IJBPAS, August, 2012, 1(7): 1003-1011

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

THE GELATINASES AND THEIR ROLE IN TUMOUR INVASION

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ABSTRACT

The term cancer covers a variety of conditions characterized by uncontrolled cellular proliferation involving formation of primary tumours, followed by metastasis of cells to distant sites to form secondary tumours. Key players in this cascade of events are the matrix metalloproteinases (MMPs), a family of endopeptidases. Several MMPs, particularly gelatinases A and B (MMP-2 and -9 respectively) play important roles in a variety of physiological and pathological conditions including wound healing, tissue remodeling and cancer. MMP-2 and MMP-9 are upregulated in almost every type of cancer, and play important roles in several steps of tumour progression including disruption of the basement membrane, degradation of ECM components, directed pericellular proteolysis and angiogenesis. This review deals with the gelatinases and their biological roles in the disease cancer.

Keywords : Gelatinases, Matrix Metalloproteinases, MMP-2, MMP-9, Tumour Invasion, Angiogenesis

INTRODUCTION

The matrix metalloproteinases (MMPs) are a family of zinc dependant endopeptidase enzymes which can collectively degrade almost all components of the extracellular matrix (ECM) **[1, 2]**. Till date, 24 MMPs have been discovered in vertebrates of which 23 are found in humans **[3]**. MMPs play important roles in numerous biological processes including wound healing, embryogenesis and angiogenesis as well as in various cancers **[1-4]**.

MMPs can be divided into a number of structural classes on the basis of their domain organization and substrate specificity. These

include the collagenases (MMP-1, MMP-8, MMP-13 and MMP-18 (*Xenopus*)) which cleave the triple helical domains of fibrillar collagens, the gelatinases (MMP-2 and MMP-9) which digest denatured collagens and gelatin, the stromelysins (MMP-3, MMP-10 and MMP-11), the matrilysins (MMP-7 and MMP-26) and the membrane-type MMPs (MT1-MMP/ MMP-14, MT2-MMP/ MMP-15, MT3-MMP/ MMP-16, MT4-MMP/ MMP-17, MT5-MMP/ MMP-24 and MT6-MMP/ MMP-25) which bear a sequence that anchors the MMP to the plasma membrane at the C-terminus **[2, 3]**. The other MMPs (MMP-12, MMP-19, MMP-20, MMP-21, MMP-23, MMP-27 and MMP-28) which fall outside these conventional categories are often grouped together in a heterogenous group **[2, 3]**.

Among the various MMPs, the role of the gelatinases (MMP-2 and MMP-9) have been intensively studied. Increased gelatinase activity has been detected in a range of pathological conditions including inflammation, cancers and cardiovascular diseases and under physiological conditions like wound healing **[1, 3-6]**.

Almost all MMPs possess an N-terminal predomain, followed by a prodomain, a catalytic metalloproteinase domain of about 170 amino acids, a hinge domain and a haemopexin-like domain **[2, 3]**. Additionally, MT-MMPs possess a C-terminal sequence that anchors the enzyme to the plasma membrane while matrilysins lack the hinge and haemopexin-like domains **[2,3]**. The catalytic domain contains a catalytic zinc ion, a structural zinc ion, and generally three calcium ions. The zinc binding motif HEXXHXXGXXH in the catalytic domain, and the ‘‘cysteine switch’’ motif PRCGXPD in the propeptide are structural components common to the matrix metalloproteinase group **[2, 3]**. The gelatinases (MMP-2 and MMP-9) bear also three repeats of a fibronectin type II domain within their catalytic domain, which are involved in binding of substrates including gelatin, elastin and thrombospondins **[2-4]**. MMP-2 (gelatinase A/ 72 kDa type IV collagenase) is nonglycosylated while MMP-9 (gelatinase B/ 92 kDa type IV collagenase) bears two N-glycosylated sites in the prodomain and the catalytic domain.MMP-9 additionally has a unique collagen V-like insertion between the catalytic domain and the C-terminal domain, the function of which is unknown **[7]**.

Gelatinase Activation

MMPs are synthesized as inactive zymogens. Interactions between the cysteine in the propetide and the catalytic zinc ion maintains pro-MMPs in an inactive state **[2, 8]**. MMPs

can be activated by proteinases or *in vitro* by chemical agents such as thiol-modifying agents like 4-aminophenylmercuric acetate (APMA), oxidized glutathione and SDS **[2]**. Chemical agents work by disturbing the cysteine-zinc interaction of the "cysteine switch" while proteinases (extracellular or intracellular) activate MMPs by cleavage of the prodomain **[1, 2, 8, 9]**. While most pro-MMPs are secreted from cells and activated extracellularly by proteinases like plasmin, pro-MMP-2 is not readily activated by general proteinases **[1-3]**. Strongin *et al* (1995) first elucidated the mechanism of activation of MMP-2 in HT1080 fibrosarcoma cells **[10]**. Subsequently, a number of workers, including our group, have provided further evidence that activation of MMP-2 occurs through a membrane associated activation complex comprising MT1-MMP, TIMP-2 and pro-MMP-2 **[11-13]**. The basic mechanism involves MT1-MMP binding pro-MMP-2 using TIMP-2 as an adaptor protein. The N-terminal domain of TIMP-2 binds to and inhibits the catalytic domain of MT1-MMP. The C-terminal domain of TIMP-2 binds to the haemopexin domain of pro-MMP-2. An adjacent MT1-MMP molecule then cleaves the propeptide domain of pro-MMP-2 (72 kDa) in this tri-molecular complex to generate active MMP-2 (68-62 kDa) which may subsequently undergo further cleavage **[2, 10-13]**.

Endogenous physiological inhibitors of MMPs, including gelatinases, include α2-macroglobulin and the tissue inhibitors of MMPs (TIMPs) **[1-4]**. Interestingly, although TIMPs are inhibitors of MMPs, activation of pro-MMP-2 by MT1-MMP requires the presence of small (catalytic) amounts of TIMP-2 **[1, 2, 10, 14]**. Further studies seem to indicate that MT2-, MT3- and MT5-MMPs can also activate pro-MMP-2 although the activation by MT2-MMP does not apparently involve TIMP-2 **[15, 16]**. Extracellular matrix metalloproteinase inducer (EMMPRIN) can stimulate the expression of a number of MMPs, including MMP-2 but not MMP-9 **[17]**.

Role of Gelatinases In Cancer

Role of Gelatinases in Promoting Invasion

Several reports indicate that MMPs are upregulated in almost every type of human cancer, and increase in expression and activity of MMPs is correlated with advanced tumour stage and shortened survival **[1-6, 9, 15]**. Initially it was believed that the principal role of MMPs lay in the extensive ECM degradation during metastasis but further investigations indicate that MMPs are important in several other steps of tumour progression as well **[1-4]**. Several

experimental and biochemical studies indicate that MMP-2 and MMP-9 are important mediators of tumour invasion and metastasis in cancers. While some MMPs may be expressed mainly by the cancer cells, the gelatinases are principally synthesized by tumour stromal cells, including fibroblasts **[1, 6]**. Brown *et al* (1993) demonstrated that activation ratios of pro-MMP-2 correspond to increased lymph node metastasis in breast and lung carcinomas **[18, 19]**; this was also subsequently demonstrated in many other human cancers including thyroid carcinomas, stomach carcinomas, oral carcinomas, breast carcinomas and melanomas **[1, 4, 6, 9, 15, 20]**.

During metastasis, cancer cells need to pass through the basement membrane, invade the surrounding stroma, enter blood or lymph vessels, extravasate and establish secondary tumours. Gelatinases (MMP-2 and MMP-9) cleave type IV collagen of basement membrane thus leading to disruption of basement membrane integrity, allowing cancer cells to spread locally or migrate to secondary sites **[1, 2]**. MMP-2 plays an important role in the extensive ECM degradation that occurs during cancer invasion and metastasis **[1, 2, 4-6, 18-20]**. The cell surface activation of pro-MMP-2 by the pro-MMP-2/MT1-MMP/ TIMP-2 "activation complex" enables MMP-2 to participate in local degradation of the ECM, providing directionality to invading cells **[1, 4, 12, 13, 15]**. Additionally, cleavage of laminin-5 by MMP-2 reveals a cryptic site that triggers cell motility **[1, 20]**. MMPs also participate in intravasation, survival and extravasation. MMP-9 increases intravasation and may also decrease cancer cell apoptosis but may often cause apoptosis in normal cells **[1]**.

Role of Gelatinases in Angiogenesis

Angiogenesis is essential for tumour growth and development. MMP-2 deficient mice show decreased angiogenesis in tumours compared to normal mice when injected with B16BL6 melanoma cells **[21]**. Cleavage of type IV collagen by MMP-2 also exposes a cryptic integrin $\alpha v\beta 3$ binding site, blockage of which by an antibody decreases angiogenesis **[15, 22]**. Expression of MMP-2 has also been shown to correlate with increased metastasis in tumours **[23]**. MMP-9 upregulates angiogenesis by increasing availability of the pro-angiogenic factor vascular endothelial growth factor (VEGF) **[1, 2, 15]**. VEGF increases angiogenesis *in vivo* by promoting endothelial cell survival by inducing Bcl-2 expression **[1, 15]**. MMP-2 and MMP-9 expression are correlated to VEGF expression, probably indicating a close

link between MMP expression and angiogenesis **[1, 15, 23]**.

Localization of Gelatinases on the Migration Front

Localization of MMPs on cellular surface is necessary to allow proteolytic activity to be directed towards the migration front around invasive cells regulating matrix degradation and promoting directed cellular invasion and metastasis. MMP-2 can localize to the surface of cells including endothelial cells and tumour cells by binding to integrin αvβ3 **[15, 24]**. Previous experiments, including our own, have indicated that pro-MMP-2 from other biological sources can be activated on tumour cell surface by MT1-MMP **[12-15]**. In many tumours, stromal fibroblasts produce large amounts of MMP-2; capture and subsequent activation of this MMP-2 by MT1-MMP on the cell surface of tumour cells would enable carcinomas to utilize MMP-2 (or pro-MMP-2) secreted by other cells to accomplish matrix digestion, cell translocation and metastasis **[3, 12, 13, 15]**. MT1-MMP cleaves CD44, the major hyaluronan receptor and a spliced portion of CD44 binds MMP-9 and acts as its cell surface receptor, thereby localizing the enzyme to the cell surface **[8, 15, 25]**. This localization is necessary for MMP-9 to promote tumour invasion and angiogenesis. Disruption of CD44/ MMP-9 complexes cause inhibition of tumour invasiveness and angiogenesis *in vivo* **[8, 25]**.

Laboratory Methods for Detection for Gelatinase Activity

As proteolysis by MMP-2 and MMP-9 is important at several stages of cancer and metastasis, detection of gelatinase activity is important in the laboratory. Gelatinase expression can be detected by Western blot or ELISA using specific antibodies and gelatinase activity can be detected by means of gelatin zymography. Zymography is an electrophoretic technique, based on SDS-PAGE that includes a substrate co-polymerized with the polyacrylamide gel for the detection of enzyme activity. Gelatin zymography involves electrophoretic separation of proteins under denaturing but non-reducing conditions through a polyacrylamide gel copolymerized with gelatin. The proteolytic activity is inhibited by SDS during electrophoresis and the enzymes are subsequently renatured by incubating in a non-ionic detergent, eg. Triton-X-100, which leads to exchange of the denaturing SDS by the said non-ionic detergent. The gel is then incubated in an appropriate buffer for the gelatinases under study for a period of time and visualized by staining with Coomassie Blue **[26-28]**. Gelatinase activities can be detected as clear bands against a blue

background of undegraded gelatin **(Figure 1)** and can be quantified by densitometry scanning techniques.

Gelatinases can be concentrated from conditioned media or cell extracts by binding to gelatin sepharose 4B beads, or purified by gelatin sepharose chromatography followed by elution with appropriate buffers **[2, 28]**. The elution buffers usually serve as the electrophoresis loading buffer and contain Tris-HCl, SDS, glycerol and bromophenol blue **[28]**. The activity of eluted gelatinases may then be assayed by zymography. By including dilutions of standard enzyme preparations, estimation of gelatinase activity *in vitro,* may also be performed.

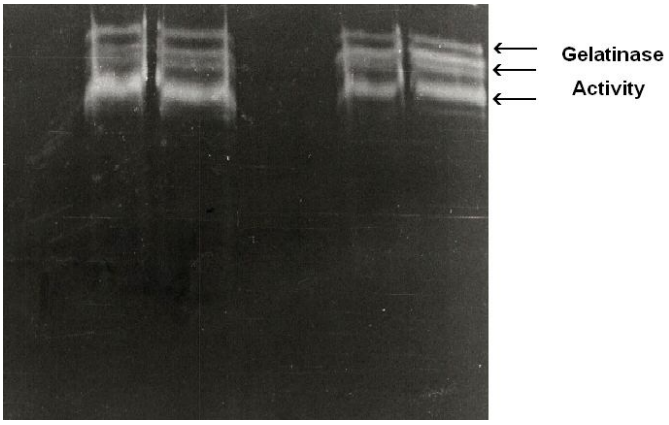

Figure 1: A gelatin zymogram. Cell extracts were incubated with sample buffer for 45 mins at 37°C and then subjected to gelatin zymography on 12.5 % SDS-PAGE co-polymerized with 0.1% gelatin. Gels were stained with 0.25% Coomassie Blue in 40% methanol and 10% glacial acetic acid.

CONCLUSION

The gelatinases, MMP-2 and MMP-9 are potent ECM degrading enzymes which play important roles during cancer and tumour invasion. During metastasis, the gelatinases, particularly MMP-2, localize to the leading edge of migrating cells and degrade complex ECM components in the basement membrane and stroma promoting cellular invasion and metastasis. The gelatinases also play important roles in tumour angiogenesis and modulate migratory properties of tumour cells. Thus, pericellular proteolysis by MMP-2 and MMP-9 play important roles in tumour invasion and cancer progression in several types of neoplasias.

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