Moonlighting Metalloproteinase

Subjects: Biochemistry & Molecular Biology | Pathology
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Protein moonlighting a.k.a. gene sharing has been defined as the concept that one protein processes multiple tasks and plays multiple roles [1]. Thus, multifunctional proteins are designated as moonlighting proteins. Matrix metalloproteinases play multiple roles in extracellular proteolysis and intracellular gene regulation [2,3], prompting us to propose a new definition of Moonlighting Metalloproteinase (MMP).

Keywords: Moonlighting Metalloproteinase ; Matrix Metalloproteinase ; Moonlighting Protein ; Protein Moonlighting ; Extracellular Matrix ; Extracellular Vesicles ; Transcription ; Cell Death

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1. Structures of MMPs [1]

The matrix metalloproteinase family consists of **about 30 members** that share similarities in their structure, regulation, and function [2]. Earlier studies showed MMPs constitute a large family of zinc/calcium-dependent endopeptidases. All MMPs have principal domains, including (1) A **signal peptide (SP) sequenc**e found at the very N-terminus of all MMPs, (2) a **pro-domain** that functions as an intramolecular inhibitor to maintain the enzyme in an inactive state, (3) a **metalloproteinase catalytic domain** that can exert the proteolytic activity, (4) A **linker sequence** connecting the catalytic domains with a following domain, and (5) a **hemopexin-like repeat (PEX or HPX) domain**, which interacts with other molecules and determines the substrate specificity. The PEX domain is present in all MMPs except for MMP-7, -23, and -26.

Proline residues in the middle of the SP can structurally weaken the secretory activities of MMPs [3][4] and thus generate intracellular MMPs. Besides, human MMP-3 contains six **nuclear localization signals (NLS)** composed of basic amino acid clusters [5]. MMP3 thus has both extracellular and nuclear functions.

Additionally, gelatinases (MMP-2, -9) contain the **fibronectin type II inserts** in the middle of the catalytic domain. Membrane type (MT) -MMPs contain **type I transmembrane (TM) domains** followed by cytoplasmic tails at the C-terminus. MT-MMPs are composed of MMP-14, -15, -16, and -24. Only MMP-23 contains a cysteine array region and an IgG-like domain.

2. Extracellular roles of MMPs

MMPs cleave substrate proteins in the extracellular space. MMP-dependent proteolysis of **extracellular matrix (ECM)** and **intercellular adhesion molecules** enable cells to migrate and invade tissue **microenvironment**. Proteolysis of ECM also triggers the **release of cytokines, chemokines, and growth factors** that activate their **receptors** and intracellular signaling pathways. In addition, MMPs also directly cleave and alter activities of growth factors, cytokines, chemokines, and their receptors. For example, MMPs can alter the activity of connective tissue growth factor (CTGF, recently known as cellular communication network factor 2 (CCN2)) by direct cleavage [5]. MMP cleavage of CCNs alters the angiogenic activities of CCNs and VEGF. A disintegrin and metalloproteinases (ADAMs) family members as well as MMPs cleave membrane-bound heparin-binding EGF-like growth factor (HB-EGF) and then release soluble HB-EGF, which stimulates EGFR/ERBB signaling [6].

Extracellular MMPs are known to be components of the **senescence-associated secretory phenotype (SASP)** that includes interleukins (IL-1β, IL-1α, IL-6, IL-8) and chemokines (CCL2) as well [7].

3. Intracellular roles of MMPs

Intracellular and intranuclear roles for MMPs have also been discovered. MMP-3 possesses several **NLSs** and can translocate into cellular nuclei, at which site MMP-3 can **bind to DNA and chromatin proteins** leading to **transcriptional regulation** of *CTGF/CCN2* gene [3][8]. Promoter analysis of the *CCN2/CTGF* gene revealed a *cis*-element, designated transcriptional enhancer dominant in chondrocytes (TRENDIC) [3][9]. One of the TRENDIC-binding proteins was identified to be MMP-3 [3]. MMP-3 overexpression enhanced *CCN2/CTGF* promoter activity in human chondrosarcoma-derived chondrocytic cell line HCS-2/8 and non-basal type, triple-negative breast cancer cell line MDA-MB-231 [3]. **Intranuclear**

translocation of recombinant MMP-3, as well as endogenous MMP-3, were observed under confocal laser scanning microscopy (CLSM) [3]. **DNA-binding** of MMP-3 was demonstrated by gel shift and chromatin immunoprecipitation (ChIP) assays. An MMP-3 specific inhibitor inhibited the activity of the *CCN2/CTGF* promoter [3], suggesting that MMP-3 proteolytic activity was partly involved in the transcriptional role for this enzyme, although the general MMP inhibitor GM6001 or an MMP2/9 inhibitor were each ineffective in this regard [3]. MMP-3 was strongly immunostained in cell nuclei in **cartilage tissues in the normal and arthritic mouse model** [3]. These findings demonstrated the role of MMP-3 in **gene regulation**.

MMPs also undergo endogenous **auto-cleavage** MMPs themselves to generate fragments containing the PEX domain. The **PEX domain** transcriptionally activates some members of **heat shock protein (HSP)** genes [10], a process that can contribute to anti-apoptosis and drug resistance. MMP-3 directly interacts with **heterochromatin proteins (HP1)**, members of the **chromobox protein (CBX)** family that involve transcriptional and chromosomal control [3][4][10].

MMPs also impact **oxidative stress, DNA damage**, and **chromosome instability** in cell nuclei. Intranuclear activities of MMP-2 and MMP-9 were shown to cleave PARP-1 and XRCC1, nuclear matrix proteins, promoting **oxidative DNA damage**, and apoptosis in an ischemic injury model [11].

4. Extracellular vesicle-associated MMPs

Members of the MMP family are often associated with **extracellular vesicles (EVs)** [12]. MMP3-rich EVs enhance cancer cell migration and invasion, molecular transmission, and gene activation while **CRISPR/Cas9**-based knockout of MMP3 reduced these pro-tumorigenic roles of EVs [8][13]. **EV-associated MMP3** was transmissive into recipient cell nuclei, trans-activated the *CCN2/CTGF* promoter, and induced CCN2/CTGF production in vitro [8].

The CRISPR/Cas9-mediated knockout of *Mmp3* gene significantly **reduced 3D-tumoroid formation** in vitro, reduced the levels of tetraspanins (CD9 and CD63) in EVs, and resulted in **destabilizing EV structural integrity** [13]. Indeed, the *Mmp3* gene loss was associated with abnormal, disorganized shapes of EVs such as crescent moon-like and broken EVs [13]. MMP3-enriched EVs were highly **penetrative** and transferred deeply into the recipient MMP3-null tumoroids [13]. The addition of MMP3-rich EVs fostered the tumorigenicity and increased the proliferation of MMP3-null cells as judged by the highly significant increase in Ki-67 expression index. Thus, MMP3-rich EVs were highly **transmissive and pro-tumorigenic** in vitro [13].

5. Roles of MMPs in cancers

MMPs represent the most prominent family of proteinases involved in **tumor progression** and are regulators of the **tumor microenvironment** [14][15]. MMPs have also been reported to be potent biomarkers of tumor progression as well constituting some of the causal factors that promote multiple processes of tumorigenesis, including oxidative stress-dependent DNA damage and chromosomal instability, **epithelial-to-mesenchymal transition (EMT)** [16], migration and invasion of cancer cells [17], **angiogenesis**, and **metastasis** [14][15][18]. MMP3-induced EMT and genomic instability are mediated by the **small GTPase Rac1b** and a **reactive oxygen species (ROS)** in breast adenocarcinoma and pancreatic cancer [16][19], indicating potent roles for MMPs in proteotoxic and genotoxic stress.

MMPs appear to be appropriate target molecules in treatments of aggressive types of cancers. Although more than 50 types of MMP inhibitors have been investigated in clinical trials for various cancers, all of those trials have so far failed [18]. The involvement of **intracellular and non-proteolytic roles for MMPs** in cancer has, however, not been well-investigated yet.

High expression of *MMP3* mRNA was **prognostically unfavorable** in particular types of cancers including head and neck, lung, pancreatic, cervical, stomach, and urothelial cancers [8]. The **RNA interference (RNAi)**-mediated knockdown of MMP-3 and MMP-9 **significantly inhibited tumor growth and metastasis** in the tumor allograft mouse model [20]. **CRISPR/Cas9**-based knockout of MMP3 revealed that MMP3 is essential for the integrities of tumors and their EVs [13].

References

1. Jeffery, C.J. Protein species and moonlighting proteins: Very small changes in a protein's covalent structure can chang e its biochemical function. Journal of proteomics 2016, 134, 19-24, doi:10.1016/j.jprot.2015.10.003.
2. Laronha, H.; Caldeira, J. Structure and Function of Human Matrix Metalloproteinases. Cells 2020, 9, doi:10.3390/cells9 051076.

3. Eguchi, T.; Kubota, S.; Kawata, K.; Mukudai, Y.; Uehara, J.; Ohgawara, T.; Ibaragi, S.; Sasaki, A.; Kuboki, T.; Takigawa, M. Novel transcription-factor-like function of human matrix metalloproteinase 3 regulating the CTGF/CCN2 gene. Mol C ell Biol 2008, 28, 2391-2413, doi:10.1128/MCB.01288-07.
4. Eguchi, T.; Kubota, S.; Kawata, K.; Mukudai, Y.; Uehara, J.; Ohgawara, T.; Ibaragi, S.; Sasaki, A.; Kuboki, T.; Takigawa, M. Novel transcriptional regulation of CCN2/CTGF by nuclear translocation of MMP3. In CCN Proteins in Health and Di sease, Perbal, A., Takigawa, M., Perbal, B., Eds. Springer Netherlands: 2010; 10.1007/978-90-481-3779-4_19pp. 255-264.
5. Hashimoto, G.; Inoki, I.; Fujii, Y.; Aoki, T.; Ikeda, E.; Okada, Y. Matrix metalloproteinases cleave connective tissue growt h factor and reactivate angiogenic activity of vascular endothelial growth factor 165. J Biol Chem 2002, 277, 36288-362 95, doi:10.1074/jbc.M201674200.
6. Prenzel, N.; Zwick, E.; Daub, H.; Leserer, M.; Abraham, R.; Wallasch, C.; Ullrich, A. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. Nature 1999, 402, 884-888, doi:10.103 8/47260.
7. Eguchi, T.; Taha, E.A. Extracellular Vesicle-associated Moonlighting Proteins: Heat Shock Proteins and Metalloproteina ses. In Heat Shock Proteins, Asea, A.A.A., Kaur, P., Eds. Springer, Dordrecht: 2020; 10.1007/7515_2020_25pp. 1-18.
8. Okusha, Y.; Eguchi, T.; Tran, M.T.; Sogawa, C.; Yoshida, K.; Itagaki, M.; Taha, E.A.; Ono, K.; Aoyama, E.; Okamura, H., et al. Extracellular Vesicles Enriched with Moonlighting Metalloproteinase Are Highly Transmissive, Pro-Tumorigenic, a nd Trans-Activates Cellular Communication Network Factor (CCN2/CTGF): CRISPR against Cancer. Cancers (Basel) 2 020, 12, doi:10.3390/cancers12040881.
9. Eguchi, T.; Kubota, S.; Kawata, K.; Mukudai, Y.; Ohgawara, T.; Miyazono, K.; Nakao, K.; Kondo, S.; Takigawa, M. Differ ent transcriptional strategies for ccn2/ctgf gene induction between human chondrocytic and breast cancer cell lines. Bio chimie 2007, 89, 278-288, doi:10.1016/j.biochi.2006.12.006.
10. Eguchi, T.; Calderwood, S.K.; Takigawa, M.; Kubota, S.; Kozaki, K. Intracellular MMP3 Promotes HSP Gene Expressio n in Collaboration With Chromobox Proteins. J Cell Biochem 2017, 118, 43-51, doi:10.1002/jcb.25607.
11. Yang, Y.; Candelario-Jalil, E.; Thompson, J.F.; Cuadrado, E.; Estrada, E.Y.; Rosell, A.; Montaner, J.; Rosenberg, G.A. In creased intranuclear matrix metalloproteinase activity in neurons interferes with oxidative DNA repair in focal cerebral is chemia. Journal of Neurochemistry 2010, 112, 134-149, doi:10.1111/j.1471-4159.2009.06433.x.
12. Shimoda, M.; Khokha, R. Metalloproteinases in extracellular vesicles. Biochimica et biophysica acta. Molecular cell res earch 2017, 1864, 1989-2000, doi:10.1016/j.bbamcr.2017.05.027.
13. Taha, E.A.; Sogawa, C.; Okusha, Y.; Kawai, H.; Oo, M.W.; Elseoudi, A.; Lu, Y.; Nagatsuka, H.; Kubota, S.; Satoh, A., et al. Knockout of MMP3 Weakens Solid Tumor Organoids and Cancer Extracellular Vesicles. Cancers (Basel) 2020, 12, doi:10.3390/cancers12051260.
14. Kessenbrock, K.; Dijkgraaf, G.J.; Lawson, D.A.; Littlepage, L.E.; Shahi, P.; Pieper, U.; Werb, Z. A role for matrix metallo proteinases in regulating mammary stem cell function via the Wnt signaling pathway. Cell Stem Cell 2013, 13, 300-313, doi:10.1016/j.stem.2013.06.005.
15. Kessenbrock, K.; Plaks, V.; Werb, Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 2010, 1 41, 52-67, doi:10.1016/j.cell.2010.03.015.
16. Radisky, D.C.; Levy, D.D.; Littlepage, L.E.; Liu, H.; Nelson, C.M.; Fata, J.E.; Leake, D.; Godden, E.L.; Albertson, D.G.; Nieto, M.A., et al. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 20 05, 436, 123-127, doi:10.1038/nature03688.
17. Sato H; Takino T; Okada Y; Cao J; Shinagawa A; Yamamoto E; Seiki, M. A matrix metalloproteinase expressed on the s urface of invasive tumour cells. Nature 1994, 370, 61-65, doi:10.1038/370061a0.
18. Vandenbroucke, R.E.; Libert, C. Is there new hope for therapeutic matrix metalloproteinase inhibition? Nat Rev Drug Di scov 2014, 13, 904-927, doi:10.1038/nrd4390.
19. Mehner, C.; Miller, E.; Khauv, D.; Nassar, A.; Oberg, A.L.; Bamlet, W.R.; Zhang, L.; Waldmann, J.; Radisky, E.S.; Crawf ord, H.C., et al. Tumor cell-derived MMP3 orchestrates Rac1b and tissue alterations that promote pancreatic adenocar cinoma. Mol Cancer Res 2014, 12, 1430-1439, doi:10.1158/1541-7786.MCR-13-0557-T.
20. Okusha, Y.; Eguchi, T.; Sogawa, C.; Okui, T.; Nakano, K.; Okamoto, K.; Kozaki, K. The intranuclear PEX domain of MM P involves proliferation, migration, and metastasis of aggressive adenocarcinoma cells. J Cell Biochem 2018, 119, 736 3-7376, doi:10.1002/jcb.27040.