinoma patients.[16,7] The origin of the elevated ribonuclease in serum of pancreatic adenocarcinoma patients is not known, but the results presented in this article describing the production of RNase 1 by pancreatic tumor cells may help to explain these observations. The enzyme may be shed by the tumor into the blood stream. If this is true, then the RNase 1 expression in pancreatic adenocarcinoma tissues likely correlates with the RNase 1 found in the blood.

Conversely, RNase 1 expression in pancreatic adenocarcinoma tissues corroborates our previous studies that described the expression of RNase 1 at the mRNA and protein levels in several human pancreatic adenocarcinoma cell lines with different degrees of differentiation.[8–10] These results also revealed an al-

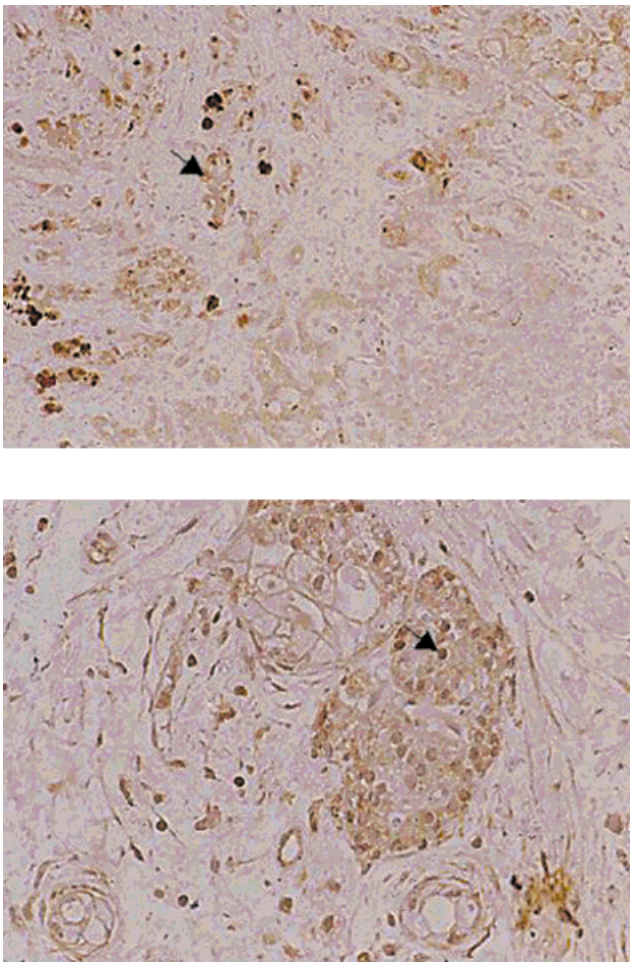

**FIGURE 3.** Ribonuclease 1 (RNase 1) expression in metastasis and islet cells of pancreatic adenocarcinomas. (Top) RNase 1 was present in tumor cells (arrow) from a liver metastasis sample. (Bottom) RNase 1 immunostaining in islet cells (arrow) from a well differentiated adenocarcinoma (original magnification, ×500).

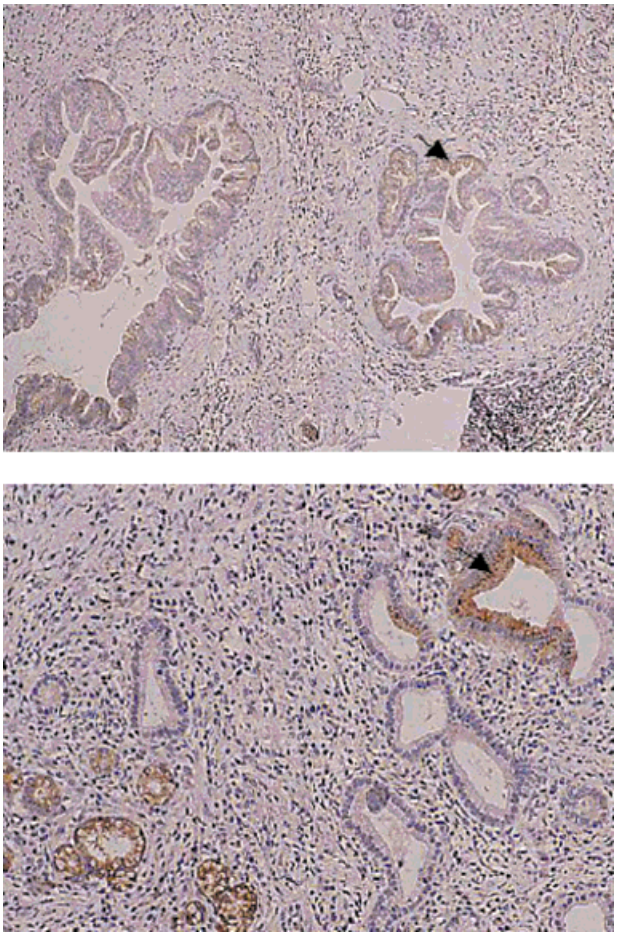

**FIGURE 4.** Ribonuclease 1 (RNase 1) expression in hyperplastic ducts of pancreatic adenocarcinoma and in pancreatitis tissues. (Top) Staining also was observed in hyperplastic ducts (arrow) of a well differentiated carcinoma. (Bottom) In pancreatitis tissues, some smaller ducts (arrow) were stained (original magnification, ×200 on the top, ×500 on the bottom).

tered glycosylation pattern of the protein.[10] The carbohydrate antigen found in RNase1 from Capan-1, sialyl Lewis X, is associated with a tumor situation, including pancreatic carcinoma.[17,18] Immunohistochemical studies aimed at elucidating the glycosylation of RNase 1 in pancreatic tumor tissues would be of great value in determining the possible use of this enzyme as a tumor marker of pancreatic adenocarcinoma.

The expression of acinar markers in ductal type pancreatic adenocarcinomas has been reported previously. Approximately 20% of pancreatic adenocarcinomas express acinar proteins like trypsin and lipase.[19] In this study, however, a different pattern of expression for another acinar protein, RNase 1, is described. This protein was expressed much more frequently (by ≈80% of pancreatic adenocarcinomas) than other acinar proteins. The high percentage of tumors that express RNase 1 compared with other acinar proteins suggests that the RNase 1 gene is not inactivated in them like some of the other genes are. Altered patterns of gene expression caused by the molecular changes may accompany the process of tumorigenesis.

It is believed generally that adenocarcinomas in the human pancreas are of ductal origin. However, other possibilities have been raised. In transgenic mice that overexpress transforming growth factor-$\alpha$ (TGF-$\alpha$),[20–22] pancreatic acinar cells may have a role in the development of pancreatic carcinoma. In this TGF-$\alpha$ transgenic mice model, an acinar-ductal carcinoma sequence is suggested, because there is a possibility that acinar cells retrodifferentiate or transdifferentiate.[22] In a hamster model, the earliest lesions include the centroacinar cells or cells of acinar ori-

gin.[23] The expression of acinar proteins like RNase 1 in ductal cell type carcinomas suggests that transformed cells may arise from protodifferentiated or pluripotent stem cells that have the capability of expressing genes for different pancreatic cell types.[10,19,24] These cells represent the progenitor population in the pancreas, and they can give rise to endocrine, acinar, and duct cells during pancreatic ontogenesis and in the early postnatal period.[25] Their existence, however, in the mature pancreas is only indirect.[22] All of these observations demonstrate that considerable doubts remain about the cell of origin of this neoplasm.

We observed RNase 1 not only in neoplastic cells but also in islets that were present in some of the tumor samples analyzed. Islets of Langerhans persist in carcinomas of the pancreas in many conditions associated with fibrosis,[26] although we did not expect that some of those islets would express an acinar protein, RNase 1. This abnormal RNase 1 expression in islets may have been induced by factors present in the tumor microenvironment. Pour et al.[27] recently suggested that some components of islets, most probably stem cells, have the capacity to develop or differentiate into any pancreatic cellular type. Pour and colleagues suggested that these islet cells are the origin of ductal type adenocarcinomas in Syrian hamsters. Determining the mechanisms of RNase 1 expression in ductal and islet cells may allow us to understand the origins of the cells that express RNase 1.

In this report, chronic pancreatitis tissues also were analyzed from patients who did not show any sign of pancreatic carcinoma. RNase 1 was expressed in some squamous metaplastic ducts and islets of the tissue. The expression level correlated positively with the degree of fibrosis. Fibrosis is found in both chronic pancreatitis tissues and pancreatic adenocarcinoma, and, in pancreatic carcinoma, fibrosis is a consequence of the pronounced desmoplastic reaction that causes a formation of connective tissue in its surroundings.[28] Although RNase 1 expression is not as common in chronic pancreatitis tissues as it is in ductal carcinomas, the current data show that RNase 1 also was expressed in nonneoplastic pancreatic pathologic states. RNase 1, therefore, not only was expressed in neoplastic stages but also was expressed in other pathologic stages that may be related to pancreatic carcinoma. Epidemiologic studies have provided strong evidence linking chronic pancreatitis with pancreatic carcinoma.[11] Whether RNase 1 can serve as an early marker for cells that may eventually develop into adenocarcinomas remains to be determined. In summary, the immunohistochemistry and ISH studies presented here demonstrated that neoplastic transformation of the exocrine pancreas generally was accompanied by RNase 1 expression and probably explains the high levels of RNase 1 protein detected in the serum of most pancreatic adenocarcinoma patients.

## REFERENCES


1. Gordis L, Gold EB. Epidemiology and etiology of pancreatic cancer. In: Go VLW, Gardner JD, Brooks FP, Lebenthal E, DiMagno EP, Scheele GA, et al., editors. The pancreas: biology, pathobiology and disease, 2nd ed. New York: Raven Press, Ltd., 1993:837.
2. Parkar SL, Tong T, Bolden S, Wingo PA. *CA Cancer J Clin* 1997;47:5–27.
3. Hall PA, Lemoine NR. Models of pancreatic cancer. *Cancer Surveys* 1993;16:135–55.
4. Futami J, Tsushima Y, Murato Y, Tada H, Sasaki J, Seno M, et al. Tissue-specific expression of pancreatic-type RNases and RNase inhibitor in humans. *DNA Cell Biol* 1997;16: 413–9.
5. Weickmann JL, Olson EM, Glitz DG. Immunological assay of pancreatic ribonuclease in serum as an indicator of pancreatic cancer. *Cancer Res* 1984;44:1682–7.
6. Kurihara M, Ogawa M, Ohta T, Kurokawa E, Kitahara T, Murata A, et al. Radioimmunoassay for human pancreatic ribonuclease and measurement of serum immunoreactive pancreatic ribonuclease in patients with malignant tumors. *Cancer Res* 1984;44:2240–3.
7. Kobayashi T, Kawakubo T. Prospective investigation of tumor markers and risk assessment in early cancer screening. *Cancer* 1994;73:1946–53.
8. Fernandez E, Fallon MJM, Frazier ML, de Llorens R, Cuchillo CM. Expression of acinar and ductal products in Capan-1 cells growing in synthetic serum and serum-free media. *Cancer* 1994;73:2285–95.
9. Frazier ML, Fernandez E, de Llorens R, Brown NM, Pathak S, Cleary KR, et al. Pancreatic adenocarcinoma cell line, MDAPanc-28, with features of both acinar and ductal cells. *Int J Pancreatol* 1996;19:31–8.
10. Fernandez E, Peracaula R, Frazier ML, de Llorens R. Ribonucleases expressed by human pancreatic adenocarcinoma cell lines. *Eur J Biochem* 2000;267:1484–94.
11. Lowenfels AB, Maisonneuve P, Cavallini G, Amman RW, Lankisch PG, Andersen JR, et al. International Pancreatitis Study Group: pancreatitis and the risk of pancreatic cancer. *N Engl J Med* 1993;328:1433–7.
12. Ekbom A, McLaughlin JK, Karlsson B-M, Nyrén, O, Gridley G, Adami H-O, et al. Pancreatitis and pancreatic cancer: a population-based study. *J Natl Cancer Inst* 1994;86:625–7.
13. Futami J, Seno M, Kasaka M, Tada H, Seno S, Yamada H. Recombinant human pancreatic ribonucleases produced in *E. coli*: importance of the amino terminal sequence. *Biochem Biophys Res Commun* 1995;216:406–13.
14. Haugg M, Schein CH. The DNA sequences of the human and hamster secretory ribonucleases determined with the polymerase chain reaction. *Nucleic Acids Res* 1992;20:612.
15. Komminoth P. Digoxigenin as an alternative probe labeling for in situ hybridization. *Diagn Mol Pathol* 1992;1:142–50.
16. Kemmer TP, Malfertheiner P, Büchler M, Kemmer ML, Ditschuneit H, Serum ribonuclease activity in the diagnosis of pancreatic disease. *Int J Pancreatol* 1991;8:23–33.
17. Kim YS, Itzkowitz SH, Yuang M, Chung Y, Satake K, Umeyama J, et al. $Le^x$ and $Le^y$ antigen expression in human pancreatic cancer. *Cancer Res* 1988;48:475–82.

18. Satomura Y, Sawabu N, Takemori Y, Ohta H, Watanabe H, Okai T, et al. Expression of various sialylated carbohydrate antigens in malignant and non-malignant pancreatic tissues. *Pancreas* 1991;6:448–58.
19. Kim J-H, Ho SB, Montgomery CK, Kim YS. Cell lineage markers in human pancreatic cancer. *Cancer* 1990;66:2134–43.
20. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RP, Lee DC. Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* 1990;61:1121–35.
21. Sandgren EP, Luetteke NC, Qui TH, Palmiter RD, Brinster RP, Lee DC, et al. Transforming growth factor alpha dramatically enhances oncogene-induced carcinogenesis in transgenic mouse pancreas and liver. *Mol Cell Biol*1993;13:320–30.
22. Schmid RM, Klöppel G, Adler G, Wagner M. Acinar-ductal-carcinoma sequence in transforming growth factor-$\alpha$ transgenic mice. *Ann NY Acad Sci* 1999;880:219–30.
23. Pour PM. Mechanism of pseudoductular (tubular) formation during pancreatic carcinogenesis in the hamster model. *Am J Pathol* 1988;130:335–44.
24. Frazier ML, Fernandez E. Gene expression in the developing pancreas and pancreatic adenocarcinoma. *Cancer Bull* 1994;46:485–91.
25. Githens S. Differentiation and development of the pancreas in animals. In: Go VLW, Gardner JD, Brooks FP, Lebenthal E, DiMagno EP, Scheele GA, et al., editors. The pancreas: biology, pathobiology and disease, 2nd ed. New York: Raven Press, Ltd., 1993:21–48.
26. Cruickshank AH, Benbow EW. Solid carcinomas of the exocrine pancreas. In: Pathology of the pancreas, 2nd ed. London: Springer-Verlag, 1995:143–84.
27. Pour PM, Weide L, Liu G, Kazakoff K, Scheetz M, Tohskov I, et al. Experimental evidence for the origin of ductal-type adenocarcinoma from the islets of Langerhans. *Am J Pathol* 1997;150:2167–80.
28. Kern HF, Elsässer H-P. fine structure of human pancreatic adenocarcinoma. In: Go VLW, Gardner JD, Brooks FP, Lebenthal E, DiMagno EP, Scheele GA, et al., editors. The pancreas: biology, pathobiology and disease, 2nd ed. New York: Raven Press, Ltd., 1993:857–9.