

Matrix Metalloproteinases and Their Role in Pancreatic Cancer: A Review of Preclinical Studies and Clinical Trials

Mark Bloomston, MD, Emmanuel E. Zervos, MD, and Alexander S. Rosemurgy II, MD

Abstract: Matrix metalloproteinases (MMPs) have received much attention in recent years for their role in a variety of malignancies. Pancreatic cancer is no exception; MMP-2 and MMP-9 show high levels of expression in clinical and experimental models. Inhibition of MMPs has shown great promise with synthetic inhibitors, such as BB-94, as tumorostatic agents in preclinical models, particularly when these are combined with gemcitabine. These findings have led to several clinical trials using the MMP inhibitors Marimastat and BAY12-9566. Herein, we discuss the roles of MMPs and their inhibition in pancreatic cancer.

Key Words: Pancreatic cancer—Matrix metalloproteinase—MMP—TIMP.

The hallmarks of pancreatic cancer are growth of tumor into surrounding vascular and visceral structures and distant tumor spread that precludes operative extirpation. These processes occur relatively early in the biological behavior of pancreatic cancer, making it the fifth most common cause of cancer-related deaths in the United States.¹ These phenomena require degradation of the surrounding extracellular matrix. Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes capable of degrading the extracellular matrix. To date, 18 different subtypes have been identified, and these are divided into 5 groups: stromelysins, collagenases, gelatinases, membrane types, and others (Table 1).^{2–5}

MMPs occur naturally and have normal physiologic roles, including, but not limited to, organogenesis, fertilization, wound healing, and inflammation.^{6–8} Recently, MMPs have been investigated for their role in tumorigenesis and cancer progression.^{9,10}

MMP EXPRESSION

MMP expression is regulated at the transcriptional level and can be induced by a variety of growth factors, oncogenes, hormones, and cytokines.^{2,11,12} Recent studies have shown that transcriptional activation is dependent on the binding of heterodimers of *c-fos* and *c-jun* proto-oncogene products to the activator protein-1 (AP-1) site.^{13,14} This association allows for maximal activation of the promoters of the inducible MMPs (MMP-1, MMP-3, MMP-7, MMP-9, MMP-10, MMP-12, and MMP-13).^{15,16} As such, studies using gene co-transfection to overexpress *c-jun* and *c-fos* have shown enhancement of MMP-1 promoter activity while anti-sense messenger RNA expression for *c-jun* attenuates MMP-1 expression.^{13,17,18}

Mitogen-activated protein kinases (MAPKs) are intricately involved in the expression of the components involved in MMP promoter induction via AP-1 and its association with *c-jun* and *c-fos*. In particular, three specific MAPK classes have been implicated: extracellular signal-regulated kinase, stress-activated protein kinase/Jun N-terminal kinases, and p38 MAPK.^{19–21} It is generally thought that the balance between these MAPK pathways regulates cell growth, differentiation, survival, and death. In tumorigenesis, however, these pathways act synergistically to upregulate MMP expression in response to a variety of stimuli (e.g., cytokines, growth factors, and cellular stress).²²

Received January 10, 2002; accepted April 25, 2002.

From the Department of Surgery, University of South Florida, Tampa, Florida.

Address correspondence and reprint requests to: Alexander S. Rosemurgy II, MD, Department of Surgery, University of South Florida, PO Box 1289, Room F-145, Tampa, FL 33601; Fax: 813-844-7396; E-mail: arosemur@hsc.usf.edu.

TABLE 1. Family of matrix metalloproteinases (MMPs)

Stromelysins	Membrane-type MMPs
MMP-3	MMP-14 (MT-MMP1)
MMP-7 (Matrilysin)	MMP-15 (MT-MMP2)
MMP-10	MMP-16 (MT-MMP3)
MMP-11	MMP-17 (MT-MMP4)
MMP-12 (Metalloelastase)	MMP-24 (MT-MMP5)
Collagenases	Other MMPs
MMP-1	MMP-18 (Xenopus MMP)
MMP-8	MMP-19
MMP-13	MMP-20 (Enamelysin)
Gelatinases	
MMP-2	
MMP-9	

MMP ACTIVATION

MMPs are produced and secreted in latent forms that require extracellular activation. This regulatory step can be accomplished in a variety of ways. Organomercurials, such as p-aminophenylmercuric acetate, can be used in vitro to bind to the conserved cysteine in the propeptide, releasing its covalent bond to the catalytic zinc ion and thus promote autocatalytic cleavage of the proform of MMP to form active MMP.^{23,24} In vivo, the propeptide can be cleaved in a similar fashion by a host of extracellular proteinases (e.g., plasmin, serine proteases, and other MMPs). Pro-MMP-11, for example, can also be activated intracellularly by the Golgi-associated proteinase, furin.²⁵

Another example of MMP activity induction is the complex activation of MMP-2, which involves membrane-bound MMP type 1 (MT-MMP1) at the cell surface. This interaction requires a tissue inhibitor of MMP, TIMP-2, to bind to and inactivate MT-MMP1; this allows pro-MMP-2 to bind and form a complex on the cell surface which acts as a substrate for a second MT-MMP1 molecule. The result is cleavage of the propeptide of MMP-2 to produce the active form.²⁶⁻²⁸ This complex formation intimately associates MMP-2 with the cell

surface, potentially involving it in the process of cell invasion.

MMPs IN PANCREATIC CANCER

MMP expression is upregulated in a variety of malignancies and correlates with the invasive and metastatic potential of thyroid, prostate, ovarian, gastric, lung, head and neck, and colorectal carcinomas.^{3,4} MMPs also seem to play an important role in the progression of pancreatic cancer. Bramhall et al.²⁹ evaluated the resected tumors of 17 patients with pancreatic cancer for MMP-2, MMP-3, MMP-7, and MMP-11 by Northern blot analysis and in situ hybridization. MMP-2 messenger RNA was the most commonly expressed MMP in tumor specimens (93%) but was not seen in normal pancreas from 17 transplant donor patients. It is clear that MMPs, particularly MMP-2 and, to a lesser extent, MMP-9, play an important role in the pathogenesis of pancreatic cancer,³⁰⁻³² but their exact role and correlation with clinicopathologic characteristics and patient outcomes is yet to be fully elucidated (Table 2). The high degree of MMP expression in pancreatic cancer, along with universally poor survival associated with even relatively early disease, makes such clinicopathologic correlations very difficult.

TISSUE INHIBITORS OF METALLOPROTEINASES

TIMPs are smaller (22-30 kDa), naturally occurring proteins capable of binding and inactivating MMPs. Four TIMPs have been identified (TIMP-1, TIMP-2, TIMP-3, and TIMP-4), each with its own physiologic role.^{33,34} The common thread within this family of enzymes is their ability to form noncovalent bonds with the latent and active forms of MMPs with a 1:1 stoichiometry.

TABLE 2. MMP expression in pancreatic cancer

y	Study	No. Patients	MMP	Findings
1995	Gress et al. ³¹	8	2, 9	Correlation with degree of desmoplasia
1998	Koshiba et al. ³²	33	2, 9	Correlation with tumor extent, nodal status, metastases, and recurrence rate (MMP-2 only)
1999	Kuniyasu et al. ⁵⁸	22	2, 9	No correlations
1999	Ito et al. ⁵⁹	46	1	Correlation with survival
2000	Gong et al. ⁶⁰	15	2, 9	Correlation with nodal status
2000	Määttä et al. ⁶¹	35	2, 9, MT1	No correlations
2000	Ellenrieder et al. ⁶²	18	2, MT1, MT2, MT3	Correlation with degree of desmoplasia
2001	Fukushima et al. ⁶³	70	7	Correlation with tumor extent, nodal status, and TNM classification
2001	Yamamoto et al. ⁶⁴	70	7	Correlation with tumor extent, nodal status, TNM classification, and survival

MMP, matrix metalloproteinase; MT, membrane-bound; TNM, tumor, node, metastasis.

Once bound to the latent form of MMP, TIMP delays its activation but does not permanently inhibit it.³⁵ TIMP can also bind to the active site of activated MMP, thus inhibiting matrix degradation.^{36,37}

The exact role of TIMPs in cancer progression remains poorly understood. Although TIMPs, by definition, are involved in the inhibition of MMPs, thereby imparting an antitumoral effect, they are also involved in the activation of MMPs, thus potentially promoting tumor progression. The literature is replete with studies attempting to correlate TIMP expression, particularly TIMP-1 and TIMP-2, with clinicopathologic characteristics (Table 3). Nonetheless, the exact role of TIMPs in tumorigenesis is not completely understood.

MMP INHIBITION IN PANCREATIC CANCER

Preclinical Studies

There has been much interest in the utility of MMP inhibition as antitumoral therapy in several malignancies, particularly in pancreatic cancer. The synthetic MMP inhibitor BB-94 (Batimastat, British Biotech, Oxford, United Kingdom) has been shown to inhibit MMP activity in a pancreatic cancer cell line in two ways: by preventing the activation of pro-MMP and by directly binding the catalytic site of activated MMP.³⁸ These findings translated into a decrease in pancreatic cancer cell invasion through a reconstituted matrix in a dose-dependent fashion without affecting cell proliferation *in vitro*.^{39,40} In an orthotopic nude mouse model in which tumor cells were injected directly into the head of the pancreas after celiotomy, fewer tumor implantations and metastases were seen, ultimately imparting a survival advantage in the treated animals.⁴¹ The protective effect

of BB-94 seems to be the result of preferential MMP-2 (vs. MMP-9) inhibition.⁴² The survival benefit of MMP inhibition is enhanced by the addition of the cytotoxic agent gemcitabine compared with either agent alone, even after tumor implantation.⁴³

Clinical Trials

The encouraging preclinical benefits of MMP inhibition have given rise to several clinical pancreatic cancer trials using the orally bioactive synthetic MMP inhibitors BB-2516 (Marimastat, British Biotech, Oxford, United Kingdom) and BAY12-9566. In a phase I study in healthy male volunteers, Marimastat was determined to be safe at doses as high as 100 mg twice daily (BID), with few side effects, which were similar to those with placebo.⁴⁴ Because Marimastat is tumorostatic rather than tumoricidal, it was predicted that chronic dosing would be necessary and that reductive tumor responses in patients with advanced disease would be subtle and difficult to detect. For this reason, in a series of six subsequent disease-specific phase I/II trials for pancreatic, prostate, ovarian, and colorectal cancers, changes in measurements of tumor markers (cancer antigen [CA] 19-9, prostate-specific antigen, CA 125, and carcinoembryonic antigen, respectively) were used as determinants of tumor response.⁴⁵ Specifically, patients with a $\geq 25\%$ increase in serum tumor markers over the preceding 4-week screening period were eligible for enrollment. The end point of tumor response was determined as a decrease in the rate of tumor marker increase over the 28-day treatment period.⁴⁵ The combined results of these studies demonstrated a dose-dependent decrease in the rate of rise of serum tumor markers. This translated into

TABLE 3. Expression of tissue inhibitors of matrix metalloproteinase (TIMP) in various malignancies

y	Study	Cancer type	No. Patients	TIMP	Findings
1997	Ree et al. ⁶⁵	Breast	34	1, 2	Positive correlation with nodal status (TIMP-1 only), metastases (TIMP-1,2); negative correlation with survival (TIMP-2 only)
2002	Nakopoulou et al. ⁶⁶	Breast	136	2	Positive correlation with differentiation and survival
1998	Ko et al. ⁶⁷	Gastric	45	2	Negative correlation with nodal status
2000	Joo et al. ⁶⁸	Gastric	65	1, 2	Positive correlation with tumor extent and TNM classification (TIMP-1 only); negative correlation with survival (TIMP-1 only)
1999	Moser et al. ⁶⁹	Cervical	154	2	No correlations
1999	Davidson et al. ⁷⁰	Cervical	49	2	Positive correlation with TNM classification; Negative correlation with survival
1999	Joo et al. ⁷¹	Colorectal	54	1, 2	Positive correlation with nodal status and TNM classification (TIMP-1 only); negative correlation with survival (TIMP-1 only)
2000	Holten-Andersen et al. ⁷²	Colorectal	588 (serum)	1	Negative correlation with survival
1995	Gress et al. ³¹	Pancreatic	8	1, 2	Positive correlation with degree of desmoplasia
2000	Gong et al. ⁶⁰	Pancreatic	15	1	Negative correlation with nodal status and differentiation

TNM, tumor, node, metastasis.

a survival benefit in responders compared with nonresponders, although survival was not a specific end point of the studies. Side effects of therapy were predominantly musculoskeletal in nature, with 4% of patients experiencing dose-limiting events, particularly at doses >50 mg BID. These dose-dependent musculoskeletal side effects were dependent on duration, because 21% of patients continuing therapy beyond 28 days developed dose-limiting musculoskeletal side effects.⁴⁵ In the 64 patients in the study with advanced pancreatic cancer, no dose-limiting musculoskeletal side effects occurred during the 28-day treatment period.⁴⁶ In the 30 patients with pancreatic cancer who elected to continue therapy beyond 28 days, however, significant musculoskeletal side effects were noted in 10 (33%), 5 of whom (17%) required dose reduction or discontinuation.⁴⁶

In a recent phase II study of 113 patients with advanced pancreatic cancer that used radiological (computed tomography) response to therapy in addition to the changes in serum CA 19-9 levels, 30% showed stabilization or decrease in serum CA 19-9 levels, and 49% had stable disease by computed tomography over the initial 28-day study period.⁴⁷ Also, 51% of patients had a decrease or stabilization of pain, mobility, and analgesia scores. Patients who showed a serologic response (i.e., decreased CA 19-9) to treatment had a significantly improved survival compared with nonresponders (245 vs. 128 days). No difference in survival was seen in patients with or without radiological response to therapy. Musculoskeletal side effects were noted in 18% of patients during the 28-day treatment period and in 68% of patients who continued treatment beyond 28 days. These side effects were dose limiting in 27%.

On the basis of the findings of phase I and II trials for Marimastat in advanced pancreatic cancer, a large multi-institutional prospective randomized trial was undertaken that compared three different doses of Marimastat with gemcitabine.⁴⁸ This study randomized 414 patients with unresectable pancreatic cancer due to local invasion or distant metastases to one of three different doses of Marimastat (5, 10, or 25 mg BID) or gemcitabine; the primary end point was survival. Survival was not significantly different between patients who received Marimastat at 25 or 10 mg BID and those who received gemcitabine, although the median survival was slightly longer for patients who received gemcitabine. The 1-year survival for patients who received gemcitabine was 19%, which was not statistically different from that of patients who received Marimastat 25 mg BID (19%), 10 mg BID (14%), or 5 mg BID (14%). Survival time in patients with metastatic disease who received gemcitabine was similar to that of patients with nonmetastatic disease who

received gemcitabine (160 vs. 169 days). Patients with nonmetastatic disease who received Marimastat, however, lived significantly longer than patients with metastases who received Marimastat (200 vs. 89 days). Although there was a slightly improved 1-year survival in patients with nonmetastatic disease who received Marimastat compared with gemcitabine (30% vs. 25%), this was not statistically significant. Secondary end points of improvement in pain, mood, and performance status tended to favor patients treated with gemcitabine. Severe nonlaboratory toxicities were more common in patients who received gemcitabine (22%) than Marimastat (13%). Overall, musculoskeletal side effects were the most common reported side effects in patients who received Marimastat, occurring in 44%. These were generally reported as joint pain and resolved with discontinuation of the drug. Dupuytren's contracture developed in one patient.

A similar trial sponsored by the National Cancer Institute of Canada compared gemcitabine with a selective oral MMP inhibitor, BAY12-9566, in patients with advanced pancreatic cancer.⁴⁹ This study was designed with two interim analysis points to allow for early termination. The first interim analysis was after 30 patients were randomized to each arm, with plans to terminate the study if response to MMP inhibition was not seen in at least six patients after 2 months of therapy. Continuation of the trial was recommended at that point. The second interim analysis was to be after the deaths of 140 patients. At the time of this second analysis, 277 patients had been enrolled. A significant overall survival disadvantage was seen in patients who received the MMP inhibitor (median, 3.2 months) compared with those who received gemcitabine (median, 6.4 months), and the trial was terminated before reaching its intended accrual goal of 350 patients. The median progression-free survival for patients who received gemcitabine was 3.5 months, compared with 1.8 months for patients who received BAY12-9566 ($P = .01$).

The failure to show the superiority of Marimastat over gemcitabine in improving survival in patients with advanced pancreatic cancer⁴⁸ or the early termination of the Canadian trial⁴⁹ should not suggest that MMP inhibition is ineffective against pancreatic cancer. These trials have a common design flaw in that they include a large number of patients with metastatic disease (65% in the Marimastat trial and 82% in the BAY12-9566 trial). The tumorostatic nature of MMP inhibitors suggests that patients with extrapancreatic disease, where the metastatic process is already established, may not derive the same benefit as those with localized disease. This is suggested in the data from the Marimastat trial that compared

survival in patients with metastatic versus nonmetastatic disease who took Marimastat.⁴⁸ This opens the door for future studies that focus on patients with localized non-resectable disease or adjuvant therapy trials. As such, patient accrual onto a Marimastat adjuvant therapy trial is complete, and results should be forthcoming in the near future. This trial (British Biotech Study 183) was recently evaluated, and it was decided to continue because “we cannot rule out some potential benefit of Marimastat in this setting” (British Biotech press release, May 2, 2001; available at <http://www.britishbiotech.com/news/173,183analysis.txt>). Continued research using these novel agents is warranted and anticipated.

TIMP GENE THERAPY

Although the side effect profiles of the synthetic MMP inhibitors remain an issue in clinical trials for pancreatic cancer, the encouraging preclinical data associated with MMP inhibition have focused some investigators on manipulating the TIMP activity in various cancers.³³ To use such novel therapy, it is important to know the source of MMP and TIMP in cancers (i.e., tumor- vs. host-derived). We have seen that when the poorly differentiated human pancreatic adenocarcinoma cancer line PANC-1 is implanted into the pancreata of nude mice, the vast majority of MMP-2 and TIMP-1 expression in the resultant tumors and surrounding stroma is human (i.e., tumor-derived).⁵⁰

Gene transfections have been undertaken in a variety of cancers to produce cell lines that overexpress TIMP-1, -2, -3, or -4. Such gene transfections have been shown to decrease the malignant potential of colorectal,⁵¹ breast,⁵² gastric,⁵³ and melanoma cell lines.⁵⁴ In pancreatic cancer, we have shown that overexpression of TIMP-1 in transfected PANC-1 cells resulted in decreased cell invasion in vitro without affecting cell proliferation, implying that TIMP-1 activity is important in limiting the invasive potential of malignant cells.⁵⁵ Also, tumor implantation, growth, and metastasis were attenuated by TIMP-1 overexpression in a nude mouse model. Finally, angiogenesis was decreased in tumors overexpressing TIMP-1 relative to wild-type PANC-1 cells.⁵⁶

Although TIMP-1 overexpression favorably affects the malignant potential of pancreatic cancer, we have seen that underexpression of TIMP-1 has even a more profound effect on tumor growth.⁵⁷ Using the same pancreatic cancer cell line as previously (PANC-1), we undertook full-length TIMP-1 antisense gene transfections to produce cell lines that underexpress TIMP-1. These cells showed decreased invasion in vitro and attenuated tumor growth in vivo compared with both wild-

type PANC-1 cells and TIMP-1 transfected cells (i.e., TIMP-1-overexpressing cells).⁵⁷

CONCLUDING REMARKS

The involvement of MMPs in various malignancies, including pancreatic cancer, makes them attractive as potential pharmacological or genetic targets for antitumor therapies. Although the jury is still out on whether MMP inhibition will prove to be more effective than traditional cytotoxic agents, it seems to show clinical activity in pancreatic cancer. Yet, the attractiveness of an orally bioavailable cancer therapeutic agent such as Marimastat is undeniable. It does seem that the disruption of the MMP/TIMP balance, at least in early preclinical work, is effective in limiting pancreatic cancer aggressiveness. Although the leap to clinical practice of TIMP transfections in the face of established tumor remains elusive, gene therapy targeting TIMP is attractive and warrants further investigation.

REFERENCES

1. Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. *CA Cancer J Clin* 2001;51:15–36.
2. Johansson N, Ahonen M, Kahari VM. Matrix metalloproteinases in tumor invasion. *Cell Mol Life Sci* 2000;57:5–15.
3. Shapiro SD. Matrix metalloproteinase degradation of extracellular matrix: biological consequences. *Curr Opin Cell Biol* 1998;10:602–8.
4. Kahari VM, Saarialho-Kere U. Matrix metalloproteinases and their inhibitors in tumour growth and invasion. *Ann Med* 1999;31:34–45.
5. Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem* 1999;274:21491–4.
6. Matrisian LM. The matrix-degrading metalloproteinases. *Bioessays* 1992;14:455–63.
7. Agren MS, Taplin CJ, Woessner JF Jr, et al. Collagenase in wound healing: effect of wound age and type. *J Invest Dermatol* 1992;99:709–14.
8. Reponen P, Sahlberg C, Huhtala P, et al. Molecular cloning of murine 72-kDa type IV collagenase and its expression during mouse development. *J Biol Chem* 1992;267:7856–62.
9. Tryggvason K, Hoyhtya M, Pyke C. Type IV collagenases in invasive tumors. *Breast Cancer Res Treat* 1993;24:209–18.
10. Mignatti P, Rifkin DB. Biology and biochemistry of proteinases in tumor invasion. *Physiol Rev* 1993;73:161–95.
11. Jones L, Ghaneh P, Humphreys M, Neoptolemos JP. The matrix metalloproteinases and their inhibitors in the treatment of pancreatic cancer. *Ann N Y Acad Sci* 1999;880:288–307.
12. Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet* 1990;6:121–5.
13. Benbow U, Brinckerhoff CE. The AP-1 site and MMP gene regulation: what is all the fuss about? *Matrix Biol* 1997;15:519–26.
14. Karin M, Liu Z, Zandi E. AP-1 function and regulation. *Curr Opin Cell Biol* 1997;9:240–6.
15. Wasyluk C, Gutman A, Nicholson R, Wasyluk B. The c-Ets oncoprotein activates the stromelysin promoter through the same elements as several non-nuclear oncoproteins. *EMBO J* 1991;10:1127–34.
16. Westermarck J, Seth A, Kahari VM. Differential regulation of

- interstitial collagenase (MMP-1) gene expression by ETS transcription factors. *Oncogene* 1997;14:2651–60.
17. Vincenti MP, White LA, Schroen DJ, et al. Regulating expression of the gene for matrix metalloproteinase-1 (collagenase): mechanisms that control enzyme activity, transcription, and mRNA stability. *Crit Rev Eukaryot Gene Expr* 1996;6:391–411.
 18. Kerr LD, Miller DB, Matrisian LM. TGF-beta 1 inhibition of transin/stromelysin gene expression is mediated through a Fos binding sequence. *Cell* 1990;61:267–78.
 19. Westermarck J, Kahari VM. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* 1999;13:781–92.
 20. Robinson MJ, Cobb MH. Mitogen-activated protein kinase pathways. *Curr Opin Cell Biol* 1997;9:180–6.
 21. Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. *Adv Cancer Res* 1998;74:49–139.
 22. Simon C, Goepfert H, Boyd D. Inhibition of the p38 mitogen-activated protein kinase by SB 203580 blocks PMA-induced Mr 92,000 type IV collagenase secretion and in vitro invasion. *Cancer Res* 1998;58:1135–9.
 23. Nagase H. Activation mechanisms of matrix metalloproteinases. *Biol Chem* 1997;378:151–60.
 24. Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci U S A* 1990;87:5578–82.
 25. Pei D, Weiss SJ. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature* 1995;375:244–7.
 26. Strongin AY, Collier I, Bannikov G, et al. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. *J Biol Chem* 1995;270:5331–8.
 27. Zucker S, Drews M, Conner C, et al. Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase 1 (MT1-MMP). *J Biol Chem* 1998;273:1216–22.
 28. Kinoshita T, Sato H, Okada A, et al. TIMP-2 promotes activation of progelatinase A by membrane-type 1 matrix metalloproteinase immobilized on agarose beads. *J Biol Chem* 1998;273:16098–103.
 29. Bramhall SR, Neoptolemos JP, Stamp GW, Lemoine NR. Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. *J Pathol* 1997;182:347–55.
 30. Satoh K, Ohtani H, Shimosegawa T, et al. Infrequent stromal expression of gelatinase A and intact basement membrane in intraductal neoplasms of the pancreas. *Gastroenterology* 1994;107:1488–95.
 31. Gress TM, Muller-Pillasch F, Lerch MM, et al. Expression and in-situ localization of genes coding for extracellular matrix proteins and extracellular matrix degrading proteases in pancreatic cancer. *Int J Cancer* 1995;62:407–13.
 32. Koshiba T, Hosotani R, Wada M, et al. Involvement of matrix metalloproteinase-2 activity in invasion and metastasis of pancreatic carcinoma. *Cancer* 1998;82:642–50.
 33. Baker AH, Ahonen M, Kahari VM. Potential applications of tissue inhibitor of metalloproteinase (TIMP) overexpression for cancer gene therapy. *Adv Exp Med Biol* 2000;465:469–83.
 34. Birkedal-Hansen H, Moore WG, Bodden MK, et al. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;4:197–250.
 35. Bode W, Reinemer P, Huber R, et al. The X-ray crystal structure of the catalytic domain of human neutrophil collagenase inhibited by a substrate analogue reveals the essentials for catalysis and specificity. *EMBO J* 1994;13:1263–9.
 36. Howard EW, Bullen EC, Banda MJ. Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2. *J Biol Chem* 1991;266:13070–5.
 37. Willenbrock F, Murphy G. Structure-function relationships in the tissue inhibitors of metalloproteinases. *Am J Respir Crit Care Med* 1994;150:S165–70.
 38. Zervos EE, Shafii AE, Haq M, Rosemurgy AS. Matrix metalloproteinase inhibition suppresses MMP-2 activity and activation of PANC-1 cells in vitro. *J Surg Res* 1999;84:162–7.
 39. Jimenez RE, Hartwig W, Antoniu BA, et al. Effect of matrix metalloproteinase inhibition on pancreatic cancer invasion and metastasis: an additive strategy for cancer control. *Ann Surg* 2000;231:644–54.
 40. Zervos EE, Norman JG, Gower WR, et al. Matrix metalloproteinase inhibition attenuates human pancreatic cancer growth in vitro and decreases mortality and tumorigenesis in vivo. *J Surg Res* 1997;69:367–71.
 41. Zervos EE, Franz MG, Salhab KF, et al. Matrix metalloproteinase inhibition improves survival in an orthotopic model of human pancreatic cancer. *J Gastrointest Surg* 2000;4:614–9.
 42. Zervos EE, Shafii AE, Rosemurgy AS. Matrix metalloproteinase (MMP) inhibition selectively decreases type II MMP activity in a murine model of pancreatic cancer. *J Surg Res* 1999;81:65–8.
 43. Haq M, Shafii A, Zervos EE, Rosemurgy AS. Addition of matrix metalloproteinase inhibition to conventional cytotoxic therapy reduces tumor implantation and prolongs survival in a murine model of human pancreatic cancer. *Cancer Res* 2000;60:3207–11.
 44. Millar AW, Brown PD, Moore J, et al. Results of single and repeat dose studies of the oral matrix metalloproteinase inhibitor marimastat in healthy male volunteers. *Br J Clin Pharmacol* 1998;45:21–6.
 45. Nemunaitis J, Poole C, Primrose J, et al. Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: selection of a biologically active and tolerable dose for longer-term studies. *Clin Cancer Res* 1998;4:1101–9.
 46. Rosemurgy A, Harris J, Langleben A, et al. Marimastat in patients with advanced pancreatic cancer: a dose-finding study. *Am J Clin Oncol* 1999;22:247–52.
 47. Evans JD, Stark A, Johnson CD, et al. A phase II trial of marimastat in advanced pancreatic cancer. *Br J Cancer* 2001;85:1865–70.
 48. Bramhall SR, Rosemurgy A, Brown PD, et al. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. *J Clin Oncol* 2001;19:3447–55.
 49. Moore M, Hamm J, Eisenberg P, et al. A comparison between gemcitabine and the matrix metalloproteinase inhibitor BAY12-9566 in patients with advanced pancreatic cancer (abstract 930). *Proc Am Soc Clin Oncol* 2000;19:240a.
 50. Bloomston M, Shafii A, Zervos EE, et al. MMP-2 and TIMP-1 are derived from, not in response to, pancreatic cancer. *J Surg Res* 2002;102:35–8.
 51. Yamauchi K, Ogata Y, Nagase H, Shirouzu K. Inhibition of liver metastasis from orthotopically implanted colon cancer in nude mice by transfection of the TIMP-1 gene into KM12SM cells. *Surg Today* 2001;31:791–8.
 52. Wang M, Liu YE, Greene J, et al. Inhibition of tumor growth and metastasis of human breast cancer cells transfected with tissue inhibitor of metalloproteinase 4. *Oncogene* 1997;14:2767–74.
 53. Watanabe M, Takahashi Y, Ohta T, et al. Inhibition of metastasis in human gastric cancer cells transfected with tissue inhibitor of metalloproteinase 1 gene in nude mice. *Cancer* 1996;77:1676–80.
 54. Koop S, Khokha R, Schmidt EE, et al. Overexpression of metalloproteinase inhibitor in B16F10 cells does not affect extravasation but reduces tumor growth. *Cancer Res* 1994;54:4791–7.
 55. Bloomston M, Shafii A, Zervos EE, et al. Overexpression of TIMP-1 by pancreatic cancer cells reduces in-vitro invasion and in-vivo tumor growth. *J Surg Oncol* (in press).
 56. Bloomston M, Shafii A, Zervos EE, Rosemurgy A. TIMP-1 overexpression in pancreatic cancer attenuates tumor growth, decreases implantation and metastasis, and inhibits angiogenesis. *J Surg Res* 2002;102:39–44.
 57. Bloomston M, Shafii A, Rojiani A, Rosemurgy A. TIMP-1 antisense favorably affects pancreatic cancer biology. *Surg Forum* 2001;52:228–9.
 58. Kuniyasu H, Ellis LM, Evans DB, et al. Relative expression of

- E-cadherin and type IV collagenase genes predicts disease outcome in patients with resectable pancreatic carcinoma. *Clin Cancer Res* 1999;5:25–33.
59. Ito T, Ito M, Shiozawa J, et al. Expression of the MMP-1 in human pancreatic carcinoma: relationship with prognostic factor. *Mod Pathol* 1999;12:669–74.
 60. Gong YL, Xu GM, Huang WD, Chen LB. Expression of matrix metalloproteinases and the tissue inhibitors of metalloproteinases and their local invasiveness and metastasis in Chinese human pancreatic cancer. *J Surg Oncol* 2000;73:95–9.
 61. Määttä M, Soini Y, Liakka A, Autio-Harminen H. 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. *Clin Cancer Res* 2000;6:2726–34.
 62. Ellenrieder V, Alber B, Lacher U, et al. Role of MT-MMPs and MMP-2 in pancreatic cancer progression. *Int J Cancer* 2000;85:14–20.
 63. Fukushima H, Yamamoto H, Itoh F, et al. Association of matrilysin mRNA expression with K-ras mutations and progression in pancreatic ductal adenocarcinomas. *Carcinogenesis* 2001;22:1049–52.
 64. Yamamoto H, Itoh F, Iku S, et al. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human pancreatic adenocarcinomas: clinicopathologic and prognostic significance of matrilysin expression. *J Clin Oncol* 2001;19:1118–27.
 65. Ree AH, Florenes VA, Berg JP, et al. High levels of messenger RNAs for tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) in primary breast carcinomas are associated with development of distant metastases. *Clin Cancer Res* 1997;3:1623–8.
 66. Nakopoulou L, Katsarou S, Giannopoulou I, et al. Correlation of tissue inhibitor of metalloproteinase-2 with proliferative activity and patients' survival in breast cancer. *Mod Pathol* 2002;15:26–34.
 67. Ko BK, Cho HR, Choi DW, et al. Reduced expression of tissue inhibitor of metalloproteinase in nodal metastasis of stomach cancer. *J Korean Med Sci* 1998;13:286–90.
 68. Joo YE, Seo KS, Kim HS, et al. Expression of tissue inhibitors of metalloproteinases (TIMPs) in gastric cancer. *Dig Dis Sci* 2000;45:114–21.
 69. Moser PL, Kieback DG, Hefler L, et al. Immunohistochemical detection of matrix metalloproteinases (MMP) 1 and 2, and tissue inhibitor of metalloproteinase 2 (TIMP 2) in stage IB cervical cancer. *Anticancer Res* 1999;19:4391–3.
 70. Davidson B, Goldberg I, Kopolovic J, et al. MMP-2 and TIMP-2 expression correlates with poor prognosis in cervical carcinoma—a clinicopathologic study using immunohistochemistry and mRNA in situ hybridization. *Gynecol Oncol* 1999;73:372–82.
 71. Joo YE, Seo KS, Kim J, et al. Role of tissue inhibitors of metalloproteinases (TIMPs) in colorectal carcinoma. *J Korean Med Sci* 1999;14:417–23.
 72. Holten-Andersen MN, Stephens RW, Nielsen HJ, et al. High preoperative plasma tissue inhibitor of metalloproteinase-1 levels are associated with short survival of patients with colorectal cancer. *Clin Cancer Res* 2000;6:4292–9.



LIPPINCOTT
WILLIAMS & WILKINS

**Unauthorized Use
Prohibited**