Differential Expression of Matrix Metalloproteinase (MMP)-2, MMP-9, and Membrane Type 1-MMP in Hepatocellular and Pancreatic Adenocarcinoma: Implications for Tumor Progression and Clinical Prognosis

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ABSTRACT

In the present study, we used in situ hybridization to study 36 primary hepatocellular carcinomas (HCCs) and 35 pancreatic adenocarcinomas to analyze the expressions of membrane-type 1 matrix metalloproteinase (MT1-MMP), MMP-2, and MMP-9 mRNAs. In HCCs, MT1-MMP mRNA was mainly expressed by cancer cells and to a lesser extent by stromal cells. MMP-2 mRNA was expressed predominantly by cells of tumor stroma, whereas MMP-9 mRNA was seen mainly in neoplastic epithelial cells. In pancreatic adenocarcinomas, MT1-MMP and MMP-9 mRNA were seen at moderate levels both in cancer and in stromal cells, whereas MMP-2 mRNA was predominantly expressed by the tumor stroma. Antigens of MMP-2, MMP-9, and MT1-MMP immunolocalized to the neoplastic epithelium and to the stromal cells in both tumor types. In gelatin zymography, increased amounts of latent and active MMP-2 were found in tumor samples of HCC as compared with adjacent nontumorous liver tissue. On the other hand, the latent form of MMP-9 was found in almost equal amounts both in tumor and normal liver samples, but its active form was present only in HCC.

Expression of MT1-MMP mRNA had a tendency to be associated with a lower degree of differentiation in HCC, but such association was not noticed in pancreatic tumors. Correlation to the clinical data showed that MT1-MMP expression had a strong statistical association with a poor outcome of patients (P < 0.01). A similar tendency was also observed in pancreatic adenocarcinomas, but the association did not reach statistical significance. MMP-2 and MMP-9 mRNA expression did not have significant correlation with prognosis. The results of this study support the previous suggestions of the importance of MT1-MMP for malignant growth and indicate that increased MT1-MMP mRNA expression by tumor cells in HCCs and pancreatic adenocarcinomas may have prognostic significance.

INTRODUCTION

MMPs are a group of zinc-dependent endopeptidases with different substrate specificities (1). They share many structural and functional properties and are known to take part in extracellular matrix remodeling and can be inhibited by TIMPs.

In cancer research, much interest has been devoted recently to a gelatinase subgroup of MMPs that include MMP-2 (gelatinase A, M, 72,000 type IV collagenase) and MMP-9 (gelatinase B, M, 92,000 type IV collagenase). This is partly because of their ability to degrade type IV collagen, the major structural component of BMs and because of their assumed important role in cellular invasion (2). MT1-MMP, the first member of a more recently established group of MMPs containing a membrane-spanning sequence, has been shown to have an important role in MMP-2 activation in cell membranes (3, 4). By immunohistochemistry, MMP-2 has been shown to be localized in carcinomas to the neoplastic epithelial cells (5–7), but in situ hybridization, on the other hand, has shown that most of the expression of mRNA for MMP-2 occurs in fibroblasts and endothelial cells of the tumor stroma (6, 7). In numerous studies, expression of MMP-2 has been shown to be related to invasive phenotype and metastatic potential of tumor cells (8, 9).

HCCs are relatively uncommon in Western countries but common in parts of Africa and the Far East. The most important etiological factors implicated in HCC are hepatitis B and C, abuse of alcohol, and exposure to aflatoxin. Increased prevalence of HCC is also associated with cirrhosis, and generally the clinical prognosis of HCC is very poor.

Pancreatic adenocarcinoma is another aggressive tumor type that frequently develops early regional and distant metastases and therefore has an unfavorable prognosis. Previous studies have shown that many MMPs and TIMPs are overexpressed in these two tumor types (10–14). However, results from these studies have not provided a systematic concept of how MMP-2, MMP-9, and MT1-MMP are expressed in mRNA as well as antigen levels, and moreover, what the clinical aspect of MMPs in these malignancies with poor prognosis is. Therefore, we collected a tumor series that comprised primary HCCs and...
pancreatic adenocarcinomas to study systematically MMP-2, MMP-9, and MT1-MMP expressions in these tumors. Because of some discrepancies in previous results, we wanted to clarify which cells are responsible for the production of these mRNAs and corresponding antigens and whether there are any differences in expression between tumors of high and poor differentiation. Because there are some previous reports with other malignancies that indicate the association of MMPs, especially that of MT1-MMP to poor prognosis of patients (15, 16), we also correlated our findings with the clinical outcome of the patients.

**MATERIALS AND METHODS**

**Materials.** Samples from 36 primary HCCs from patients who underwent partial hepatectomy during 1985–1997 were retrieved from the files of the Department of Pathology, Oulu University Central Hospital. There were 18 females and 18 males with a mean age of 64 years (range, 7–83 years). The average tumor size was 5.1 cm (range, 2–15 cm). There were 13 tumors of differentiation grade I, 13 of grade II, and 10 of grade III. Similarly, samples from 35 pancreatic ductal adenocarcinomas were collected from years 1985–1994. Of the tumors, 13 were of grade I, 15 of grade II, and 7 of grade III. The average tumor size was 3.4 cm (range, 1–7 cm). The mean age of the patients was 64.6 years (range, 53–73 years). Of the patients, 25 were females and 10 were males. Clinical data, including age, sex, tumor size, survival time after surgical operation, and cause of death, are presented in Tables 1 and 2. The tumor material was fixed in 10% neutral formalin and embedded in paraffin. The diagnoses of tumors were based on a light microscopic examination of H&E-stained sections. Grading was done according to the criteria of the WHO (17, 18).

**RNA Probes for Hybridization.** A 635-bp Scel-SacI fragment of the K-191 cDNA clone, for the human MMP-2 (19) was ligated into the M13 polylinker site of pSP64 and pSP65 vectors (Promega Corp., Madison, WI). A 574-bp EcoRI-HindIII fragment of human MMP-9 cDNA clone K-174.1 (20) was subcloned into the pGem 4Z vector (Promega). A 420-bp fragment from nucleotide 218 to nucleotide 638 of the human MT1-MMP cDNA (MMP-14) cDNA was amplified by PCR from human placental cDNA (21) and ligated into BluescriptSK+ vector (Stratagene, La Jolla, CA) to generate pBL420.

**In Situ Hybridization.** For in situ hybridization, the sections were treated with 0.2 M hydrochloric acid for 20 min at room temperature and washed in diethylpyrocarbonate-treated water for 5 min. Proteinase K (1 mg/ml; Roche Diagnostics, Indianapolis, IN) treatment was carried out for 30 min at 37°C, and the reaction was terminated with 0.2% glycine in PBS. Sections were fixed with 4% paraformaldehyde in PBS for 20 min and washed in PBS. Acetylation was done in 0.25–0.5% acetic anhydride in 0.1% triethanolamine for 10 min. Sections were washed in PBS and air-dried for 1–2 h at room temperature. Incubation in prehybridization mixture [10 mM DT (Sigma, St. Louis, MO), 10 mM Tris-HCl, 10 mM NaPO4, 5 mM EDTA (Sigma), 0.3 M NaCl, 1 mg/ml yeast tRNA, deionized formamide 50%, and dextran sulfate 10% (w/v); 0.02% (w/v) Ficoll (Amersham Pharmacia Biotech, Upptana, Sweden), 0.02% (w/v) polyvinylpyrrolidone, and 0.02 mg/ml BSA] was carried out for 2 h. Thereafter, the sections were washed in PBS and dehydrated. For hybridization, the probes were first denatured by boiling them for 1 min and then placed on ice. Three × 106 cpm of the 35S-labeled antisense or sense probe in 40 μl of prehybridization buffer was applied on each section, and the hybridization was carried out at 50°C overnight. The posthybridization washes were as follows: twice at 50°C for 1 h in prehybridization mixture except for dextran sulfate and tRNA, 15 min in 0.5 M NaCl in 10 mM Tris-HCl, 1 mM EDTA at 37°C, 30 min in 0.5 M NaCl containing 40 μg/ml RNase A (Sigma) at 37°C, 15 min in 0.5 M NaCl at 37°C, 15 min twice in 2× SSC, and 15 min twice in 1× SSC at 50°C. The sections were then dehydrated in ethanol containing 300 mM ammonium acetate and air-dried at room temperature for 1 h. For autoradiography, the slides were dipped in NTB-2 film emulsion (Kodak, New York, NY) and air-dried at room temperature for 1 h. Autoradiographs were developed for 3 days.
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Copenhagen, Denmark) for 30 min, followed by the avidin-biotin-peroxidase complex, and the substrate solution (3,3-diaminobenzidine tetrahydrochloride in H2O2 in Tris buffer, pH 7.4; Sigma) for 10 min. For control stains, PBS was used instead of the primary antibody.

**Gelatin Zymography.** To assess the MMP-2 and MMP-9 activity in HCC tissues, gelatin zymography modified from Heussen and Dowdle (22) was performed. Fifty μg of tumor tissue obtained from HCC and an equal amount of adjacent nontumor tissue were manually homogenized. Thirty-μl aliquots of both tumor and nontumor samples were dissolved in the SDS sample buffer in the absence of reducing agents without boiling. The samples were then run in SDS-PAGE in gels that also contained copolymerized gelatin (1.5 mg/ml). After electrophoresis, the gels were washed twice for 15 min in buffer containing 5 mM calcium chloride, 1 μM zinc chloride, 50 mM-Tris-HCl (pH 7.6), supplemented with 2.5% Triton X-100. Finally, the gel was incubated for 16 h in same buffer also containing 0.2 m NaCl, 0.02% sodium trinitrate, and 10 mM calcium chloride at 37°C. The enzyme activity was visualized by staining the gels with a solution containing 50% methanol, 10% acetic acid, and 0.1% Coomassie Brilliant Blue and destaining in 10% methanol and 10% acetic acid.

**Statistical Analysis.** Clinical data were collected from the patients’ clinical records, and survival times were counted (months) from the date of partial hepatectomy or pancreatectomy. Statistical analysis was performed by the Kaplan-Meier method with log-rank analysis. HCCs were analyzed comparing groups negative (−) for mRNA expression to positively expressing (+ through ++++) groups. Because there were only few cases of pancreas adenocarcinomas that did not express any mRNAs investigated, we combined negative (−) and low expression (+) groups and compared those with moderate (+++) and strong (++++) expressions. The results from survival analysis were expressed by reporting Ps, and values < 0.05 were regarded as significant.

**RESULTS**

Positive signals in **in situ** hybridization experiments were scored by using a semiquantitative scale. The results for HCCs are presented in Table 1 and for pancreatic adenocarcinomas in Table 2.

**MT1-MMP mRNA Expression.** mRNA for MT1-MMP was detected in 12 of 36 (33%) HCCs. Of these, the expression was seen exclusively in carcinoma cells in seven tumors. In five cases, there was also expression in fibroblasts and, more weakly, in endothelial cells. The stromal signal was generally weak and most distinct in areas of abundant fibrosis and was often localized to cells adjacent to islands of tumor cells. In some areas, positive labeling was also seen in epithelial cells of proliferating, but not of normal, bile ducts. In carcinoma cells of HCCs, the MT1-MMP mRNA expression was independent from that in stroma, and no clear coexpression with fibroblasts was noticed. The expression level in carcinoma cells varied from negative to strong positivity and had a tendency to be associated with a low degree of differentiation; 5 of 10 of grade III tumors showed positive labeling (Fig. 1a).

In pancreatic adenocarcinomas, MT1-MMP mRNA expression was seen in 34 of 35 (97%) cases. There was only one
case showing no signals for MT1-MMP mRNA in neoplastic epithelium, and the expression was weak in 18, moderate in 16, but not strong in any of the cases. Thirty-three of 35 tumors showed MT1-MMP mRNAs also in the tumor stroma, where the expression was weak in 23 cases and moderate in nine cases, and in one case the labeling was strong. Strongest stromal MT1-MMP mRNA signal was seen in the fibroblasts of newly formed fibrous tissue in the vicinity of invasive epithelial ducts or islands (Fig. 1c). Normal acinar or ductal structures were negative.

**MMP-2 mRNA Expression.** MMP-2 mRNAs were detected in 14 of 36 (38%) of HCCs. The expression occurred in stromal cells of 13 of 36 tumors. The mRNA level varied from weak to moderate. In eight tumors, mRNAs were detected also in carcinoma cells. Of stromal cells both fibroblasts and, to a lesser extent, endothelial cells showed positive signals, usually in areas of abundant fibrosis either around tumor islands or more diffusely in the tumor stroma (Fig. 2a).

Because of exhausted tumor material, *in situ* hybridization for MMP-2 mRNA was performed for 27 pancreatic adenocarcinomas. MMP-2 mRNAs were detected in 23 of 27 (85%) tumors. Stromal expression clearly predominated over that in neoplastic epithelium; it was weak in 6, moderate in 10, and strong in 6 tumors. Positive transcripts were localized to fibroblasts and endothelial cells, especially in areas of abundant fibrous desmoplasia. mRNA expression for MMP-2 by neoplastic epithelium was seen in 12 of 27 tumors, and the labeling was weak in 11 and moderate in 1 of them. Epithelial expression never occurred without simultaneous stromal positivity (Fig. 2b).

**MMP-9 mRNA Expression.** MMP-9 mRNAs were observed in 12 of 36 (33%) HCCs. Twelve of them showed expression in carcinoma cells and four also in the stromal cells. The intensity of mRNA signal varied from weak to moderate and was generally not associated with the amount of fibrous stroma. Within tumor tissue, the expression was more pronounced in the more dysplastic nodules. In stroma, both fibroblasts and endothelial cells contained the mRNA. Occasionally, mRNAs were also seen in nontumorous hepatocytes of cirrhotic areas and in cells of proliferating bile ducts (Fig. 3a).

*In situ* hybridization for MMP-9 mRNA was performed for 26 pancreatic adenocarcinomas. Twenty-three of 26 (91%) tumors expressed the mRNA. Generally, carcinoma cells and stromal cells were equally labeled for the mRNA. Twenty-two tumors showed mRNA signal in the carcinoma cells, of which 15 cases had weak and 7 moderate expression patterns. Twenty cases showed stromal labeling, and of these 11 had weak, 8 moderate, and 1 strong expression patterns. mRNAs in the tumor stroma were mainly detected in widely spread fibroblasts and to a lesser extent also in vascular endothelium (Fig. 3b).

**Immunohistochemical Staining.** Immunohistochemical staining was performed to show the corresponding MMP-2, MMP-9, and MT1-MMP protein expression in HCCs and pancreatic adenocarcinomas. Because of the shortage of antibodies, four cases of HCCs were stained. Carcinoma cells in all cases were positive for MMP-2, and a clear staining reaction was also seen in stromal fibroblasts and endothelial cells (Fig. 2c).

MMP-9 staining reaction was seen in HCCs in individual carcinoma cells scattered over the tumor area. Occasionally, stromal fibroblasts and endothelial cells were seen to express...
Fig. 2  a, in situ hybridization for MMP-2 in HCC. Positive signals are localized to the stromal fibroblasts showing elongated cell shape (arrows). b, in situ hybridization for MMP-2 mRNA in pancreatic adenocarcinoma. Positive grains are localized abundantly to the stromal fibroblasts (arrows), whereas the neoplastic gland (G) does not contain labeling (arrowhead). c, immunohistochemical staining for MMP-2 in HCC. Cancer cells contains intracytoplasmic positivity for MMP-2 antigen. d, immunohistochemical staining for MMP-2 in pancreatic adenocarcinoma. Cancer cells are strongly stained, but the stroma remains negative. a, b, and d, ×265; c, ×410.

Fig. 3  a, in situ hybridization for MMP-9 mRNA in HCCs. Labeling is strongly localized to carcinoma cells (arrows). b, in situ hybridization for MMP-9 mRNA in pancreatic adenocarcinoma. Positive grains are localized especially to the carcinoma cells of neoplastic gland (G) but are also present in stromal fibroblasts (arrows). c, immunohistochemical staining for MMP-9 in HCC. Individual carcinoma cells contain intracytoplasmic positivity for MMP-9 antigen (arrowheads). d, immunohistochemical staining for MMP-9 in pancreatic adenocarcinoma. The neoplastic epithelium contains strong intracytoplasmic positivity. ×265.
MMP-9 antigen (Fig. 3c). Because of a high content of endogenous peroxidases and biotin in liver cells, resulting in increased possibilities for nonspecific binding, the immunohistochemical stainings were not as successful for MT1-MMP in HCCs. In some grade III carcinomas, there was, however, intracytoplasmic or cell membrane staining of the carcinoma cells for MT1-MMP.

Immunohistochemical staining for the presence of MMP-2 was performed on four pancreatic adenocarcinomas. They all showed a clear positive staining reaction in carcinoma cells but also in fibroblasts and endothelial cells (Fig. 2d). Occasional staining could also be seen in cells of normal epithelial ducts of the adjacent pancreatic tissue.

MMP-9 staining was performed for eight pancreatic adenocarcinomas. They all showed a clear intracytoplasmic reaction in neoplastic epithelial cells. Overlying tumor stroma remained, in most of the cases, negative, and only occasional tumor fibroblasts and endothelial cells were seen to express MMP-9 antigen (Fig. 3d).

MT1-MMP staining was performed on eight pancreatic adenocarcinomas. In all cases, intracellular staining was seen in both carcinomas and also to a lesser extent in stromal cells. In some areas, a positive staining reaction could also be seen in cell membranes of the neoplastic epithelial cells (Fig. 1d).

**Gelatin Zymography.** Gelatinolytic activity has been previously well documented in pancreatic adenocarcinomas (11, 14). Therefore, gelatin zymography was performed on HCCs (Fig. 4).

In normal liver tissue, there was a faint Mr 72,000 gelatinolytic band, corresponding to the latent form of MMP-2. No evidence of the molecular size of its active form was present. Tumor tissues of HCC contained the clear Mr 72,000 band, but there was also a Mr 62,000 band corresponding the molecular size of the active form of MMP-2.

Tumor and nontumor samples showed almost comparable bands for the latent Mr 92,000 MMP-9, but the active, Mr 82,000 form was seen only in HCC samples.

**Statistical Analysis.** To analyze whether the expression of MMPs was associated with the outcome of the patients, the semiquantitative results of *in situ* hybridization were compared with the survival data of the patients. A significant association was seen between the expression of MT1-MMP mRNA by carcinoma cells of HCC and shortened survival of patients ($P = 0.01$, log rank; Fig. 5). No such association was seen in cases where the mRNA expression was seen present only in stromal cells ($P = 0.81$, log rank). Generally, there was a tendency of increased MT1-MMP mRNA expression in grade III tumors when compared with grade I tumors, but the difference was not statistically significant ($P = 0.07$).

In HCCs, there was no statistically significant difference in survival between patients showing negative or positive results for the presence of MMP-2 or MMP-9 mRNA in carcinoma cells ($P = 0.24$, log rank, and $P = 0.97$, log rank, respectively) or in stromal cells ($P = 0.94$, log rank, and $P = 0.70$, log rank, respectively). There was, however, a tendency that positive expression was associated with a poorer prognosis of patients.

In pancreatic adenocarcinomas when compared with prognosis MT1-MMP mRNA expression by neoplastic cells did not associate with survival ($P = 0.32$, log rank), but the Breslow test gave a significant value ($P = 0.04$). No significant association was seen when correlated to the mRNA expression by the stroma ($P = 0.12$, log rank). Neither was there any difference in the mRNA expression between various differentiation grades (I–III) of pancreatic adenocarcinomas.

Because only one of the pancreatic tumors expressed moderately or strongly mRNA for MMP-2 by carcinoma cells, statistical analysis was not performed. The association of stromal expression to the patients’ outcome did not show statistical significance ($P = 0.27$, log rank). Nor was there any significant association between the patients’ prognosis and the expression of mRNA for MMP-9 by cancer cells ($P = 0.65$, log rank) or by stromal cells ($P = 0.35$, log rank). However, according to life tables, there was a tendency for a poorer prognosis of patients with increased MMP-9 mRNA expression by tumor cells.

**DISCUSSION**

In the present study, we investigated the expressions of MMP-2, MMP-9, and MT1-MMP mRNA in two different
tumor types, HCC and pancreatic adenocarcinoma, by in situ hybridization. The results show that carcinoma cells of HCCs are the main producers of MT1-MMP mRNA, and only a low level of expression could be detected in stromal cells. This points out that carcinoma cells themselves are active modulators of extracellular matrix space, especially in high-grade tumors. This result is similar to the recent study by Ogata et al. (11), who also showed correlation between increased MT1-MMP mRNA expression and tumor dedifferentiation. Our result differs from the result of Theret et al. (23), who demonstrated mRNA synthesis of MT1-MMP solely by stromal cells of HCCs, with no clear neoplastic epithelial expression. In pancreatic adenocarcinoma, MT1-MMP mRNA was almost equally expressed by neoplastic epithelium and stromal cells. Interestingly, MT1-MMP mRNA expression was equal in high- and low-grade tumors, showing fundamental differences to HCCs. The finding agrees well with the result by Inamura et al. (13) but differs from that of Bramhall et al. (12), who demonstrated mRNA synthesis predominantly in the epithelial cancer cells. In both tumor types, MT1-MMP immunolocalized both to the neoplastic epithelial cell population and occasionally to the stromal cells, indicating that cancer cells and stromal cells are producing both the mRNA and corresponding protein.

There was a statistically significant association with a poor prognosis of patients in HCCs when mRNA synthesis of MT1-MMP was present in cancer cells themselves but not when stromal cells alone expressed the mRNA. A similar tendency could also be observed in pancreatic adenocarcinomas but with no statistical significance. The association of increased MT1-MMP mRNA expression with shortened lifetime in HCCs is easily understandable by its connection to the poor differentiation grade of tumors, although it is generally difficult to assess the prognosis of patients with HCC, because some patients with large and progressive tumors have late recurrences and long survival periods. Our result agrees also with Harada et al. (24), who showed increased MT1-MMP mRNA expression in HCC to be associated with capsular infiltration. Our results also support the previous reports of the importance of MT1-MMP in malignant growth but also add evidence for its association with poor prognosis of patients with a malignant disease of the liver. MT1-MMP is one of the key enzymes among MMPs, and its overexpression seems to have a significant effect on tumor growth. To date, there is at least four mechanisms by which MT1-MMP can enhance tumor progression: it can activate MMP-2 on tumor cell membranes (3, 4, 24); it is a very effective degradative enzyme by itself, having substrates such as fibronectin, tenasin, nidogen, aggregan, and perlecan (25); MT1-MMP is a very potent regulator for neoangiogenesis (26), a phenomenon that is critical for malignant growth (reviewed in Ref. 27); and recently MT1-MMP was shown to process directly laminin-5 γ2 chain, which caused a strong migration effect by tumor cells over laminin-5 surfaces (28). Previously, we have shown that pancreatic adenocarcinomas strongly express laminin-5 γ2 chain by tumor cells and also contain laminin-5-bearing BM structures around tumor islands (29), and in a preliminary experiments, we noticed that laminin-5 γ2 chain is abundantly expressed also by HCCs. MT1-MMP and laminin-5 can trigger an important migratory effect in carcinoma cells, leading to their spreading. How these mechanisms together are operating in malignant growth remains to be clarified. It seems, however, that the association of MT1-MMP with poor prognosis of patients is rather connected to tumor type and general growth pattern than differentiation grade. Unraveling the regulatory mechanisms among the functions of MT1-MMP, MMP-2, TIMP-2, and laminin-5 and possibly some other factors may prove to be pivotal to our understanding of malignant behavior in general.

In their study, Theret et al. (23) also showed a correlation between MT1-MMP mRNA levels and MMP-2 and TIMP-2 mRNA levels, as well as with MMP-2 activation in HCCs. In the present study, simultaneously increased MMP-2 and MT1-MMP expression by carcinoma cells was not observed. Gelatin zymography showed that although latent MMP-2 was present both in tumor and adjacent non-tumor liver tissues, active MMP-2 was only seen in tumor tissue of HCC. This finding is in agreement with the result by Ogata et al. (11), who also showed increased MMP-2 activation in tissue samples of HCC.

MMP-2 has been suggested to have a very important role in controlling tumor cell invasion because it is very commonly overexpressed in tumor masses and has a high ability to degrade BM macromolecules. In HCCs and pancreatic adenocarcinomas, MMP-2 mRNA synthesis occurred mainly by stromal fibroblasts and endothelial cells and only to a lesser extent by carcinoma cells. This result is similar to what has been reported previously in many tumor types such as breast, ovary, and colon carcinomas (5, 6, 7). Bramhall et al. (12) also reported MMP-2 mRNA synthesis to be more abundantly present in the tumor stroma of pancreatic adenocarcinoma. It seems likely that the type or amount of stromal stroma is an important modulator for MMP-2 expression, because there was higher MMP-2 production in pancreatic tumors when compared with liver tumors, which are not usually accompanied by fibrosis so abundant as in pancreatic adenocarcinomas. Tumor stromal cells are also known to express many different types of regulatory factors that up-regulate MMP expression (30). Although MMP-2 was abundantly synthesized by stromal cells, it immunolocalized mainly to tumor cells in HCCs and pancreatic adenocarcinoma.

In HCCs, MMP-9 mRNA was found to be expressed mainly by tumor cells. There was no clear association with the prognosis in mRNA expressions of MMP-9 or MMP-2. However, there was a tendency to have a strong expression for both mRNAs in tumors with a poorer survival of patients. In a previous study on HCCs, it was shown that increased MMP-9 mRNA expression, as studied by Northern hybridization, was associated with capsular infiltration (10). Elevated plasma levels of MMP-9 in patients with HCC have also been observed, especially in patients having macroscopic portal vein invasion (31). It is notable that in some cases of this study, MMP-9 mRNA expression varied from nodule to nodule, being stronger in the more dysplastic ones. Gelatin zymography showed the presence of M, 92,000-sized MMP-9 in almost equal amounts in

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4 Unpublished data.
MMPs investigated is up-regulated in HCCs and pancreatic adenocarcinomas but are differentially expressed by intensity and a cellular origin. Furthermore, MT1-MMP produced by carcinoma cells of HCCs was associated with a poor prognosis of patients, and a similar tendency was also present in pancreatic adenocarcinomas. The results support previous suggestions that MT1-MMP could be one of the key enzymes among MMPs in the process of invasion. Additionally, the uncontrolled production of MT1-MMP alone could be of significance for malignant growth and spread.

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