Role of MMP-9 in metastasis: an overview

Priyanki Bose and Rina Rani Ray[2]

R-45/1, Baishnabghata-Patuli Township, Kolkata- 700094

Email – prnk.bose@gmail.com

[2]Associate Professor, Post Graduate Department of Zoology, Bethune College,

181, Bidhan Sarani, Kolkata- 700006

Abstract

Matrix metalloproteinases of the MMP family are involved in degradation and remodeling of the extracellular matrix and basement membrane that, in turn, modulate cell division, migration and angiogenesis. The degradation of the extracellular matrix allows migration of metastatic cells as well as facilitates enhanced tumor growth by providing necessary space. Moreover, with the tumor progression of invasive cancers, the ratio of active to latent form of MMP-9 also increased. MMP-9 not only promotes metastasis but also has an active angiogenic property, required for tumor cell survival.

This review article is mainly focused on the basic background of the MMP gene family, especially MMP-9 and their role in metastasis. This article also enlightens the recent researches on their application to live subjects. Findings indicate different functional mechanisms for MMP-9 during distinct steps of the metastatic cascade and their pro-metastatic as well as anti-metastatic roles.

Matrix metalloproteinases are considered as promising targets for the treatment of cancer due to their strong involvement in malignant pathologies. Clinical/preclinical studies on MMP inhibition in tumor models brought positive results raising the idea that the development of strategies to inhibit MMPs may be proved to be a powerful tool to fight against cancer.

Key words: MMPs, tumour, metalloproteinases, angiogenic.

Matrix metalloproteinase 9 (MMP-9) is one of the most studied enzymes in cancer which can cleave proteins of the extracellular matrix and a large number of receptors and growth factors. Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes. Most MMP's are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases, enzyme. The enzyme encoded by this gene degrades type IV and V collagens.

MMP-9 gene:

This gene can be found on Chromosome 26 at location: 44,070,954 - 44,078,606. The DNA sequence contains 13 exons and the transcript length: 2,335 bps translated to a 707 residues protein. MMP-9 expression is regulated by several cytokines and growth factors, including interleukins, interferons, EGF (Epidermal growth factor), NGF (Nerve growth factor), basic FGF (Fibroblast growth factor), VEGF (Vascular endothelial growth factor), PDGF (Platelet derived growth), TNF-a (Tumor necrosis factor), TGF-b (Tranforming growth factor), the extracellular matrix metalloproteinase inducer EMMPRIN and also osteopontin. Many of these stimuli induce the expression and/or activation of c-fos and c-junproto-oncogene products, which heterodimerize and bind activator protein-1 (AP-1) sites within of MMP9 gene promoters.

Primary function is degradation of proteins in the extracellular matrix. It proteolytically digests decorin, elastin, fibrillin, laminin, gelatin (denatured collagen), and types IV, V, XI and XVI collagen and also activates growth factors like proTGFb and proTNFa. Physiologically, MMP-9 in coordination with other MMPs, play a role in normal tissue remodeling events such as neurite gowth, embryonic development, angiogenesis, ovulation, mammary gland involution and wound healing. MMP-9 with other MMPs is also involved in osteoblastic bone formation and/or inhibits osteoclastic bone resorption. [Atlas of Genetics and Cytogenetics in Oncology and Haematology, 2009]

Expression of MMP-9 in tumors

In many cancers, elevated plasma levels of soluble gelatinases have been positively correlated with higher incidence of metastases in different cancer types and considered as a valuable prognostic factor. Thus, high serum levels of MMP-9 are linked to rapid progression, poor overall survival and secondary metastasis in cancer patients with melanoma. In an

experimental model for spontaneous metastasis of rat mammary carcinoma, serum and plasma levels ofMMP-9 were associated with the development and extent of metastases in the lung and lymph nodes.
High metastatic potential of rat osteosarcoma cell lines also has been associated with overexpression of MMP-9.
A strong link between MMP expression and malignant progression is further demonstrated in genetically engineered mice, which develop spontaneous or chemically induced tumors and therefore reproduce the natural steps of the metastatic cascade. Thus, in transgenic mice carrying murine mammary tumor virus promoter linked to polyoma middle T antigen and MMP-9 promoter, MMP-9 was found expressed in the invasive mammary carcinomas, but not in carcinomas *in situ* or hyperplastic mammary glands. [Elena I. Deryugina, E.I and Quigley, J.P. (2006)]

Transcriptional Regulation of MMP-

Matrix metalloproteinase-9 (MMP-9) may play a critical catalytic role in tissue remodeling *in vivo*, but it is secreted by cells as a stable, inactive zymogen, pro-MMP-9, and requires activation for catalytic function. [Visse, R and Nagase, H.(2003)]
To examine MMP-9 activation in cellular setting cultures of human tumor cells (MDA-MB-231 breast carcinoma cells) were employed that were induced to produce MMP-9 over a 200-fold concentration range (0.03–8.1 nM). The levels of tissue inhibitors of metalloproteinase (TIMPs) in the induced cultures remain relatively constant at 1–4 nM. Quantitation of the zymogen/active enzyme status of MMP-9 in the MDA-MB-231 cultures indicates that even in the presence of potential activators, the molar ratio of endogenous MMP-9 to TIMP dictates whether pro-MMP-9 activation can progress. When the MMP-9/TIMP ratio exceeds 1.0, MMP-9 activation progresses,through an interacting protease cascade involving plasmin and stromelysin 1 (MMP-3). Although plasmin can proteolytically process pro-MMP-9 but does not yield an enzymatically active MMP-9, nor does it cause the MMP-9 to be more susceptible to activation. Plasmin, is very efficient at generating active MMP-3 (stromelysin-1) from exogenously added pro-MMP-3. The activated MMP-3 becomes a potent activator of the 92-kDa pro-MMP-9, yielding an 82-kDa species that is enzymatically active in solution and represents up to 50–75% conversion of the zymogen. The activated MMP-9 enhances the invasive phenotype of the cultured cells as their ability to both degrade extracellular matrix and transverse basement membrane is significantly increased following zymogen activation. That this enhanced tissue remodelling capability is due to the activation of MMP-9 is demonstrated through the use of a specific anti-MMP-9 blocking monoclonal antibody. In addition, overexpression of individuals MMPs is frequently accompanied by a corresponding increased expression of TIMPs. Thus, MMP-9 and TIMP-1 have been found both elevated in the serum of patients with lung carcinomas. High metastatic potential of rat osteosarcoma cell lines also has been associated with overexpression of MMP-9. Cancer progression and MMPs are closely associated with inflammation, especially with persistent inflammation. The role of inflammatory cells, including macrophages, neutrophils, mast cells, dendritic cells, and T cells in the initiation of cancer. In a related study, MMP-9, induced in the lungs by distant tumors prior to metastasis, was localized to lung endothelial cells and macrophages. Functional importance of MMP-9 induction in the pre-metastatic sites was confirmed by the reduction of lung metastasis in MMP-9- deficient hosts. [Noemi Ramos-DeSimone,et al (1999)]

Role of MMP-9 activation in tumor cell invasion and metastasis

Tumor invasion and metastasis formation are major obstacles for successful cancer therapy. Metastasis is a complex multistep process that requires sequential interactions between invasive cell and extracellular matrix. [Stetler-Stevenson, W.G., Liotta, L. A. and Kleiner Jr, D. E.(1993)]
In an experiment conducted by University of Texas, on parabiont nude mice, revealed a direct correlation between intratumoral MMP-9+/+ expressing macrophages, angiogenesis, and progressive tumor growth. Because the expression of MMP-9 by L3.6pl tumor cells was similar in all parabionts, the data clearly demonstrate a major role for host-derived MMP-9 in angiogenesis and in the growth of human pancreatic cancer in the pancreas of nude mice.
Injected L3.6pl cells into the pancreas of six MMP-9+/+ mice and six MMP-9_/_ nude mice (the available number of animals). Pancreatic tumors developed in all six MMP-9+/+ nude mice, but only three of the MMP-9_/_ nude mice developed tumors that were significantly smaller than those in MMP-9+/+ mice ($P < .01$).
MVD in L3.6pl tumors in the pancreas of MMP-9_/_ and MMP-9+/+ nude mice was determined by staining for CD31. In MMP-9+/+ nude mice, L3.6pl tumors were highly vascularized, with a median of 71 microvessels per field (range, 54–96), whereas the median number of microvessels per field was 27 (range, 21–33) in MMP-9_/_ nude mice ($P < .001$). [Nakamura,T ; Kuwai, T et al.(2007)]
MMP-9 plays a significant role in breast tumor cell invasion and metastasis and are produced by stromal cells. DNAzymes or catalytic oligonucleotides are new classes of gene targeting molecules that bind and cleave a specific mRNA, result-

ing in decreased protein expression, as in, anti-MMP-9 DNAzyme (AM9D) for the treatment of primary and metastatic breast cancer was evaluated in vitro and in vivo using MDA-MB-231 cells and the MMTV-PyMT transgenic breast cancer mouse model. AM9D specifically inhibited expression of MMP-9 in MDA-MB-231 cells resulting in reduced invasive property of these cells by 43%.This decrease in tumor growth was correlated with decreased MMP-9 protein production within the treated tumor tissues. Tumors treated with AM9D were also less vascularized and contained more apoptotic cells compared to control and untreated tumors. Thus it is seen that down regulation of MMP-9 by AM9D could prove useful as a therapy against breast carcinoma tumor growth and invasion. [**Hallett, B M. and Teng, A(2013)**]

Conclusion

MMPs are important components in many biological and pathological processes because of their ability to degrade ECM components. It has become clear that the ECM is not a mere scaffold for cells but that it also harbors cryptic biological functions that can be revealed on proteolysis. This puts a new light on the interplay between cells, the ECM, and its catabolism. Considerable advancements have been made in the understanding of biochemical and structural aspects of MMPs, including their activation and catalytic mechanisms, substrate specificity, and the mechanism of inhibition by TIMPs. Structural analyses have also led to the design of potent synthetic matrixin inhibitors, some of which have exhibited efficacy in animal models of cancer and arthritis, but unfortunately, clinical trials have shown no significant benefit. Such discrepancies may be due to the fact that the trials were conducted on patients with advanced stages of disease. [Fingleton, B (2003)]

Inhibition of matrix metalloproteinases (MMPs), a family of proteolytic enzymes linked to many aspects of cancer progression, has been explored as a therapeutic goal for almost two decades. Thus far, all tested MMP inhibitors (MMPIs) have failed to reach primary end points in Phase III clinical trials, although secondary analyses suggest benefits in particular patient groups.

Future progress in the therapeutic use of MMPIs is dependent on the ability to selectively target cancer-associated MMPs at the correct stage in tumour progression and the development of surrogate markers of in vivo efficacy.

References :-

[1] Atlas of Genetics and Cytogenetics in Oncology and Haematology,(2009) http://atlasgeneticsoncology.org/Genes/MMP9ID41408ch20q11.html

[2] Elena I. Deryugina, E.I and Quigley, J.P. (2006) Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev (2006) 25: 9–34.doi: 10.1007/s10555-006-7886-9

[3] Visse, R and Nagase, H.(2003) Matrix Metalloproteinases and Tissue Inhibitors of Metallo proteinases. Structure, Function, and Biochemistry. *Circulation Research.* **92:** 827-839 doi: 10.1161

[4] Noemi Ramos-DeSimone, Elizabeth Hahn-Dantona et al (1999) Activation of Matrix Metalloproteinase-9 (MMP-9) via a Converging Plasmin/Stromelysin-1 Cascade Enhances Tumor Cell Invasion,. *The Journal of Biological Chemistry,* 274: 13066-13076. doi: 10.1074/jbc.274.19.13066

[5] , Stetler-Stevenson, W.G., Liotta, L. A. and Kleiner Jr, D. E.(1993) Extracellular matrix 6 : role of matrix metalloproteinases in tumor invasion and metastasis., December 1993, The FASEB Journal, 7 (15): 1434- 1441.

[6] Nakamura,T ; Kuwai, T et al.(2007) Stromal Metalloproteinase-9 Is Essential to Angiogenesis and Progressive Growth of Orthotopic Human Pancreatic Cancer in Parabiont Nude Mice; Neoplasia, 9 (11): 979 – 986.

[7] **Hallett, B M. and Teng, A(2013)** Anti-matrix metalloproteinase-9 DNAzyme decreases tumor growth in the MMTV-PyMT mouse model of breast cancer, **,** *Breast Cancer Research* , **15**:R12,

[8] Fingleton, B (2003) Matrix metalloproteinase inhibitors for cancer therapy: the current situation and future prospects;. 7(3) : 385-397 doi:10.1517/14728222.7.3.385.