Matrix metalloproteinases and bladder cancer

Hiro-omi Kanayama

Department of Urology, The University of Tokushima School of Medicine, Tokushima, Japan

Abstract : Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes which degrade the extracellular matrix or components of the basement membrane. They have essential roles in tumor invasion and metastasis. In bladder cancer, elevated MMP-2 and MMP-9 expression in tumor tissues, correlated with tumor stage, grade or prognosis, were reported in several studies. Moreover, high levels of serum or urine MMP and TIMP were observed in patients with bladder cancer especially in advanced cases. However, the true roles of MMPs and TIMPs in bladder cancer progression are not yet clarified. Here, we discuss the roles and clinical implications of MMPs in bladder cancer. J. Med. Invest. 48 : 31-43, 2001

Keywords **:** *matrix metalloproteinase, tumor invasion, metastasis, progression, bladder cancer*

INTRODUCTION

The process of cancer progression consists of multisteps, which can be rate limiting since a failure or an insufficiency at any of the steps aborts the process (1-3). The outcome of the process is dependent on both the intrinsic properties of the tumor cells and the responses of the host. The steps or events required for the formation of tumor invasion and metastasis are the same in all tumors (Fig. 1). The major steps in tumor progression are as follows : 1) After the initial transformation, tumor cells grow at the primary site. 2) Neovascularization must occur when the tumor mass forms 2 mm more in diameter (4). Several angiogenic factors play key roles to establish neovascularization (5-7). 3) Local invasion of the basement membrane and degradation of the stroma are necessary for migration from the primary site (8-11). Matrix metalloproteinases may play the most important role in these steps. 4) Thin-walled venules, like lymphatic channels or small capillaries, must be penetrated by tumor cells for tumor cell entry into the circulation. Some carcinomas metastasize and grow via the lymphatic system, and others spread via the hematogeneous route. 5) After the circulating tumor cells attach to the epithelium of venules, extravasation occurs via a similar mechanism as the initial invasion. 6) Tumor cells grow at distant sites with neovascularization, similar to primary site, and metastatic tumors can be established. Then the metastatic process can be completed. To produce detectable lesions, the metastases must develop neovascularization, evade the host immune system (12), and respond to organ-specific factors that influence their growth (13-17). Many factors have essential roles in this metastatic process, and the matrix metalloproteinases (MMPs) must be one of the most important factors in several steps.

Received for publication December 1, 2000 ; accepted January 19, 2001.

Address correspondence and reprint requests to Hiro-omi Kanayama, M.D., Ph. D., Department of Urology, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-633-7160.

MATRIX METALLOPROTEINASES (MMPs) : STRUCTURE, FUNCTION AND REGULATION

In many physiological states or processes, degradation of extracellular matrix is very important and essential, for example, during development, growth, and repair or remodeling of organ tissues (18-21). However, excessive degradation of tissues or proteolysis causes several pathological conditions, for example, rheumatoid arthritis, osteoarthritis, autoimmune disorders of skin, and others (18, 22, 23). In addition, in tumor invasion, metastasis and angiogenesis, degradation of the extracellular matrix is an essential steps and increased expression levels of matrix metalloproteinases (MMPs) is associated with tumor invasion and metastasis with different histogenetic origin (19, 24).

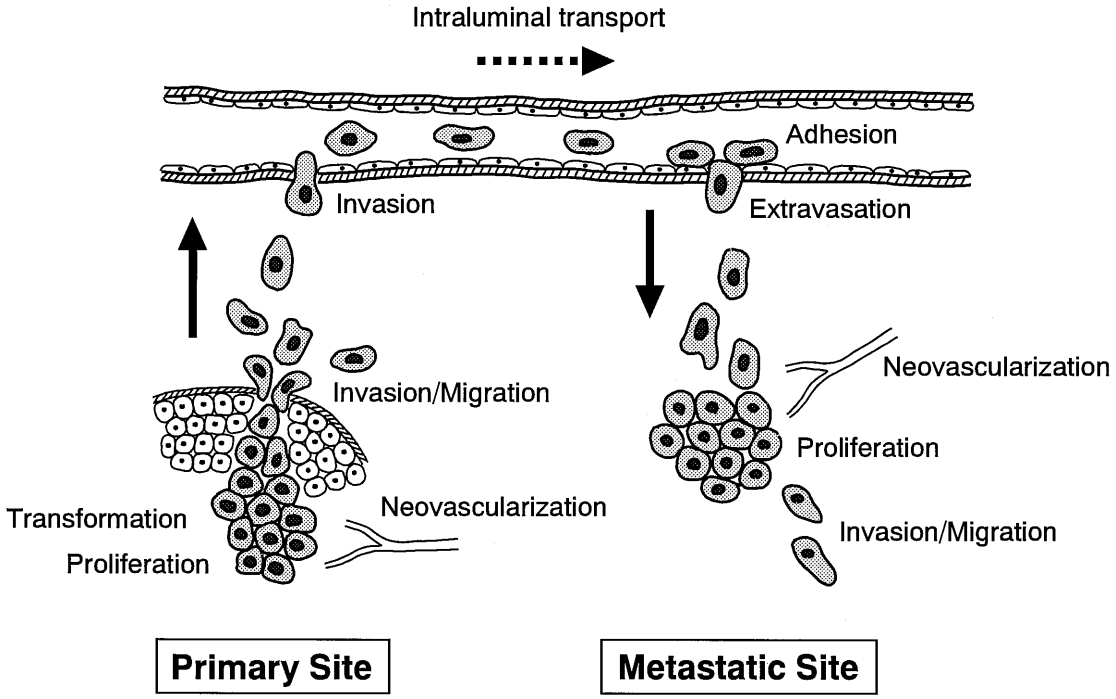

Fig. 1. Multisteps of tumor invasion and metastasis. Tumor cells must complete a series of sequential steps for production of metastasis. Many factors are associated with these process, for example, MMPs, angiogenic factors, and adhesion molecules. MMPs have essential roles in these multisteps.

(1) Structure

MMPs are a family of at least 20 human zinc-dependent endopeptidases, which, collectively capable of degrading extracellular matrix components (19, 22, 23, 25-27). These members of the MMP gene family can be classified into subgroups of collagenases, stromelysins, gelatinases, membrane-type MMPs, and other MMPs according to their substrate specificity and structure (Table 1). In general, MMPs contain a signal/propeptide domain, a catalytic domain with the highly conserved zinc binding site, hinge region, and a hemopexin-like domain (Fig. 2). In addition, gelatinase-A (MMP-2) and gelatinase-B (MMP-9) contain a gelatin-binding site as fibronectin type II inserts within the catalytic domain, and MT-MMPs contain a transmembrane domain in the c-terminal of the hemopexin-like domain. A hemopexin-like domain is absent in matrilysin (MMP-7), the smallest MMP (18, 27). The substrate specificity of MMPs has been determined by their ability to degrade different components of extracellular matrix *in vitro*, however, no direct evidence was obtained *in vivo*.

(2) Function

Collagenase, including interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13) are the secreted neutral proteinases capable of degradation of native fibrillar collagens of types I, II, III (18, 23). These collagenases play a crucial role in degradation of collagenous extracellular matrix in various physiological and pathological situations (28-30). Gelatinase-A (MMP-2, 72 kDa Type IV collagenase) is expressed by a variety of normal and transformed cells. Gelatinase-B (MMP-9, 92 kDa Type IV collagenase) is produced by monocytes, alveolar macrophages, and various malignant cells (18, 23). In addition, MMP-2 and MMP-9 can degrade gelatin, laminin, and MMP-2 has also been reported to degrade native type I collagen and activate MMP-9 and MMP-13 (24, 31). The stromelysin subgroup contains stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), and stromelysin-3 (MMP-11) (18, 23). MMP-3 and MMP-10 are expressed by fibroblastic cells and by normal and transformed squamous epithelial cells (28, 30). Stromelysins degrade basement membrane components, type IV collagen, and fibronectin (18, 23). The cDNA of stromelysin-3 (MMP-11) was cloned from invasive breast cancer tissue (32). The predicted structure of MMP-11 resembles that of other stromelysins and collagenases (18, 23). The smallest MMP-7, matrilysin can degrade gelatin I, III, IV, V and some other extracellular matrix including fibronectin. Moreover, both matrilysin and macrophage metalloelastase have the ability to degrade elastin (18, 22, 23). The first membrane-type MMP (MT 1-MMP, MMP-14) was cloned from invasive lung cancer cells (33) and revealed a typical

Table 1. MMP and TIMP family

MMP group and TIMP	MMP	Name(Enzyme)	Molecular weight		Substrates
			Latent	Active	
Collagenases	1	Interstitial collagenase	55000	45000	Fibrillary collagens I, II, III, VI, IX, Proteoglycans
	8	Neutrophil collagenase	75000	58000	Collagen type I, II, III
	13	Collagenase 3	60000	48000	Collagen type I, II, III
Gelatinases	2	Gelatinase A 72 kD Gelatinase 72 kD Type IV collagenase	72000	66000	Gelatin type I, II, III, Collagen type IV, V, VII, X, Fibronectin, Elastin
	9	Gelatinase B 92 kD Gelatinase 92 kD Type IV collagenase	92000	86000	Gelatin type I, V, Collagen type IV, V
Stromelysins	3	Stromelysin-1 Procollagenase	57000	45000	Cartilage proteoglycans, Fibronectin, Laminin, Gelatin type I, III, IV, V, Collagen type III, IV, V, IX, Procollagenase
	10	Stromelysin-2	57000	44000	Gelatin type I, III, IV, V, Collagen III, IV, V, Procollagenase, Fibronectin
	11	Stromelysin-3	51000	44000	Casein
Membrane-type MMPs	14	MT1-MMP	66000	56000	ProMMP-2, Collagen I, II, III, Gelatin, ProTNF
	15	MT2-MMP	72000		ProMMP-2
	16	MT3-MMP	64000	52000	ProMMP-2
	17	MT4-MMP			
Others	7	Matrilysin or PUMP-1	28000	19000	Gelatin I, III, IV, V, Cartilage Proteoglycan, Fibronectin, Procollagenase, ProTNF, Collagen IV
	12	Macrophage metalloelastase	54000	45000/22000	Elastin
Tissue inhibitors of MMPs		TIMP-1	28500(glycosylated)		All MMPs except MMP-14, MMP-19, Binds to ProMMP-9
		TIMP-2	21000(unglycosylated)		All MMPs, Binds to ProMMP-2
		TIMP-3	27000(glycosylated) 24000(unglycosylated)		All MMPs, Binds to ProMMP-2 and ProMMP-9
		TIMP-4	23000(unglycosylated)		MMP-1, 2, 3, 7, 9, Binds to ProMMP-2

five-domain modular structure resembling collagenases and stromelysins;it also contains an additional short carboxyl-terminal transmembrane domain. Three other MT-MMPs ; MT2-MMP (MMP-15), MT3-MMP (MMP-16), and MT4-MMP (MMP-17) were cloned. Active MT1-MMP serves as a cell membrane receptor for the complex formed of latent MMP-2 (proMMP-2) and tissue inhibitor of metalloproteinases-2 (TIMP-2). The complex of MT1-MMP and MT2-MMP works at the cell surface as an activator for proMMP-2.

Most MMPs are secreted as latent proenzyme that are proteolytically activated in the extracellular space, with the exception of MMP-11 and MT1-MMP, which are activated prior to secretion intracellularly by furin-like proteases (18, 22, 23, 31). The activity of MMPs in the extracellular space is specifically inhibited by tissue inhibitors of metalloproteinases (TIMPs), which bind to the highly conserved zinc binding site of active MMPs at molar equivalence. The TIMP gene family consists of four structurally related members, TIMP-1, -2, -3, and -4, which show 30 to 40% identity at the amino acid level and possess 12 conserved cysteine residues (34). TIMP-1, -2, and -4 are secreted in soluble form whereas TIMP-3 is associated with extracellular matrix. TIMPs have biological effects that extend beyond their role as inhibitors of MMP activity (35). They induce changes in cell morphology, stimulate growth of several cell types, and TIMP-2 is also involved in activation of MMP-2 (31).

(3) Regulation

Regulation of the MMPs is exerted at many levels and involves both transcriptional and post-transcriptional

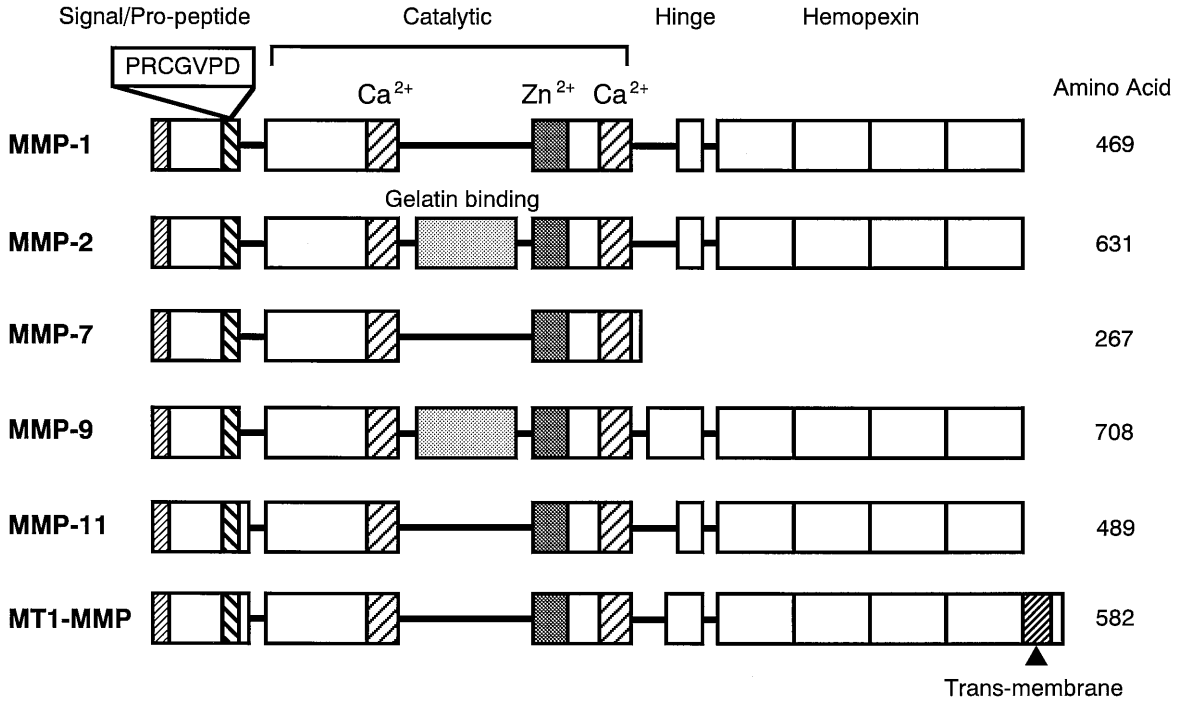

Fig. 2. The structure of MMPs. MMPs consist of the N-terminal signal/propeptide, catalytic, hinge, and C-terminal hemopexin domains. Gelatinases include the gelatin-binding domain, MMP-7 lacks the hemopexin domain, and MT-MMP has transmembrane domain. ProMMPs can be activated by cleavage of the propeptide domain which includes PRCGVPD.

mechanisms (25, 36). Many different factors have been shown to influence the transcription of MMPs, including hormones, growth factors, oncogenes, and cytokines (19, 37, 38). The mechanism of transcriptional activation has been extensively studied and the AP-1 binding site has been the focus of much recent research (36). The AP-1 binding site is located upstream from the transcriptional activation site and has been considered to play an important role in the transcriptional activation of the MMP promoters. From a recent study, the interaction of this site with other cis-acting elements is necessary for basal transcription and induction by cytokines and growth factors (39, 40). The AP-1 site is also involved in the down-regulation of MMPs by transforming growth factor beta, retinoids and glucocorticoids. Several findings suggested that the MMP subgroup was independently regulated (41, 42). In a recent study, a single nucleotide polymorphism in the MMP-1 promoter increased the transcription rate of the MMP-1 gene (43). The increased frequency of the GG polymorphism in several tumor-derived cell lines may be associated with increased matrix degradation (43). The soluble MMPs, such as collagenases, gelatinases, and stromelysins, are secreted as proenzymes, requiring activation. Acquisition of proteolytic activity is associated with the loss of the amino-terminal pro-domain (18, 23). It is becoming apparent that the MMPs interact with other proteases, for example, plasmin and MT1-MMP (44).

THE ROLE OF MMPs IN TUMOR INVASION AND METASTASIS

(1) MMPs expression in malignant tumors

Liotta *et al.* described the role of MMPs in cancer in 1980 (45). They identified a type IV collagenase involved in melanoma invasion and metastasis, and suggested that proteolysis was essential step in tumor invasion. From the cloning of the type IV collagenase, this activity has been considered to be attributed to MMP-2 or MMP-9 (46, 47). Although it was initially suggested that the tumor cells produced these MMPs, it has become clear that the interaction between host stromal cells and tumor cells was important for the induction of MMPs (48). The concept of stromal cell expression of MMPs has been acceptable by the identification of stromelysin-3 as a stromal metalloproteinase associated with breast cancer (32). *In situ* hybridization for MMP revealed that the expression of MMPs in stromal cell is more common than in tumor cells. Many MMPs are induced in connective tissue cells, including fibroblasts and inflammatory cells. There is some evidence that the mRNA of MMP-2 is produced by stroma cells, but the protein is located in tumor cells, especially in the invasive front of tumor

tissue (49). In addition, matrilysin is commonly expressed in the epithelial component of adenocarcinomas (50).

There is a general correlation between the stage of tumor progression and the level of MMPs expression (26, 27). In a murine system of squamous cell carcinomas, high levels of stromelysin-1 in highly metastatic spindle-cell carcinomas and very low levels in benign papillomas were observed (51). The expression of MMP-9 is associated with melanoma growth and subsequent metastasis (52), and high expression of MMP-2 is associated with high tumor grade (53). MMP-2 is widely expressed in breast cancers, however, the ratio of the active form of MMP-2 is increased in advanced disease. In addition, malignant tumors tend to express various MMPs rather than benign tissues (54, 55). Colon adenocarcinomas express matrilysin, stromelysin-1, stromelysin-3, MMP-2, and collagenase-1 (56). From these findings, there is a general positive correlation between tumor aggressiveness and the expression of MMPs.

The ability of the diagnostic or prognostic value of the expression of the MMPs or TIMPs were observed in some studies (26). Expression of stromelysin-3 has been associated only in malignant breast tumors, and it is not expressed in benign tissues (57). Although some other studies have reported associations between stromelysin-3 expression and lymph node metastasis, and, thus, shorter survival in patients with infiltrating ductal carcinoma of the breast, larger studies are necessary to clarify the true value (58-61). In addition, high levels of serum MMP-2 were observed in the patients with prostate cancer (62). Another study mentioned that tissue levels of active MMP-2 were associated with Gleason score and lymph node metastases, and high levels of serum MMP-2 were also found in patients with prostate cancer (63). Similar findings about plasma TIMP-1 were also reported in prostate cancer (64, 65). In colon cancer, immunohistochemical detection of interstitial collagenase is associated with a poor prognosis (66). Matrilysin expression, measured using reverse transcriptase polymerase chain reaction (RT-PCR), has also been suggested to have prognostic value in colon and esophageal cancer (67). In a study of esophageal carcinoma, patients with tumors that demonstrated no matrilysin expression had a better disease-free and overall survival (68).

(2) The role of MMPs and TIMPs in tumor progression

The increased expression of MMPs in advanced tumors and the ability of these enzymes to degrade extracellular matrix barriers suggested that these enzymes have important roles in tumor invasion and metastasis (9, 10, 27). This hypothesis was supported by the findings from experimental and spontaneous metastasis models (69). Recombinant TIMP-1 decreased the number of lung nodules of B 16-F 10 melanoma cells (70). Increased establishment of lung metastases after intravenous injection of several cancer cells were associated with MMP-2, MMP-9 and MT1-MMP (71-73). It has also been shown that MMP-2, MMP-9, and matrilysin metastasize to appropriate target organs from their primary sites in orthotopic models of bladder, fibrosarcoma, and colon cancer (71, 72, 74). In addition, B16-F10 melanoma cells transfected with TIMP-1 produced significantly fewer metastatic nodules than control cells (75). These findings suggest that MMPs and TIMPs have some roles for extravasation to target organs from vessels. However, the role of MMPs in tumor cell intravasation, or entry into the vessels, appears to be better established. TIMPs have been shown to inhibit tumor cell invasion in *in vitro* invasion assay (69). Matrilysin was observed to promote invasion of DU145 prostate cells into the diaphragm of nude mice (76). Recently, an *in vivo* model of tumor cell intravasation using the chick chorioallantoic membrane demonstrated that the intravasation of human epidermoid carcinoma cells was associated with the production of MMP-9 (77). The ability of tumor cells to penetrate the epithelial basement membrane, migrate through stroma, and enter vessels appears to be dependent on matrix-degrading proteases, including the MMPs. However, the process of tumor cell invasion and metastasis is tightly coupled to neovascularization, and MMPs have also been implicated in the process of angiogenesis (78). Based on recent studies with MMP-knock-out mice, MMP-2 or MMP-9 may be associated with neovascularization (79, 80). One of the primary effects of MMPs on tumor progression also appears to be the ability to create the space for tumor growth (19). The effect of TIMP on the establishment of distant metastases was its ability to inhibit the growth of tumor cells at metastatic sites (81). In addition, MMPs also appear to contribute to the establishment and growth of primary tumors (82-84). Thus, MMPs appear to be able to alter the extracellular environment and induce tumor cell establishment and growth.

MMPs IN BLADDER CANCER

(1) Expression of MMPs in tumor tissues

In bladder cancer patients, the presence of deep muscle invasion, infiltrative proliferation, or infiltration to vessels is associated with a high recurrence rate and poor prognosis (85). We evaluated the expression of MMP-2, MMP-9 and its inhibitors TIMP-1 and TIMP-2 in 22 bladder cancer tissues by Northern blot and slot blot analysis and High MMP-2, TIMP-1, and TIMP-2 expression levels were observed in advanced tumor tissues (86). Davies *et al*. reported that MMP-2 and MMP-9 activities quantitated by gelatin zymography correlated with tumor grade and invasion in bladder cancer (87). Grignon *et al*. showed that high levels of TIMP-2 expression were associated with poor outcome in bladder cancer patients undergoing radical cystectomy (88).

We also evaluated MMP-2, TIMP-2 and MT1-MMP expression in bladder cancer tissues using RT-PCR analysis (89). MMP-2 and TIMP-2 expression levels were strongly associated with tumor stage and prognosis. High levels of expression of MMP-2 and TIMP-2 were observed in muscle invasive ≧pT2 bladder cancer tissues compared with low stage pTa-1 tumors. However, MT1-MMP expression was not correlated with tumor invasion. Although, the levels of MT1-MMP was not associated with tumor invasion, it was associated with patient outcome. Other studies have shown high expression of MT1-MMP in cancer tissues and association with invasiveness of cervical cancer cells (90) or lymph node metastases in lung cancers (91). Thus, there may be some tissue specificity regarding the involvement of MT1-MMP in tumor invasion or metastasis. In bladder cancer, MT1-MMP may involve distant metastasis directly but not tumor invasion.

The relatively activated MMP-2 expression can be measured by gelatin zymography which is capable of highly sensitive differentiation of latent and activated forms of gelatinases. Therefore, we determined activation of MMP-2 using gelatin zymography (92) (Fig. 3). The expression of activated MMP-2 and the expression of total MMP-2 in invasive tumors (≧pT 2) were both significantly higher than in superficial tumors (pTa-1). These findings indicated that MMP-2 expression in urothelial tumors was highly correlated with tumor invasion, and showed a strong correlation between the levels of activated MMP-2 and those of total MMP-2. Both expression levels were associated in tumor invasion, but the findings suggested that the activated form of MMP-2 expression was a better indicator of tumor invasion. Moreover, we observed that the high expression groups of the activated form of MMP-2 and total MMP-2 showed significantly worse cause-specific survival than did the low expression groups. High expressions of activated MMP-2 were more strongly linked with an unfavorable prognosis than was total MMP-2 expression. We also reported MMP-2 and MT1-MMP expression in invasive urothelial tumor tissues transplanted in SCID mice (93).

In bladder cancer, the pathologic stage or tumor grade is associated with patient survival. However, even within patients of the same stage or grade, there are some differences in patient survival (85). Therefore, there is a need to identify other predictors for bladder cancer patients. We examined the survival of patients according to the levels of expression of MMP-2, TIMP-2, and MT1-MMP to evaluate the usefulness of these proteins as predictors. Patients with high expression levels of MMP-2, TIMP-2, or MT1-MMP showed worse cause-specific survival, even within the

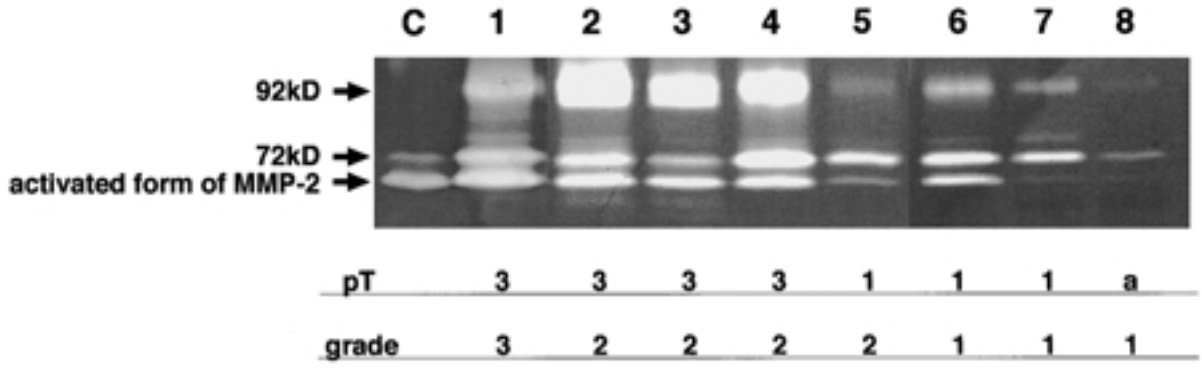

Fig. 3. Gelatin zymography of bladder cancer tissue extract. Eight urothelial cancer tissues (lanes 1 to 8) were obtained operatively from the patients with bladder cancer. lane C : UCT-2 tumor line tissue extract was used as the standard in each gel. pT : pathological stage of bladder tumor. grade : histological grade of tumor cells. 72 kilodaiton (kDa) : latent form of MMP-2. Lowest band : activated form of MMP-2.92 kDa : latent form of MMP-9.

group of patients with muscle invasive ≧pT2 tumors resected radically (89). However, differences in the MMP-2/TIMP-2 ratios did not affect survival. In our study, the expression levels of MMP-2 was correlated with those of TIMP-2. Therefore, the ratio of MMP-2/TIMP-2 might not affect patient outcome. Grignon *et al.* showed that high levels of TIMP-2, as assessed by immunohistochemical staining, were associated with poor outcome in patients with invasive bladder cancer treated by cystectomy but that MMP-2 expression did not affect survival (88). However, it is complicated to evaluate MMP-2 or TIMP-2 expression by immunohistochemical staining methods. Moreover, evaluation of expression by gelatin zymography or Northern blot analysis is also difficult to use in clinical settings. Our results demonstrated that levels of MMP-2, TIMP-2, or MT1-MMP in tumor tissues obtained by operation or biopsy may be useful for prognosis, even in patients with muscle invasive tumors (89, 92). It is possible that adjuvant chemotherapy can be avoided in patients with low levels of MMP-2 or TIMP-2 whose muscle invasive bladder cancers have been radically resected.

Recently, several studies reported MMP-2 and MMP-9 in bladder cancer. Papathoma *et al.* reported that zymographical analysis of the levels of MMP-9 and active MMP-2 showed a significant increase with tumor grade and invasiveness, however, the correlation between the levels of both gelatinases with recurrence in superficial tumors or progression in invasive tumors was not significant (94). We evaluated MMP-9 expression in bladder tumor tissues using gelatin zymography (92), however, the correlation between MMP-9 expression levels and tumor invasion was weak and not significant. The background of patients might have been different in those two studies. Ozdemir *et al.* reported a strong correlation of basement membrane degradation with p53 inactivation and/or MDM2 overexpression in superficial urothelial carcinomas, and they suggested that MMP-9 plays a key role in the invasion step of superficial urothelial carcinomas (95, 96). Kitagawa *et al.* reported that MT1-MMP and MT2-MMP may play an important role in the development and multifocal occurrence of urothelial cancer (97).

(2) Serum MMPs and TIMPs

Recently, some studies evaluated serum levels of MMPs and TIMPs, and their clinical usefulness. Gohji *et al.* reported the prognostic significance of serum MMPs and TIMPs, and the imbalance between serum matrix metalloproteinase-2 and its inhibitor as a predictor of recurrence of urothelial cancer (98-100). Measurement of plasma/serum MMP and TIMP levels may provide important information for selecting and following patients considered for treatment with drugs that interfere with MMP activity (101). We also observed high levels of serum TIMP-1 in patients with advanced bladder cancers (102).

(3) Urinary MMPs and TIMPs

Moses *et al.* evaluated the incidence of matrix metalloproteinases in urine of cancer patients (103). They detected three molecular weight classes of urinary MMPs, MMP-2, MMP-9, and high molecular weight (Mr≧150,000) species, correlated with disease status. The presence of biologically active MMP-2 or MMP-9 was an independent predictor of organ-confined cancer, and the high molecular weight species was an independent predictor of metastatic cancer. Monier *et al.* detected 72 kDa proMMP-2 and its activated 68 kDa form, a 92 kDa proMMP-9, and a higher molecular weight complex (115 kDa) which was identified as proMMP-9 (104). MMPs in urine can be used as predictors of bladder cancer diagnosis and prognosis. Sier *et al.* also reported that urinary MMP-2 and MMP-9 activity levels were significantly correlated with each other, and they concluded that enhanced urinary MMP activity or especially in combination with other markers, might be useful as a marker for superficial bladder carcinoma (105). Detection of proMMP-9 in bladder washes also may be a novel approach for the identification of patients with more aggressive forms of bladder cancer (106).

(4) Regulation of MMP-2 and MMP-9 in bladder cancer cells by cytokines

Shin *et al.* observed that MMP-2 and MMP-9 expression was up-regulated by tumor necrosis-alpha or interferon-gamma, and they suggested BCG immunotherapy may enhance the invasiveness of bladder cancer in certain conditions with induction of MMPs (107). Kageyama *et al.* also suggested the possibility that BCG promotes invasion of bladder cancer cells because of the induced secretion of MMP-9 from peripheral mononuclear cells by BCG (108). Basic fibroblast growth factor also induces MMP-2 and MMP-9 secretion from bladder cancer cells (109). However, Slaton *et al.* observed interferon-alpha down regulated the production of basic fibroblast growth factor and MMP-9 from invasive bladder cancer cells (110). From these findings, certain cytokines may be useful for treatment of invasive bladder cancer by regulating MMPs.

CONCLUSIONS

It has been clarified that MMPs have important roles in tumor invasion and metastasis. Large numbers of studies have examined the presence of individual MMPs in different types of cancer, using various methods. In bladder cancer, although these studies suggested the clinical usefulness of MMP-2 and MMP-9 in tissues, serum and urine for further diagnosis of tumor biological activities or patient status, further examinations of large numbers of patients are necessary. In the near future, it may be possible to use MMPs or TIMPs for clinical applications as MMPs expression levels in biopsied cancer tissues, serum or urine MMPs and TIMPs obtained from patients with bladder cancer. The development of antibodies that distinguish between pro-enzymes and activated enzymes, and between free MMP and MMP complexed with TIMP may provide new approaches for bladder cancer diagnosis or treatment. Moreover, MMP-2 or MMP-9 would be the target for therapy in especially advanced bladder cancer patients. With the development of MMP inhibitors, anti-invasion or metastasis therapy may be useful clinically.

REFERENCES

1. Poste G, Fidler IJ : The pathogenesis of cancer metastasis. Nature 283 : 139-146, 1979.
2. Fidler IJ : Critical factors in the biology of human cancer metastasis : twenty-eighth G. H. A. Clowes Memorial Award Lecture. Cancer Res 50 : 6130-6138, 1990.
3. Aznavoorian A, Murphy AN, Stetler-Stevenson WG, Liotta LA : Molecular aspects of tumor cell invasion and metastasis. Cancer 71 : 1368-1383, 1993.
4. Folkman J : Angiogenesis : initiation and modulation. In : Nicolson GL, Milas L, eds. Cancer Invasion and Metastasis : Biologic and Therapeutic Aspects. Raven Press, New York, 1984, pp. 201-209
5. Folkman J : How is blood vessel growth regulated in normal and neoplastic tissue? G. H. A. Clowes Memorial Awards Lecture. Cancer Res 46 : 467-473, 1986.
6. Folkman J, Klagsburn M : Angiogenic factors. Science 235 : 444-447, 1987.
7. Ellis LM, Fidler IJ : Angiogenesis and metastasis. European Journal of Cancer 32A : 2451-2460, 1996.
8. Gabbert H : Mechanisms of tumor invasion : evidence from in vivo observations. Cancer Metastasis Rev 4 : 283-310, 1985.
9. Liotta LA : Tumor invasion and metastasis-role of the extracellular matrix. Rhoads Memorial Award Lecture. Cancer Res 46 : 1-7, 1986.
10. Liotta LA, Rao CN, Barsky SH : Tumor invasion and the extracellular matrix. Lab Invest 49 : 636-649, 1983
11. Mareel M : Invasion in vitro:methods of analysis. Cancer Metastasis Rev 2 : 201-209, 1983.
12. Fidler IJ, Gersten DM and Hart IR : The biology of cancer invasion and metastasis. Adv Cancer Res 28 : 149-250, 1978
13. Nicolson GL : Cancer Metastasis : tumor cell and host organ properties important in metastasis to specific secondary sites. Biochim Biophys Acta 948 : 175-224, 1988.
14. Horak E, Darling DL, Tarin D : Analysis of organ-specific effects on metastatic tumor formation by studies in vivo. J Natl Cancer Inst 76 : 913-922, 1986.
15. Naito S, Giavazzi R, Fidler IJ : Correlation between the in vitro interaction of tumor cells with an organ environment and metastatic behavior in vivo. Invasion Metastasis 7 : 16-29, 1987.
16. Price JE, Tarin D, Fidler IJ : The influence of organ microenvironment on pigmentation of metastatic murine melanoma. Cancer Res 48 : 2258-2264, 1988.
17. Price JE, Naito S, Fidler IJ : The role of the organ microenvironment in the selective process of metastasis. Clin Exp Metastasis 6 : 91-102, 1988.
18. Shapiro SD : Matrix metalloproteinase degradation of extracellular matrix : biological consequences. Curr Opin Cell Biol 10 : 602-608, 1998.
19. Johnsen M, Lund LR, Romer J, Almholt K, Dano K : Cancer invasion and tissue remodeling : com mon themes in proteolytic matrix degradation. Curr Opin Cell Biol 10 : 667-671, 1998.
20. Rudolph-Owen LA, Matrisian LM : Matrix metalloproteinases in remodeling of the normal and neoplastic mammary gland. J Mammary Gland Biol Neoplasia 3 : 177-189, 1998.
21. Ravanti L, Kahari V : Matrix metalloproteinases in wound repair. Int J Mol Med 6 : 391-407, 2000.
22. Kahari V-M, Saarialho-Kere U : Matrix metalloproteinases in skin. Exp Dermatol 6 : 199-213, 1997.
23. Woessner JF : The matrix metalloproteinase family. In : Parks WC, Mecham RP eds. Matrix Metalloproteinases. Academic Press, San Diego, 1998, pp. 1-14
24. Basset P Okada A, Chenard M-P, Kannan R,

Stoll I, Anglard P, Bellocq J-P, Rio M-C : Matrix metalloproteinases as stromal effectors of human carcinoma progression : therapeutical implications. Matrix Biol 15 : 535-541, 1997.
25. Westermarck J, Kahari VM : Regulation of matrix metalloptroteinase expression in tumor invasion. FASEB J 13 : 781-792, 1999.
26. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM : Matrix metalloproteinases : biologic activity and clinical implications. J Clin Oncol 18 : 1135-1149, 2000.
27. Curran S, Murray GI : Matrix metalloproteinases. molecular aspects of their roles in tumor invasion and metastasis. Eur J Cancer 36 : 1621-1630, 2000.
28. Airola K, Johansson N, Kariniemi A-L, Kahari V-M, Saarialho-Kere U : Human collagenase- 3 is expressed in malignant squamous epithelium of the skin. J Invest Dermatol 109 : 225-231, 1997.
29. Johansson N, Vaalamo M, Grenman S, Hietanen S, Klemi P, Saarialho-Kere U, Kahari V-M : Collagenase-3 (MMP-13) is expressed by tumor cells in invasive vulvar squamous cell carcinomas. Am J Pathol 154 : 469-480, 1999.
30. Uria JA, Balbin M, Loez JM, Alvarez J, Vizoso F, Takigawa M, Loez-Otim C : Collagenase-3 (MMP-13) expression in chondrosarcoma cells and its regulation by basic fibroblast growth factor. Am J Pathol 153 : 91-101, 1998.
31. Murphy G, Knauper V : Relating matrix metalloproteinase structure to function : why the "hemopexin" domain?. Matrix Biol 15 : 511-518, 1997.
32. Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Limacher JM, Podhajcer OL, Chenard MP, Rio MC, Chambon P : A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 348 : 699-704, 1990.
33. Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M : A matrix metalloproteinase expressed on the surface of invasive tumor cells. Nature 370 : 61-65, 1994.
34. Sato H, Kinoshita T, Takino T, Nakayama K, Seiki M : Activation of a recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2. FEBS Lett 393 : 101-104, 1996.
35. Gomez DE, Alonso DF, Yosiji H, Thorgeirsson UP : Tissue inhibitors of metalloproteinases : structure, regulation and biological functions Eur J Cell Biol 74 : 111-122, 1997.
36. Jones JL, Walker RA : Control of matrix metalloproteinase activity in cancer. J Pathol 183 : 377-379, 1997.
37. Atula S, Grenman R, Syrjanen S : Fibroblast can modulate the phenotype of malignant epithelial cells in vitro. Exp Cell Res 235 : 180-187, 1997.
38. Skobe M, Fusenig NE : Tumorigenic conversion of immoral human keratinocytes through stromal cell activation. Proc Natl Acad Sci USA 95 : 1050-1055, 1998.
39. Benbow U, Brinckerhoff CE : The AP-1 site and MMP gene regulation : what is all the fuss about? Matrix Biol 15 : 519-526, 1997.
40. Crawford HC, Matrisian LM : Mechanisms controlling the transcription of matrix metalloproteinase genes in normal and neoplastic cells. Enzyme Protein 49 : 20-37, 1996.
41. Brown PD, Levy AT, Margulies IMK, Liotta LA, Stetler-Stevenson WG : Independent expression and cellular processing of Mr 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines. Cancer Res 50 : 6184-6191, 1990.
42. Vincenti MP, Coon CI, Mengshol JA, Yocum S, Mitchell P, Brinckerhoff CE : Cloning of the gene for interstitial collagenase-3 (matrix metalloproteinase-13) from rabbit synovial fibroblasts : differential expression with collagenase-1 (matrix metalloproteinase-1). Biochem J 331 : 341-346, 1998.
43. Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, Brinekerhoff CE : A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 58 : 5321-5325, 1998.
44. Okumura Y, Sato H, Seiki M, Kido H : Proteolytic activation of the precursor of membrane type1 matrix metalloproteinase by human plasmin. A possible cell surface activator. FEBS Lett 102 : 181-184, 1997.
45. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S : Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 284 : 67-68, 1980.
46. Matrisian LM, Glaichenhaus N, Gesnel MC, Breathnach R : Epidermal growth factor and oncogenes induce transcription of the same cellular mRNA in rat fibroblasts. EMBO J 4 : 1435-1440, 1985.
47. Goldberg G, Wilhelm SM, Kronberger A, Bauer EA, Grant GA, Eisen AZ : Human fibroblast collagenase : Complete primary structure and homology to an oncogene transformation-induced rat protein. J Biol Chem 261 : 6600-6605, 1986.
48. Nabeshima K, Lane WS, Biswas C : Partial sequenc-

ing and characterization of the tumor cell-derived collagenase stimulatory factor. Arch Biochem Biophys 285 : 90-96, 1991.
49. Monsky WL, Kelly T, Lin C-Y, Yeh Y, Stetler-Stevenson WG, Mueller SC, Chen WT : Binding and localization of (M)r 72,000 matrix metalloproteinase at cell surface invadopodia. Cancer Res 53 : 3159-3164, 1993.
50. McDonnell S, Navre M, Coffey RJ, Matrisian LM : Expression and localization of the matrix metalloproteinase pump-1 (MMP-7) in human gastric and colon carcinomas. Mol Carcinog 4 : 527-533, 1991.
51. McDonnell S, Matrisian LM : Stromelysin in tumor progression and invasion. Cancer Metastasis Rev 9 : 305-319, 1991.
52. MacDougall JR, Bani MR, Lin Y, Rak J, Korbel RS : The 92-kDa gelatinase B is expressed by advanced stage melanoma cells : Suppression by somatic cell hybridization with early stage melanoma cell. Cancer Res 55 : 4174-4181, 1995.
53. Vaisanen A, Tuominen H, Kallioinen M, Turpeenniemi-Hujanen T : Matrix metalloproteinase-2 (72 kd type iv collagenase) expression occurs in the early stage of human melanocytic tumor progression and may have prognostic value. J Pathol 180 : 283-289, 1996.
54. Davies B, Miles DW, Happerfield LC, Naylor MS, Bobrow LG, Rubens RD, Balkwill FR : Activity of type IV collagenases in benign and malignant breast disease. Br J Cancer 67 : 1126-1131, 1993.
55. Brown PD, Bloxidge RE, Anderson E, Howell A : Expression of activated gelatinase in human invasive breast carcinoma. Clin Exp Metastasis 11 : 183-189, 1993.
56. Newell KJ, Witty JP, Rodgers WH, Matrisian LM : Expression and localization of matrix-degrading metalloproteinases during colorectal tumorigenesis. Mol Carcinog 10 : 199-206, 1994.
57. Wolf C, Rouyer N, Lutz Y, Adida C, Loriot M, Bellog JP, Chambon P, Basset P : Stromelysin 3 belongs to a subgroup of proteinases expressed in breast carcinoma fibroblastic cells and possibly implicated in tumor progression. Proc Natl Acad Sci USA 90 : 1843-1847, 1993.
58. Ahmad A, Hanby A, Dublin E, Poulsom R, Smith P, Barnes D, Rubens R, Anglard P, Hart I : Stromelysin-3 : An independent prognostic factor for relapse-free survival in node-positive breast cancer and demonstration of novel breast carcinoma cell expression. Am J Pathol 152 : 721-728, 1998.
59. Chenard MP, O'Siorain L, Shering S, Rouyer N, Lutz Y, Wolf C, Basset P, Bellocq JP, Duffy MJ : High levels of stromelysin-3 correlate with poor prognosis in patients with breast carcinoma. Int J Cancer 69 : 448-451, 1996.
60. Kawami H, Yoshida K, Ohsaki A, Kuroi K, Nishiyama M, Toge T : Stromelysin-3 mRNA expression and malignancy : Comparison with clinicopathological features and type IV collagenase mRNA expression in breast tumors. Anticancer Res 13 : 2319-2324, 1993.
61. Tetu B, Brisson J, Lapointe H, Bernard P : Pro gnostic significance of stromelysin 3, gelatinase A, and urokinase expression in breast cancer. Hum Pathol 29 : 979-985, 1998.
62. Gohji K, Fujimoto N, Hara I, Fujii A, Gotoh A, Okada H, Arakawa S, Kitazawa S, Miyake H, Kamidono S, Nakajima M : Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. Int J Cancer 79 : 96-101, 1998.
63. Stearns ME, Stearns M : Immunohistochemical studies of activated matrix metalloproteinase-2 (MMP-2a) expression in human prostate cancer. Oncol Res 8 : 63-67, 1996.
64. Jung K, Nowak L, Lein M, Priem F, Schnorr D, Loening SA : Matrix metalloproteinases 1 and 3, tissue inhibitor of metalloproteinase-1 and the complex of metalloproteinase-1 tissue inhibitor in plasma of patients with prostate cancer. Int J Cancer 74 : 220-223, 1997.
65. Baker T, Tickle S, Wasan H, Docherty A, Isenberg D, Waxman J : Serum metalloproteinases and their inhibitors : Markers for malignant potential. Br J Cancer 70 : 506-512, 1994.
66. Murray GI, Duncan ME, O'Neil P, Melvin WT, Fothergill JE : Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. Nat Med 2 : 461-462, 1996.
67. Ichikawa Y, Ishikawa T, Momiyama N, Yamaguchi S, Masui H, Hasegawa S, Chishima T, Takimoto A, Kitamura H, Akitaya T, Hosokawa T, Mitsuhashi M, Shimada H : Detection of regional lymph node metastases in colon cancer by using RT-PCR for matrix metalloproteinase-7, matrilysin. Clin Exp Metastasis 16 : 3-8, 1998.
68. Yamamoto H, Adachi Y, Itoh F, Iku S, Matsuno K, Kusano M, Arimura Y, Endo T, Hinoda Y, Hosokawa M, Imai K : Association of matrilysin expression with recurrence and poor prognosis in human esophageal squamous cell carcinoma.

Cancer Res 59 : 3313-3316, 1999.
69. Chambers AF, Matrisian LM : Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 89 : 1260-1270, 1997.
70. Schultz RM, Silberman S, Persky B, Bajkowski AS, Carmichael DF : Inhibition by human recombinant tissue inhibitor of metalloproteinases of human amnion invasion and lung colonization by murine B16-F10 melanoma cells. Cancer Res 48 : 5539-5545, 1988.
71. Kawamata H, Kameyama S, Kawai K, Tanaka Y, Nan L, Barch DH, Stetler-Stevenson WG, Oyasu R : Marked acceleration of the metastatic phenotype of a rat bladder carcinoma cell line by the expression of human gelatinase A. Int J Cancer 63 : 568-575, 1995.
72. Bernhard EJ, Gruber SB, Muschel RJ : Direct evidence linking expression of matrix metalloproteinase 9 (92-kDa gelatinase/collagenase) to the metastatic phenotype in transformed rat embryo cells. Proc Natl Acad Sci USA 91 : 4293-4297, 1994.
73. Tsunezuka Y, Kinoh H, Takino T, Watanabe Y, Okada Y, Shinagawa A, Sato H, Seiki M : Expression of membrane-type matrix metalloproteinase 1 (MT1-MMP) in tumor cells enhances pulmonary metastasis in an experimental metastasis assay. Cancer Res 56 : 5678-5683, 1996.
74. Hasegawa S, Koshikawa N, Momiyama N, Moriyama K, Ichikawa Y, Ishikawa T, Mitsuhashi M, Shimada H, Miyazaki K : Matrilysin-specific oligonucleotide inhibits liver metastasis of human colon cancer cells in a nude mouse model. Int J Cancer 76 : 812-816, 1998.
75. Khokha R, Zimmer MJ, Wilson SM, Chambers AF : Up-regulation of TIMP-1 expression in B16-F10 melanoma cells suppresses their metastatic ability in chick embryo. Clin Exp Metastasis 10 : 365-370, 1992.
76. Powell WC, Knox JD, Navre M, Grogan TM, Kittelson J, Nagle RB, Bowden GT : Expression of the metalloproteinase matrilysin in DU-145 cells increases their invasive potential in severe combined immunodeficient mice. Cancer Res 53 : 17-422, 1993.
77. Kim J, Yu W, Kovalski K, Ossowski L : Requirement for specific proteases in cancer cell intravasation as revealed by a novel semi-quantitative PCR-based assay. Cell 94 : 353-362, 1998.
78. McCawley LJ, Matrisian LM : Matrix metalloproteinases : multifunctional contributors to tumor progression. Mol Med Today 6 : 149-156, 2000.
79. Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S : Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res 58 : 1048-1051, 1998.
80. Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z : MMP-9/Gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 93 : 411-422, 1998.
81. Koop S, Khokha R, Schmidt EE, MacDonald IC, Morris VL, Chambers AF, Groom AC : Overexpression of metalloproteinase inhibitor in B16F10 cells does not affect extravasation but reduces tumor growth. Cancer Res 54 : 4791-4797, 1994.
82. Witty JP, McDonnell S, Newell K, Cannon P, Navre M, Tressler RJ, Matrisian LM : Modulation of matrilysin levels in colon carcinoma cell lines affects tumorigenicity in vivo. Cancer Res 54 : 4805-4812, 1994.
83. Noel AC, Lefebvre O, Maquoi E, VanHoorde L, Chenard MP, Mareel M, Foidart JM, Basset P, Rio MC : Stromelysin-3 expression promotes tumor take in nude mice. J Clin Invest 97 : 1924-1930, 1996.
84. Masson R, Lefebvre O, Noel A, Fahime ME, Chenard MP, Wendling C, Kebers F, LeMeur M, Dierich A, Foidart JM, Basset P, Rio MC : In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. J Cell Biol 140:1535-1541, 1998.
85. Liponen PK, Eskelinen MJ, Kiviranta J, Pesonen E : Prognosis of transitional cell bladder cancer : A multivariate prognostic score for improved prediction. J Urol 146 : 1535-1540, 1991.
86. Naruo S, Kanayama H, Aki M, Kagawa S : Gene expressions of type IV collagenase and tissue inhibitor of metalloproteinases (TIMP) in human bladder cancers. Jpn J Urol 84 : 841-850, 1993
87. Davies B, Waxman J, Wasan H, Abel P, Williams G, Krausz T, Neal D, Thomas D, Hanby A, Balkwill F : Levels of matrix metalloproteases in bladder cancer correlate with tumor grade and invasion. Cancer Res 53 : 5365-5369, 1993.
88. Grignon DJ, Sakr W, Toth M, Ravery V, Angulo J, Shamsa F, Pontes JE, Crissman JC, Fridman R : High levels of tissue inhibitor of metalloproteinase-2 (TIMP-2) expression are associated with poor outcome in invasive bladder cancer. Cancer Res 56 : 1654-1659, 1996.
89. Kanayama H, Yokota K, Kurokawa Y, Murakami Y, Nishitani M, Kagawa S : Prognostic values of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 expression in bladder cancer.

Cancer 82 : 1359-1366, 1998.
90. Gilles C, Polette M, Piette J, Munaut C, Thompson EW, Birembaut P, Foidart JM : High level of MT-MMP expression is associated with invasiveness of cervical cancer cells. Int J Cancer 65 : 209-13, 1996.
91. Tokuraku M, Sato H, Murakami S, Okada Y, Watanabe Y, Seiki M. Activation of the precursor of gelatinase A/72kDa type IV collagenase/MMP-2 in lung carcinomas correlates with the expression of membrane-type matrix metalloproteinase (MT-MMP) and with lymph node metastasis. Int J Cancer 64 : 355-9, 1995.
92. Kanda K, Takahashi M, Murakami Y, Kanayama H, Kagawa S : The role of the activated form of matrix metalloproteinase-2 in urothelial cancer. BJU Int 86 : 553-557, 2000.
93. Furukawa A, Tsuji M, Nishitani M, Kanda K, Inoue Y, Kanayama H, Kagawa S : Role of the matrix metalloproteinase and tissue inhibitors of metalloproteinase families in noninvasive and invasive tumors transplanted in mice with severe combined immunodeficiency. Urology 51 : 849-853, 1998.
94. Papathoma AS, Petraki C, Grigorakis A, Papakonstantinou H, Karavana V, Stefanakis S, Sotsiou F, Pintzas A : Prognostic significance of matrix metalloproteinases 2 and 9 in bladder cancer. Anticancer Res 20 : 2009-2013, 2000.
95. Ozdemir E, Kakehi Y, Okuno H, Habuchi T, Okada Y, Yoshida O : Strong correlation of basement membrane degradation with p53 inactivation and/or MDM2 overexpression in superficial urothelial carcinomas. J Urol 158 : 206-211, 1997.
96. Ozdemir E, Kakehi Y, Okuno H, Yoshida O : Role of matrix metalloproteinase-9 in the basement membrane destruction of superficial urothelial carcinomas. J Urol 161 : 1359-1363, 1999.
97. Kitagawa Y, Kunimi K, Ito H, Sato H, Uchibayashi T, Okada Y, Seiki M, Namiki M : Expression and tissue localization of membrane-types 1, 2, and 3 matrix metalloproteinases in human urothelial carcinomas. J Urol 160 : 1540-1545, 1998
98. Gohji K, Fujimoto N, Komiyama T, Fujii A, Ohkawa J, Kamidono S, Nakajima M : Elevation of serum levels of matrix metalloproteinase-2 and -3 as new predictors of recurrence in patients with urothelial carcinoma. Cancer 78 : 2379-2387, 1996
99. Gohji K, Fujimoto N, Fujii A, Komiyama T, Okawa J, Nakajima M : Prognostic significance of circulating matrix metalloproteinase-2 to tissue inhibitor of metalloproteinases-2 ratio in recurrence of urothelial cancer after complete resection. Cancer Res 56 : 3196-3198, 1996.
100. Gohji K, Fujimoto N, Ohkawa J, Fujii A, Nakajima M : Imbalance between serum matrix metalloproteinase-2 and its inhibitor as a predictor of recurrence of urothelial cancer. Br J Cancer 77 : 650-655, 1998.
101. Zucker S, Hymowitz M, Conner C, Zarrabi HM, Hurewitz AN, Matrisian L, Boyd D, Nicolson G, Montana S : Measurement of matrix metalloproteinases and tissue inhibitors of metalloproteinases in blood and tissues. Clinical and experimental applications. Ann NY Acad Sci 878 : 212-227, 1999.
102. Naruo S, Kanayama H, Takigawa H, Kagawa S, Yamashita K, Hayakawa T : Serum levels of a tissue inhibitor of metalloproteinases-1 (TIMP-1) in bladder cancer patients. Int J Urol 1 : 228-231, 1994.
103. Moses MA, Wiederschain D, Loughlin KR, Zurakowski D, Lamb CC, Freeman MR : Increased incidence of matrix metalloproteinases in urine of cancer patients. Cancer Res 58 : 1395-1399. 1998.
104. Monier F, Surla A, Guillot M, Morel F : Gelatinase isoforms in urine from bladder cancer patients. Clin Chim Acta 299 : 11-23, 2000.
105. Sier CF, Casetta G, Verheijen JH, Tizzani A, Agape V, Kos J, Blasi F, Hanemaaijer R : Enhanced urinary gelatinase activities (matrix metalloproteinases 2 and 9) are associated with early-stage bladder carcinoma : a comparison with clinically used tumor markers. Clin Cancer Res 6 : 2333-2340, 2000.
106. Bianco FJ Jr, Gervasi DC, Tiguert R, Grignon DJ, Pontes JE, Crissman JD, Fridman R, Wood DP Jr : Matrix metalloproteinase-9 expression in bladder washes from bladder cancer patients predicts pathological stage and grade. Clin Cancer Res 4 : 3011-3016, 1998.
107. Shin KY, Moon HS, Park HY, Lee TY, Woo YN, Kim HJ, Lee SJ, Kong G : Effects of tumor necrosis factor-alpha and interferon-gamma on expressions of matrix metalloproteinase-2 and -9 in human bladder cancer cells. Cancer Lett 159 : 127-134, 2000.
108. Kageyama Y, Kawakami S, Fujii Y, Kihara K, Oshima H : Bacillus Calmette-Guerin enhances production and secretion of type IV collagenases in peripheral blood mononuclear cells. Jpn J Cancer Res 88 : 281-828, 1997.
109. Miyake H, Yoshimura K, Hara I, Eto H, Arakawa S, Kamidono S : Basic fibroblast growth factor regulates matrix metalloproteinases production and in vitro invasiveness in human bladder can-

cer cell lines. J Urol 157 : 2351-2355, 1997.
110. Slaton JW, Perrotte P, Inoue K, Dinney CP, Fidler IJ : Interferon-alpha-mediated down-regulation of angiogenesis-related genes and therapy of bladder cancer are dependent on optimization of biological dose and schedule. Clin Cancer Res 5 : 2726-2734, 1999.