Bratisl Lek Listy 2011; 112 (8)
435 – 440

REVIEW

Clinical importance of matrix metalloproteinases

Krizkova S[1], Zitka O[1], Masarik M[2], Adam V[1], Stiborova M[3], Eckschlager T[4], Hubalek J[5], Kizek R[1]

Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic.
kizek@sci.muni.cz

Abstract: This review gives a brief summary on clinical applications of MMPs and their determination. Primarily, the activity of MMPs in cancer formation, development and metastasis is discussed. Further, survey on methods including fluorimetric methods, zymographies, Western-blotting, immunocapture assay, enzyme-linked immunosorbent assay, immunocytochemistry and immunohistochemistry, phage display, multiple-enzyme/multiple-reagent system, activity profiling, chronopotentiometric stripping analysis and imaging methods for detection and determination of MMPs follows *(Fig. 3, Ref. 100)*. Full Text in free PDF *www.bmj.sk.*
Key words: matrix metalloproteinases, clinical diagnostics, zymography, cancer.

Matrix metalloproteinases (MMPs) were identified in vertebrates in 1962 by Jerome Gross and Charles M. Lapiere who both studied the degradation of triple-helical collagen during tadpole tail metamorphosis (1). Since the discovery of these proteins, more then thirty thousand papers have been published according to the Web of Science on MMPs and have included the term "metalloproteinase*" within article titles, keywords and abstracts. Much attention is associated with the extensive number of MMPs families throughout the prokaryotic and eukaryotic systems. Their phylogenetic origin is attributed to *Bacteroides fragilis* (2). MMPs family members reveal up to 40 % in primary structural similarity. Approximately 20 types of MMPs have been identified, which are classified according to the pre-synthetic region on chromosomes and substrate specificities. They are labelled with numbers ranging from MMP-1 to MMP-28 (3). They are classified into five sub-groups according to functionality: collagenases, stromelysins, matrilysins, gelatinases, membrane-associated MMPs and MMPs with no group designation. X-ray crystallography and nuclear magnetic resonance (NMR) studies have made it possible to determine the structures of many MMPs (4). Though structural differences exist, all MMPs require zinc and calcium ions to support their enzymatic activity (Fig. 1). The enzyme itself is divided into three domains: N-terminal propeptide, catalytic domain and C-terminal domain (5).

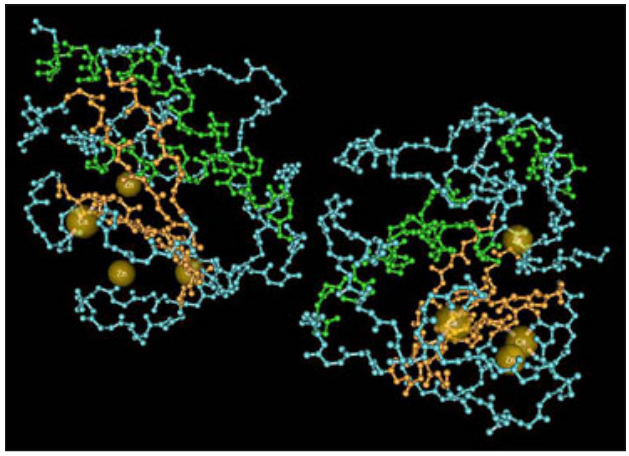

Fig. 1. Human MMP-8 (PDB ID: 2OY4) contains two domains (2OY4_A and 2OY4_F) each bound to two calcium and zinc ions. The structure is shown with a trace protein backbone without helix and strand objects in ball and sticks style. Figure prepared using Cn3D software from National Centre for Biotechnology Information.

The N-terminal propeptide contains approximately 80 amino acids and ensures enzyme latency. The most important functional amino acid within the N-terminal propeptide is cysteine, which interacts with catalytic zinc ions through the thiol group and constitutes the cysteine switch (6). In the propeptide, a highly conserved sequence (Pro-Arg-Gly-Cys-X-Pro-Asp, where X represents any amino acid) is present. Cleavage of the propeptide activates MMP from proMMP (7).

The C-terminal domain (or hemopexin-like domain) is structurally similar to proteins of the hemopexin family. The domain has a relatively large surface area for protein-protein interactions e.g. cell membrane receptors. It is ellipsoid-shaped with

[1]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic, [2]Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno-Bohunice, Czech Republic, [3]Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic, [4]Department of Paediatric Haematology and Oncology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic, [5]Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Brno, Czech Republic

Address for correspondence: R. Kizek, Dept of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic.
Phone: +420.5.45133350, Fax: +420.5.45212044

Acknowledgements: Financial support from GA AV IAA401990701, GACR 301/09/P436, IGAMZ 10200-3, NANOSEMED GA AV KAN208130801 and GACR P301/10/0356 is highly acknowledged.

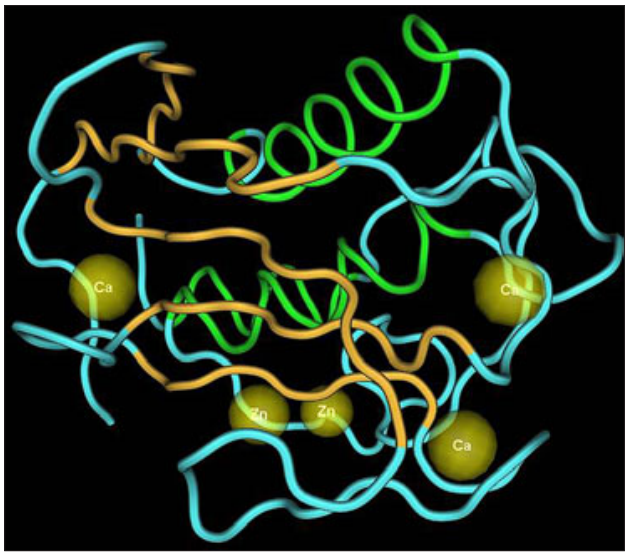

Fig. 2. The Human MMP-12 (PDB ID: 2OXU). Domain (2OXUA) bounds three calcium ions and two zinc ions. The structure is shown without helix and strand objects, worms style. Figure prepared using the program Cn3D of the National Centre for Biotechnology Information.

propeller-like subdomain, where each leaf of the "propeller" is composed of 4 antiparallel β-sheets and one α-helix. The first and fourth leave are linked by a disulfide bridge (7). As published for collagenase-1, the catalytic and C-terminal domains (7), are freely attached by a flexible proline-rich peptide linker (hinge). The length of the hinge is extremely variable, from 16 amino acid residues in collagenase to 65 amino acid residues in MMP-15.

The catalytic domain consists of five β-sheets, three α-helices and connecting loops. It is composed of 170 amino acids and contains zinc-binding motif (His-Glu-His-XX-XX-XX-Gly-His, where X represents any amino acid) associated with methionine, which forms a unique structure known as the methionine loop. The catalytic domain contains two zinc ions and two or three calcium ions (Fig. 2). The first Zn^{2+} ion present in the active site directly participates in catalytic processes. The second Zn^{2+} ion (also called structural) and Ca^{2+} ions are approximately 12 nm far from the Zn^{2+} ion in the catalytic site (8). Calcium ions are necessary to stabilize the domain structure (9).

There are several differences in the structure and function of the domains among MMPs. The hemopexin-like domain is characteristic for collagenase and it is necessary for the degradation of specific amino acid sequences in interstitial collagen. The catalytic domain of MMPs has proteolytic activity (10). Matrilysin MMPs, however, do not contain this domain. MT-MMPs possess this catalytic domain. However, deletion of the hemopexin domains in MT1-, MT2-, MT3-, MT5-, and MT6-MMP does not impair their abilities to activate proMMP2 (11). This transmembrane domain contains one hydrophobic chain composed of approximately 25 amino acids with a purine-like convertase specific recognition motif (Arg-X-Lys-Arg, where X represents any amino acid), except MT4-MMP and MT6-MMP, which are connected to

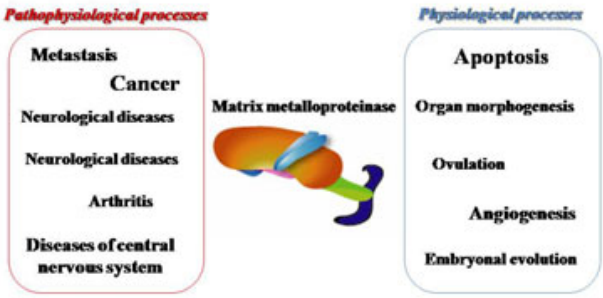

Fig. 3. A summary of important pathological and physiological processes of MMPs.

the cell surface by a glycosylphosphatidylionositol (GPI) transmembrane anchor (9, 12–14). Gelatinases contain domains showing structural similarities with matrix proteins, three tandem copies of the domain with a sequence similarity to fibronectin type II (58 aminoacid moieties long) are present in all gelatinases (15). Matrilysins (MMP-7 and MMP-26) belong to the smallest members of MMPs and do not contain the hemopexin-like domain. For MMP-23 the hydrophobic N-terminal signal anchor is specific (16, 17). MMP-19, MMP-20, MMP-27 and MMP-12 are considered to be the first members of a new MMPs group. Structures of other domains of matrilysins are similar to other MMPs, i.e. a signal sequence, the latent domain, catalytic domain and C-terminal propeptide-like hemopexin, but lack structures specific for other groups (18, 19). Some MMPs, such as MT-MMPs and stromelysin-3, contain protein convertase specific recognition motifs (Arg-X-Arg-X-Lys-Arg, where X represents any amino acid) (20).

MMPs overall thriving popularity among scientists is due to their roles in many physiological and pathological processes (Fig. 3). The best-known physiological role these proteins play is cleaving and rebuilding connective tissues such as collagen and elastin, which are components of extracellular matrix providing structural support to the animal cells and also performing various other important functions. Numerous of matrix and non-matrix proteins are both potential substrates for MMPs (21). MMPs ability to cleave and remodel surrounding tissues components effects activities such as cell migration, differentiation, growth, inflammatory processes, neovascularization, wound healing, apoptosis, uterine cycle, embryonic development and ovulation (6). MMPs also play a wide and complex role in angiogenesis. Many types of MMPs are produced by endothelial cells and have been described to be important in the formation of new blood vessels under physiological conditions (22). MMPs, furthermore, play a role in a number of pathological processes such as arthritis, Alzheimer's disease, atherosclerosis, vascular disease, gastritis ulcer disease, central nervous system disease, liver cirrhosis, and pro-angiogenic activities in malignant tumours (23–25). This review gives a brief summary on clinical applications of MMPs and their determination.

MMPs and cancer

MMPs and their impact on tumour diseases emerged in the early nineties, when they were studied in relation to stomach,

colon (26) and prostate cancer (27). Isolating MMPs and studying their enzyme activities were published a year later (28). In the same year it was found that stromal cells synthesize MMPs along with neoplastic epithelial cells to degrade the basal membrane, a characteristic sign of invasive tumour proliferation (29). Thus, the relationship between MMPs and tumours were intensively studied (30). In 1995, MT-MMPs (membrane type) in colorectal, chest, head and neck cancer was found (31). Since then the mechanistic process of extracellular matrix degradation mediated by MMPs has been the focus of many investigations for years. Recent studies have shown the role of MMPs in cancer progression is much more complex than that derived from their direct degrading action on extracellular matrix components (32–34).

There is an increasing evidence supporting the participation of MMPs in the regulation of tumour growth by favouring the release of cell proliferation factors such as insulin-like growth factors bound to specific binding proteins (35). MMPs may also target and activate growth factors whose precursors are anchored to the cell surface or sequestered in the peritumour extracellular matrix (36). It was also found that tumours with higher concentrations of MMP-11 and MMP-13 have a significantly higher probability of relapse (37).

MMP activities have also been traditionally associated with a variety of escaping mechanisms that cancer cells develop to avoid host immune responses (25, 38, 39). Some MMPs, such as MMP-9, can suppress the proliferation of T lymphocytes through IL-2Rα signalling disruption (40). Likewise, MMP-11 decreases the sensitivity of tumour cells to natural killer cells by generating a bioactive fragment from α1-proteinase inhibitor (41). MMPs can also modulate antitumour immune reactions by efficiently cleaving several chemokines or regulating their mobilization (42–44). During tumour proliferation and developing metastases, MMPs are responsible for tissue reconstruction near proliferating cells of malignant neoplasm, and participate in tumour growth in surrounding tissue (32, 45–48).

Determination of MMPs

MMPs can be used as markers for some cancer including colorectal, thyroid, bladder and breast cancer, neurodegenerative, immune and cardiovascular diseases (49). Assays for the detection of MMPs for both clinical and research purposes are summarized in the following reviews in which different methods and applications are discussed (50–55). Enzymatic, immunochemical and fluorimetric methods are commonly used techniques in clinical research. In vivo imaging methods are of particular interest in cancer research and diagnostics (56). Immunochemical methods precede enzymatic methods, but cannot distinguish between active MMP and inactive MMP in zymogene form (50). Fluorimetric methods using fluorescently labelled substrates show low detection limits but allow to determine MMP activity quantitatively and to study target MMPs sequences (51). There are a number of other methods that are subjects of interest such as phage display, Multiple-Enzyme/Multiple-Reagent Assay System (MEMRAS) and activity based profiling.

Fluorimetric methods use fluorescently labelled substrates for detection of various MMPs. Implementation of microplate-based screening (excluding so-called Real-Time Zymography) enables to analyse a large number of samples. The availability of various fluorescent probes including near-infrared fluorescent probes (57–59) makes it possible to simultaneously detect and quantify several different types of MMPs.

Zymography and all its modifications are one of the few simple and quantifiable approaches for directly determining and detecting spatial distribution of MMPs activity in both active and inactive states (zymogene) (60). Gelatine zymography was used to identify overexpressed MMP-2 and MMP-9, during the development of glioma in rat nervous system (61). MMP-8, which is found in human saliva, was studied as a potential marker for diagnosis and monitoring of periodontitis (62). Sodium dodecyl sulfate polyacrylamide gel electrophoresis zymography was also utilized for determining gelatinase B (MMP-9) activity in serum of gastric cancer patients (63). Besides gelatine, casein (64) and collagen (65) zymography can be also used for MMPs characterization. In situ zymography was developed for localizing MMP activity in tissue slices or cells. This method is successful in detecting and localizing MMP-2, -7, -9 in tissues of different origins (66, 67). Contrary to in situ zymography, in vivo zymography was developed to study MMPs activity at the level of the whole organism. This method was utilized for studying the effects of Prinomastat, a synthetic hydroxamic acid derivative with potential antineoplastic activity able to inhibit MMPs activity, on MMP activity (68). Reverse zymography is used to analyze the activities of tissue inhibitors of metalloproteinases (TIMPs) in complex biological samples. Four homologous TIMPs (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) are major endogenous tissue regulators of MMPs with molecular weights ranging from 21 to 30 kDa and have been indentified using this method (69, 70). The experimental setup and conditions were well described by Oliver et al (71). The most recently developed methods in the zymographies are real-time zymography and reverse real-time zymography (72).

Western-blotting is also used for determination of MMPs both for clinical (73) and research (74–77) purposes. Immunocapture assay is based on the use of specific antibodies against MMPs and their ability of 4-aminophenylmercuric acetate to activate proteolytic enzymes. This method was developed to detect MMP-2 (78) and MMP-9 (79). Enzyme-linked immunosorbent assay (ELISA) protocols have been optimized to detect MMPs, pro-MMPs, TIMPs and MMPs-TIMPs complexes. ELISA was used to study the mechanism of MMP-9 action during skin inflammation (80), MMP expression in ischemic heart disease (81) and the degradation of extracellular matrix of bone in osteoporosis by osteoprotegerin and MMP-2 (82). Determination of MMP expression using immunohistochemistry has been demonstrated for several types of tumours including melanoma, breast and prostate carcinoma (75, 83–86). Studies confirmed that the overexpression of certain MMPs increased the invasion of carcinoma cells (85). Immunocytochemistry was also used to detect MMPs expression in acute myeloid leukaemia cell (87), glioma (88),

colorectal cancer (89), ovarian cancer (89) and in a number of cell lines derived from different cancers (90,91).

Monovalent and polyvalent phage displays based on M13 bacteriophages and filamentous phage (92,93) belong to other methods used in MMPs studies and are commonly for investigating substrate specificity of MMPs and their inhibitors (94,95). The requirement for selectivity and absolute substrate specificity may be compromised using a multiple-enzyme/multiple-reagent system (50, 51, 96). For MMP activity profiling, Activity-Based Proteomic Probes (97) and others (98) are used. Chronopotentiometric stripping analysis was also successfully applied to detect MMP-9 and to study its interactions with collagen (99).

Utilizing MMPs as targets for in vivo imaging is a relatively young field and much has been done over the past decade to develop probes for MMPs. MMPs imaging has been limited to optical imaging method (OIM), positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI) (56). Imaging of MMPs in cancer has many potential applications (100). It takes advantage of the catalytic nature of proteinases as a means to enhance the sensitivity of screening methods for early cancer detection (56, 57).

References

1. Gross J, Lapiere CM. Collagenolytic Activity in Amphibian Tissues - a Tissue Culture Assay. Proc Natl Acad Sci USA 1962; 48: 1014–1022.

2. Obiso RJ, Azghani AO, Wilkins TD. The Bacteroides fragilis toxin fragilysin disrupts the paracellular barrier of epithelial cells. Infect Immun 1997; 65: 1431–1439.

3. Puente XS, Sanchez LM, Overall CM et al. Human and mouse proteases: A comparative genomic approach. Nat Rev Genet 2003; 4: 544–558.

4. Bode W. Structural basis of matrix metalloproteinase function, in: Proteases and the Regulation of Biological Processes, Portland Press Ltd, London, 2003, pp. 1–14.

5. Liacini A, Sylvester J, Li WQ et al. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. Matrix Biol. 2002; 21: 251–262.

6. Zitka O, Kukacka J, Krizkova S et al. Matrix metalloproteinases. Curr Med Chem 2010; 17: 3751–3768.

7. GomisRuth FX, Gohlke U, Betz M et al. The helping hand of collagenase-3 (MMP-13): 2.7 angstrom crystal structure of its C-terminal haemopexin-like domain. J Mol Biol 1996; 264: 556–566.

8. Dhanaraj V, Ye QZ, Johnson LL et al. X-ray structure of a hydroxamate inhibitor complex of stromelysin catalytic domain and its comparison with members of the zinc metalloproteinase superfamily. Structure 1996; 4: 375–386.

9. Borkakoti N. Structural studies of matrix metalloproteinases. J Mol Med 2000; 78: 261–268.

10. Bode W. A Helping Hand for Collagenases – the Hemopexin-Like Domain. Structure 1995; 3: 527–530.

11. Wang P, Nie J, Pei DQ. The hemopexin domain of membrane-type matrix metalloproteinase-1 (MT1-MMP) is not required for its activation of proMMP2 on cell surface but is essential for MT1-MMP-mediated invasion in three-dimensional type I collagen. J Biol Chem 2004; 279: 51148–51155.

12. Buttner FH, Hughes CE, Margerie D et al. Membrane type 1 matrix metalloproteinase (MT1-MMP) cleaves the recombinant aggrecan substrate rAgg1(mut) at the ‚aggrecanase' and the MMP sites – Characterization of MT1-MMP catabolic activities on the interglobular domain of aggrecan. Biochem J 1998; 333: 159–165.

13. Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem 1999; 274: 21491–21494.

14. Ye S. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. Matrix Biol 2000; 19: 623–629.

15. Murphy G, Nguyen Q, Cockett MI et al. Assessment of the Role of the Fibronectin-Like Domain of Gelatinase-a by Analysis of a Deletion Mutant. J Biol Chem 1994; 269: 6632–6636.

16. Uria JA, Lopez-Otin C. Matrilysin-2, a new matrix metalloproteinase expressed in human tumors and showing the minimal domain organization required for secretion, latency, and activity. Cancer Res 2000; 60: 4745–4751.

17. Wilson EM, Spinale FG. Myocardial remodelling and matrix metalloproteinases in heart failure: turmoil within the interstitium. Ann Med 2001; 33: 623–634.

18. Lohi J, Wilson CL, Roby JD et al. Epilysin, a novel human matrix metalloproteinase (MMP-28) expressed in testis and keratinocytes and in response to injury. J Biol Chem 2001; 276: 10134–10144.

19. Stracke JO, Fosang AJ, Last K et al. Matrix metalloproteinases 19 and 20 cleave aggrecan and cartilage oligomeric matrix protein (COMP). FEBS Lett 2000; 478: 52–56.

20. Marchenko GN, Ratnikov BI, Rozanov DV et al. Characterization of matrix metalloproteinase-26, a novel metalloproteinase widely expressed in cancer cells of epithelial origin. Biochem J 2001; 356: 705–718.

21. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 2006; 69: 562–573.

22. Folgueras AR, Pendas AM, Sanchez LM et al. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. Int J Dev Biol 2004; 48: 411–424.

23. Zitka O, Kukacka J, Krizkova S et al. Matrix metalloproteinases. Curr Med Chem 2010; 17: 3751–3768.

24. Bovee J, Hogendoorn PCW, Wunder JS et al. Cartilage tumours and bone development: molecular pathology and possible therapeutic targets. Nat Rev Cancer 2010; 10: 481–488.

25. Coussens LM, Fingleton B, Matrisian LM. Cancer therapy – Matrix metalloproteinase inhibitors and cancer: Trials and tribulations. Science 2002; 295: 2387–2392.

26. McDonnell S, Navre M, Coffey RJ et al. Expression and Localization of the Matrix Metalloproteinase Pump-1 (MMP-7) in Human Gastric and Colon Carcinomas. Mol Carcinogen 1991; 4: 527–533.

27. Pajouh MS, Nagle RB, Breathnach R et al. Expression of Metalloproteinase Genes in Human Prostate-Cancer. J Cancer Res Clin Oncol 1991; 117: 144–150.

28. Okada Y, Gonoji Y, Naka K et al. Matrix Metalloproteinase-9 (92-Kda Gelatinase Type-IV Collagenase) from Ht-1080 Human Fibrosarcoma Cells - Purification and Activation of the Precursor and Enzymatic-Properties. J Biol Chem 1992; 267: 21712–21719.

29. Poulsom R, Pignatelli M, Stetlerstevenson WG et al. Stromal Expression of 72 Kda Type-IV Collagenase (Mmp-2) and Timp-2 Messenger-RNAS in Colorectal Neoplasia. Am J Pathol 1992; 141: 389–396.

30. Davies B, Waxman J, Wasan H et al. Levels of Matrix Metalloproteases in Bladder-Cancer Correlate with Tumor Grade and Invasion. Cancer Res 1993; 53: 5365–5369.

31. Okada A, Bellocq JP, Rouyer N et al. Membrane-Type Matrix Metalloproteinase (MT-MMP) Gene Is Expressed in Stromal Cells of Human Colon, Breast, and Head and Neck Carcinomas. Proc Natl Acad Sci USA 1995; 92: 2730–2734.

32. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2: 161–174.

33. Freije JMP, Balbin M, Pendas AM et al. Matrix metalloproteinases and tumor progression. In: A. LlombartBosch and V. Felipo (Eds.), New Trends in Cancer for the 21st Century, Kluwer Academic/Plenum Publ, New York, 2003, pp. 91–107.

34. Hojilla CV, Mohammed FF, Khokha R. Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. Br J Cancer 2003; 89: 1817–1821.

35. Manes S, Mira E, Barbacid MD et al. Identification of insulin-like growth factor-binding protein-1 as a potential physiological substrate for human stromelysin-3. J Biol Chem 1997; 272: 25706–25712.

36. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 2000; 14: 163–176.

37. Escaff S, Fernandez JM, Gonzalez LO et al. Study of matrix metalloproteinases and their inhibitors in prostate cancer. Br J Cancer 2010; 102: 922–929.

38. Coussens LM, Tinkle CL, Hanahan D et al. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 2000; 103: 481–490.

39. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420: 860–867.

40. Sheu BC, Hsu SM, No HN et al. A novel role of metalloproteinase in cancer-mediated immunosuppression. Cancer Res 2001; 61: 237–242.

41. Kataoka H, Uchino H, Iwamura T et al. Enhanced tumor growth and invasiveness in vivo by a carboxyl-terminal fragment of alpha 1-proteinase inhibitor generated by matrix metalloproteinases – A possible modulatory role in natural killer cytotoxicity. Am J Pathol 1999; 154: 457–468.

42. Li QL, Park PW, Wilson CL et al. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. Cell 2002; 111: 635–646.

43. McQuibban GA, Gong JH, Tam EM et al. Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. Science 2000; 289: 1202–1206.

44. Van den Steen PE, Dubois B, Nelissen I et al. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). Crit Rev Biochem Mol Biol 2002; 37: 375–536.

45. Stetlerstevenson WG, Aznavoorian S, Liotta LA. Tumor-Cell Interactions with the Extracellular-Matrix During Invasion and Metastasis. Annu Rev Cell Biol 1993; 9: 541–573.

46. Kopczynska E, Dancewicz M, Kowalewski J et al. Usefulness of MMP-9/TIMP-1 ratio in estimation of lung cancer invasion and metastasis. Wspotczesna Onkol 2007; 11: 355–359.

47. Wu ZS, Wu Q, Yang JH et al. Prognostic significance of MMP-9 and TIMP-1 serum and tissue expression in breast cancer. Int J Cancer 2008; 122: 2050–2056.

48. Maeshima Y, Colorado PC, Kalluri R. Two RGD-independent alpha(v)beta(3) integrin binding sites on tumstatin regulate distinct antitumor properties. J Biol Chem 2000; 275: 23745–23750.

49. Dollery CM, McEwan JR, Henney AM. Matrix Metalloproteinases and Cardiovascular-Disease. Circulat Res 1995; 77: 863–868.

50. Lombard C, Saulnier J, Wallach J. Assays of matrix metalloproteinases (MMPs) activities: a review. Biochimie 2005; 87: 265–272.

51. Cheng XC, Fang H, Xu WF. Advances in assays of matrix metalloproteinases (MMPs) and their inhibitors. J Enzym Inhib Med Chem 2008; 23: 154–167.

52. George J, Teear ML, Norey CG et al. Evaluation of an imaging platform during the development of a FRET protease assay. J Biomol Screen 2003; 8: 72–80.

53. Zucker SN, Cao J. Imaging metalloproteinase activity in vivo. Nature Med 2001; 7: 655–656.

54. Wagner S, Breyholz HJ, Faust A et al. Molecular imaging of matrix metalloproteinases in vivo using small molecule inhibitors for SPECT and PET. Curr Med Chem 2006; 13: 2819–2838.

55. Lopez-Avila V, Spencer JV. Methods for Detection of Matrix Metalloproteinases as Biomarkers in Cardiovascular Disease. Clinical Medicine Insights: Cardiology 2008; 2008.

56. Scherer RL, McIntyre JO, Matrisian LM. Imaging matrix metalloproteinases in cancer. Cancer Metastasis Rev 2008; 27: 679–690.

57. Weissleder R, Tung CH, Mahmood U et al. In vivo imaging of tumors with protease-activated near-infrared fluorescent probes. Nat Biotechnol 1999; 17: 375–378.

58. Machado DE, Berardo PT, Palmero CY et al. Higher expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) and metalloproteinase-9 (MMP-9) in a rat model of peritoneal endometriosis is similar to cancer diseases. J Exp Clin Cancer Res 2010; 29: 9.

59. Tung CH, Mahmood U, Bredow S et al. In vivo imaging of proteolytic enzyme activity using a novel molecular reporter. Cancer Res 2000; 60: 4953–4958.

60. Sakimoto T, Shoji J, Yamada A et al. Upregulation of matrix metalloproteinase in tear fluid of patients with recurrent corneal erosion. Jpn J Ophthalmol 2007; 51: 343–346.

61. Zhao JX, Yang LP, Wang YF et al. Gelatinolytic activity of matrix metalloproteinase-2 and matrix metalloproteinase-9 in rat brain after implantation of 9L rat glioma cells. Eur J Neurol 2007; 14: 510–516.

62. Herr AE, Hatch AV, Throckmorton DJ et al. Microfluidic immunoassays as rapid saliva-based clinical diagnostics. Proc Natl Acad Sci USA 2007; 104: 5268–5273.

63. Dragutinovic VV, Radovanovic NS, T Izrael-Zivkovic L et al. Detection of gelatinase B activity in serum of gastric cancer patients. World J Gastroenterol 2006; 12: 105–109.

64. Raser KJ, Posner A, Wang KKW. Casein Zymography – a Method to Study Mu-Calpain, M-Calpain, and Their Inhibitory Agents. Arch Biochem Biophys 1995; 319: 211–216.

65. Gogly B, Groult N, Hornebeck W et al. Collagen zymography as a sensitive and specific technique for the determination of subpicogram levels of interstitial collagenase. Anal Biochem 1998; 255: 211–216.

66. Kurschat P, Wickenhauser C, Groth W et al. Identification of activated matrix metalloproteinase-2 (MMP-2) as the main gelatinolytic enzyme in malignant melanoma by in situ zymography. J Pathol 2002; 197: 179–187.

67. Nemori R, Yamamoto M, Kataoka F et al. Development of in situ zymography to localize active matrix metalloproteinase-7 (Matrilysin-1). J. Histochem Cytochem 2005; 53: 1227–1234.

68. Ferrario A, Chantrain CF, von Tiehl K et al. The matrix metalloproteinase inhibitor prinomastat enhances photodynamic therapy responsiveness in a mouse tumor model. Cancer Res 2004; 64: 2328–2332.

69. Murphy G. Matrix metalloproteinases and their inhibitors. Acta Orthop Scand 1995; 66: 55–60.

70. Kerrigan JJ, Mansell JP, Sandy JR. Matrix turnover. J Orthodont 2000; 27: 227–233.

71. Oliver GW, Leferson JD, StetlerStevenson WG et al. Quantitative reverse zymography: Analysis of picogram amounts of metalloproteinase inhibitors using gelatinase A and B reverse zymograms. Anal Biochem 1997; 244: 161–166.

72. Hattori S, Fujisaki H, Kiriyama T et al. Real-time zymography and reverse zymography: A method for detecting activities of matrix metalloproteinases and their inhibitors using FITC-labeled collagen and casein as substrates. Anal Biochem 2002; 301: 27–34.

73. Rajagopalan S, Meng XP, Ramasamy S et al. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro - Implications for atherosclerotic plaque stability. J Clin Invest 1996; 98: 2572–2579.

74. Attiga FA, Fernandez PM, Weeraratna AT et al. Inhibitors of prostaglandin synthesis inhibit human prostate tumor cell invasiveness and reduce the release of matrix metalloproteinases. Cancer Res 2000; 60: 4629–4637.

75. Egawa N, Koshikawa N, Tomari T et al. Membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) cleaves and releases a 22-kDa extracellular matrix metalloproteinase inducer (EMMPRIN) fragment from tumor cells. J Biol Chem 2006; 281: 37576–37585.

76. Maeta N, Osaki M, Shomori K et al. CYR61 downregulation correlates with tumor progression by promoting MMP-7 expression in human gastric carcinoma. Oncology 2007; 73: 118–126.

77. Rawdanowicz TJ, Hampton AL, Nagase H et al. Matrix Metalloproteinase Production by Cultured Human Endometrial Stromal Cells - Identification of Interstitial Collagenase, Gelatinase-a, Gelatinase-B, and Stromelysin-1 and Their Differential Regulation by Interleukin-1-Alpha and Tumor-Necrosis-Factor-Alpha. J Clin Endocrinol Metab 1994; 79: 530–536.

78. Capper SJ, Verheijen J, Smith L et al. Determination of gelatinase-A (MMP-2) activity using a novel immunocapture assay, in: R.A. Greenwald, S. Zucker and L.M. Golub (Eds.), Inhibition of Matrix Metalloproteinases: Therapeutic Applications, New York Acad Sciences, New York, 1999, pp. 487–490.

79. Hanemaaijer R, Visser H, Konttinen YT et al. A novel and simple immunocapture assay for determination of gelatinase-B (MMP-9) activities in biological fluids: Saliva from patients with Sjogren's syndrome contain increased latent and active gelatinase-B levels. Matrix Biol 1998; 17: 657–665.

80. Purwar R, Kraus M, Werfel T et al. Modulation of keratinocyte-derived MMP-9 by IL-13: A possible role for the pathogenesis of epidermal inflammation. J Invest Dermatol 2008; 128: 59–66.

81. Kelly PJ, Morrow JD, Ning MM et al. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke - The biomarker evaluation for antioxidant therapies in stroke (BEAT-stroke) study. Stroke 2008; 39: 100–104.

82. Dai Y, Shen L. Relationships between serum osteoprotegerin, matrix metalloproteinase-2 levels and bone metabolism in postmenopausal women. Chin Med J 2007; 120: 2017–2021.

83. Hoyhtya M, Fridman R, Komarek D et al. Immunohistochemical Localization of Matrix Metalloproteinase 2 and Its Specific Inhibitor Timp-2 in Neoplastic Tissues with Monoclonal-Antibodies. Int J Cancer 1994; 56: 500–505.

84. Sroka IC, McDaniel K, Nagle RB et al. Differential localization of MT1-MMP in human prostate cancer tissue: Role of IGF-1R in MT1-MMP expression. Prostate 2008; 68: 463–476.

85. Ueno H, Nakamura H, Inoue M et al. Expression and tissue localization of membrane-types 1, 2, and 3 matrix metalloproteinases in human invasive breast carcinomas. Cancer Res 1997; 57: 2055–2060.

86. Thompson EW, Yu M, Bueno J et al. Collagen-Induced MMP-2 Activation in Human Breast-Cancer. Breast Cancer Res Treat 1994; 31: 357–370.

87. Travaglino E, Benatti C, Malcovati L et al. Biological and clinical relevance of matrix metalloproteinases 2 and 9 in acute myeloid leukaemias and myelodysplastic syndromes. Eur J Haematol 2008; 80: 216–226.

88. Rao JS, Yamamoto M, Mohaman S et al. Expression and localization of 92 kDa type IV collagenase gelatinase B (MMP-9) in human gliomas. Clin Exp Metastasis 1996; 14: 12–18.

89. Afzal S, Lalani EN, Foulkes WD et al. Matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 expression and synthetic matrix metalloproteinase-2 inhibitor binding in ovarian carcinomas and tumor cell lines. Lab Invest 1996; 74: 406–421.

90. Chambaut-Guerin AM, Herigault S, Rouet-Benzineb P et al. Induction of matrix metalloproteinase MMP-9 (92-kDa gelatinase) by retinoic acid in human neuroblastoma SKNBE cells: Relevance to neuronal differentiation. J Neurochem 2000; 74: 508–517.

91. Tomita T, Iwata K. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in colonic adenomas-adenocarcinomas. Dis Colon Rectum 1996; 39: 1255–1264.

92. Smith GP, Petrenko VA. Phage display. Chem. Rev. 1997; 97: 391–410.

93. Kehoe JW, Kay BK. Filamentous phage display in the new millennium. Chem Rev 2005; 105: 4056–4072.

94. Matthews DJ, Wells JA. Substrate Phage – Selection of Protease Substrates by Monovalent Phage Display. Science 1993; 260: 1113–1117.

95. Ohkubo S, Miyadera K, Sugimoto Y et al. Identification of substrate sequences for membrane type-1 matrix metalloproteinase using bacteriophage peptide display library. Biochem Biophys Res Commun 1999; 266: 308–313.

96. Rasmussen FH, Yeung N, Kiefer L et al. Use of a multiple-enzyme/multiple-reagent assay system to quantify activity levels in samples containing mixtures of matrix metalloproteinases. Biochem 2004; 43: 2987–2995.

97. Saghatelian A, Jessani N, Joseph A et al. Activity-based probes for the proteomic profiling of metalloproteases. Proc Natl Acad Sci USA 2004; 101: 10000–10005.

98. Freije JR, Klein T, Ooms JA et al. Activity-based matrix metalloprotease enrichment using automated, inhibitor affinity extractions. J Proteome Res 2006; 5: 1186–1194.

99. Huska D, Adam V, Zitka O et al. Chronopotentiometric Stripping Analysis of Gelatinase B, Collagen and Their Interaction. Electroanalysis 2009; 21: 536–541.

100. Perentes JY, McKee TD, Ley CD et al. In vivo imaging of extracellular matrix remodeling by tumor-associated fibroblasts. Nat Methods 2009; 6: 143–145.

Received December 5, 2010.
Accepted April 4, 2011.