Commentary

MMPs in Unusual Places

David M. Hockenbery

*From the Fred Hutchinson Cancer Research Center,
Seattle, Washington*

Enzymes typically function either intracellularly or extracellularly, but not at both locations. In this issue of *The American Journal of Pathology*, Si-Tayeb and colleagues demonstrate that an active form of the well-known extracellular protease matrix metalloproteinase-3 (MMP-3, also known as stromelysin-1) is also present in the nuclear compartment of malignant and nontransformed hepatocytes.¹ Although at first this finding might be dismissed as sloppy protein targeting, the identification of a nuclear localization signal in MMP-3 suggests that this alternate localization represents an important, heretofore underappreciated, aspect of MMP-3 function.

Matrix metalloproteinases represent a large family of vertebrate proteins best known for their functions in remodeling of extracellular matrix and for their important roles in wound healing, angiogenesis, and invasive properties of cancer cells.² Cell membrane proteins such as E-cadherin, protransforming growth factor- β , and pro-tumor necrosis factor- α have also been identified as MMP substrates, expanding the potential importance of this family to include direct effects on cell-cell signaling and intercellular interactions.³⁻⁵ MMPs are part of the larger metzincin superfamily of proteins distinguished by a catalytic zinc-binding motif with three histidines and an undergirding methionine.⁶ The MMPs are produced as inactive zymogens with a prodomain containing a "cysteine switch" that coordinates the active site zinc.⁷ Extracellular processing occurs in sequential steps, with an initial cleavage by a serine protease (or another activated MMP) within the prodomain, followed by a second intermolecular cleavage by an active MMP to generate the mature enzyme.⁸

While investigating MMP-3 expression in hepatocellular carcinoma, Si-Tayeb et al observed prominent homogeneous nuclear immunostaining for MMP-3 in 17:19 hepatocellular carcinoma specimens.¹ Adjacent hepatocytes and myofibroblasts also exhibited nuclear MMP-3 staining, although plasmacytes featured the expected cytoplasmic staining. This finding was confirmed with two MMP-3 antibodies directed at the hinge and proximal hemopexin domains but not with antibodies recognizing

N- or C-terminal regions of MMP-3. Thus, the nuclear form(s) of MMP-3 appeared to have undergone additional processing or to have concealed certain epitopes. Western blot analysis of the HepG2 hepatocellular carcinoma cell line revealed 45- and 35-kd immunoreactive MMP-3 proteins in nuclear fractions, consistent with mature (45-kd) and truncated versions of the enzyme. Surprisingly, casein zymography and casein affinity purification studies in HepG2 nuclear extracts revealed only the 35-kd form. The authors suggest that the 45-kd MMP-3 may be maintained in an inactive state by forming a complex with another protein. Notably, TIMP1 (tissue inhibitor of metalloproteinase-1) has been observed in cell nuclei.^{9,10}

There are previous examples of intracellular activation for certain MMPs due to the presence of a furin-cleavage site at the junction of the propeptide and mature enzyme in MMP-11, MMP-27, and the four membrane-type-MMPs.¹¹ Furin, a subtilisin family endopeptidase localized to the *trans*-Golgi compartment, possesses strong preference for basic amino acids at positions P4 to P1 of the cleavage site (RXXR). MMP-3 and MMP-2 have furin cleavage motifs immediately preceding the cysteine switch residue in their prodomains. Surprisingly, furin-dependent intracellular cleavage of MMP-2 was recently reported to generate an inactive enzyme in Cos cells, suggesting that additional processing was required for MMP activation.¹² The most likely candidates for secondary intracellular processing of MMP-2 and/or MMP-3 are the membrane-type-MMPs or other MMPs capable of direct activation by furin. An enhanced green fluorescent protein-tagged mature MMP-3 protein with an active site mutation retained nuclear localization in the Si-Tayeb study, suggesting that self-processing is unlikely to play a role in MMP-3 activation.¹

Si-Tayeb and colleagues identified a putative nuclear localization signal in the catalytic domain of MMP-3.¹ The addition of this domain to an enhanced green fluorescent

Accepted for publication June 8, 2006.

This commentary relates to Si-Tayeb et al, *Am J Pathol* 2006; 169:1390–1401, published in this issue.

Address reprint requests to David M. Hockenbery, Fred Hutchinson Cancer Research Center, Division of Clinical Research and Human Biology, 1100 Fairview Avenue North, C3-168, Seattle, WA 98109-1024. E-mail: dhockenb@fhcrc.org.

protein reporter increased nuclear localization, whereas mutation or partial deletion of this sequence in the enhanced green fluorescent protein-MMP-3 fusion protein interfered with nuclear localization. The absence of full-length MMP-3 in nuclear extracts raises the possibility that processing is required to expose the nuclear localization signal for nuclear transport. Golubkov et al have described trafficking of MT1-MMP to centrosomes after cell surface expression and endocytic trafficking, and this possibility has not been excluded for MMP-3.¹³

Transfection of HepG2 cells with enhanced green fluorescent protein-MMP-3 elicited an apoptotic response characterized by activated caspase-3.¹ The lack of apoptosis with the previously mentioned MMP-3 active site mutant and suppression of apoptosis with an MMP-3 inhibitor suggest that specific proteolytic targets are cleaved by MMP-3. Recent articles have demonstrated the presence of active MMP-2 in the nuclei and sarcomeres of cardiac myocytes and identified nuclear poly(ADP-ribose) polymerase as a novel MMP-2 substrate.^{14,15} Known substrates for MMP-3 include MMPs (MMP-1, -3, and -9), insulin and insulin-like growth factor binding proteins, substance P, protease inhibitors (α_2 -macroglobulin, antithrombin III, α_1 -antichymotrypsin, α_1 -proteinase inhibitor), urokinase-type plasminogen activator, and extracellular matrix components (nonfibrillar and denatured fibrillar collagens, aggrecan, fibronectin, laminin, and elastin).¹⁶ MMPs recognize at least six amino acids with different degrees of conservation at the substrate cleavage site, complicating bioinformatics efforts to identify substrates.^{16,17} Nuclear targets for proteolysis during apoptosis include poly(ADP-ribose) polymerase, lamins, inhibitor of caspase-activated DNase, and various factors involved in RNA processing and DNA repair, none of which are known to function upstream of caspase-3 in apoptosis models. In contrast, cleavage of Rb^{18,19} and several kinases with nuclear localization, including mammalian Ste20-like kinase 1-3 (Mst-1/2,3)^{20–22} and p21-activated protein kinase-2,²³ are sufficient to promote apoptosis. Another possibility is cleavage of an initiator caspase, several of which have been observed in the nucleus (caspase-2, -8, -9, and -10).^{24–26} Since processed, active MMP-3 is identified in nuclei of apparently healthy cells, MMP-3 activity may be tolerated in nonapoptotic roles.

One of the most interesting aspects of MMP activity in cancer models is the association between MMP activation and genetic instability. Several transgenic mouse models have demonstrated the ability of individual MMPs to promote early stages of carcinogenesis rather than the expected late effects on metastatic/invasive phenotypes.^{3,27,28} The p53 and c-abl responses to DNA damage are positively modulated by cell adhesion.^{29,30} Interference with this mechanism by MMP cleavage of extracellular matrix proteins may result in a failure to repair damaged DNA or eliminate cells with irreparable damage through apoptosis. A second potential mechanism for MMP-dependent genetic instability, involving oxidative stress, was identified by Radisky et al.³¹ They identified an isoform of the Rac1b small G protein that is induced in cells expressing active MMP-3 and is both

sufficient and necessary for mitochondrial ROS generation.

Last, the paper by Si-Tayeb et al provides a third potential mechanism of MMP-3-associated genetic instability. The possibility that apoptotic DNA fragmentation itself is a cause of genetic instability is doubtful, since cells deficient in the apoptotic endonuclease caspase-activated DNase have higher, not lower, levels of mutations, gene amplifications, and chromosomal aberrations.³² However, cleavage of nuclear proteins, possibly those involved in DNA repair, might lead to accumulation of DNA base lesions or strand breaks, eventually triggering apoptosis. Combined with MT1-MMP-induced centrosome dysfunction,¹³ regulation of genomic integrity by nuclear substrates of MMP-3 would expand the list of potential tumorigenic targets of MMPs. In the face of these multiple pathways, which events are most relevant to cancer models? The identification by Si-Tayeb et al of a nuclear localization signal domain in MMP-3 provides a logical means to begin dissecting such relevant downstream targets.

References

1. Si-Tayeb K, Monviesin A, Mazzocco C, Lepreux S, Decossas M, Cubel G, Taras D, Blanc J-F, Robinson DR, Rosenbaum J: Matrix metalloproteinase 3 is present in the cell nucleus and involved in apoptosis. *Am J Pathol* 2006, 169:1390–1401
2. Sternlicht MD, Werb Z: How matrix metalloproteinases regulate cell behavior. *Annu Rev Dev Biol* 2001, 17:463–516
3. Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ: Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *J Cell Biol* 1997, 139:1861–1872
4. English WR, Puente XS, Freije JM, Knauper V, Amour A, Merryweather A, Lopez-Otin C, Murphy G: Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor- α convertase activity but does not activate pro-MMP2. *J Biol Chem* 2000, 275:14046–14055
5. Yu Q, Stamenkovic I: Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- β and promotes tumor invasion and angiogenesis. *Genes Dev* 2000, 14:163–176
6. Stocker W, Bode W: Structural features of a superfamily of zinc-endopeptidases: the metzincins. *Curr Opin Struct Biol* 1995, 5:383–390
7. Van Wart HE, Birkedal-Hansen H: The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci USA* 1990, 87:5578–5582
8. Nagase H: Activation mechanisms of matrix metalloproteinases. *Biol Chem* 1997, 378:151–160
9. Zhao WQ, Li H, Yamashita K, Guo XK, Hoshino T, Yoshida S, Shinya T, Hayakawa T: Cell cycle-associated accumulation of tissue inhibitor of metalloproteinases-1 (TIMP-1) in the nuclei of human gingival fibroblasts. *J Cell Sci* 1998, 111:1147–1153
10. Ritter LM, Garfield SH, Thorgeirsson UP: Tissue inhibitor of metalloproteinases-1 (TIMP-1) binds to the cell surface and translocates to the nucleus of human MCF-7 breast carcinoma cells. *Biochem Biophys Res Commun* 1999, 257:494–499
11. Pei D, Weiss SJ: Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature* 1995, 375:244–247
12. Cao J, Rehemtulla A, Pavlaki M, Kozarekar P, Chiarelli C: Furin directly cleaves proMMP-2 in the trans-Golgi network resulting in a nonfunctioning proteinase. *J Biol Chem* 2005, 280:10974–10980
13. Golubkov VS, Chekanov AV, Doxsey SJ, Strongin AY: Centrosomal pericentrin is a direct cleavage target of membrane type-1 matrix metalloproteinase in humans but not in mice: potential implications for tumorigenesis. *J Biol Chem* 2005, 280:42237–42241

14. Kwan JA, Schulze CJ, Wang W, Leon H, Sariahmetoglu M, Sung M, Sawicka J, Sims DE, Sawicki G, Schulz R: Matrix metalloproteinase-2 (MMP-2) is present in the nucleus of cardiac myocytes and is capable of cleaving poly (ADP-ribose) polymerase (PARP) in vitro. *FASEB J* 2004, 18:690–692
15. Wang W, Schulze CJ, Suarez-Pinzon WL, Dyck JR, Sawicki G, Schulz R: Intracellular action of matrix metalloproteinase-2 accounts for acute myocardial ischemia and reperfusion injury. *Circulation* 2002, 106:1543–1549
16. Nagase H, Fields CG, Fields GB: Design and characterization of a fluorogenic substrate selectively hydrolyzed by stromelysin 1 (matrix metalloproteinase-3). *J Biol Chem* 1994, 269:20952–20957
17. Smith MM, Shi L, Navre M: Rapid identification of highly active and selective substrates for stromelysin and matrilysin using bacteriophage peptide display libraries. *J Biol Chem* 1995, 270:6440–6449
18. An B, Dou QP: Cleavage of retinoblastoma protein during apoptosis: an interleukin 1 beta-converting enzyme-like protease as candidate. *Cancer Res* 1996, 56:438–442
19. Janicke RU, Walker PA, Lin XY, Porter AG: Specific cleavage of the retinoblastoma protein by an ICE-like protease in apoptosis. *EMBO J* 1996, 15:6969–6978
20. Huang CY, Wu YM, Hsu CY, Lee WS, Lai MD, Lu TJ, Huang CL, Leu TH, Shih HM, Fang HI, Robinson DR, Kung HJ, Yuan CJ: Caspase activation of mammalian sterile 20-like kinase 3 (Mst3). Nuclear translocation and induction of apoptosis. *J Biol Chem* 2002, 277:34367–34374
21. Lee KK, Ohyama T, Yajima N, Tsubuki S, Yonehara S: MST, a physiological caspase substrate, highly sensitizes apoptosis both upstream and downstream of caspase activation. *J Biol Chem* 2001, 276:19276–19285
22. Ura S, Masuyama N, Graves JD, Gotoh Y: Caspase cleavage of MST1 promotes nuclear translocation and chromatin condensation. *Proc Natl Acad Sci USA* 2001, 98:10148–10153
23. Jakobi R, McCarthy CC, Koeppel MA, Stringer DK: Caspase-activated PAK-2 is regulated by subcellular targeting and proteasomal degradation. *J Biol Chem* 2003, 278:38675–38685
24. Alcivar A, Hu S, Tang J, Yang X: DEDD and DEDD2 associate with caspase-8/10 and signal cell death. *Oncogene* 2003, 22:291–297
25. Ritter PM, Marti A, Blanc C, Baltzer A, Krajewski S, Reed JC, Jaggi R: Nuclear localization of procaspase-9 and processing by a caspase-3-like activity in mammary epithelial cells. *Eur J Cell Biol* 2000, 79:358–364
26. Shikama Y, U M, Miyashita T, Yamada M: Comprehensive studies on subcellular localizations and cell death-inducing activities of eight GFP-tagged apoptosis-related caspases. *Exp Cell Res* 2001, 264:315–325
27. Sternlicht MD, Lochter A, Simpson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ, Werb Z: The stromal protease MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell* 1999, 98:137–146
28. Matrisian L: Cancer biology: extracellular proteinases in malignancy. *Curr Biol* 1999, 9:R776–R778
29. Truong T, Sun G, Doorly M, Wang JY, Schwartz MA: Modulation of DNA damage-induced apoptosis by cell adhesion is independently mediated by p53 and c-Abl. *Proc Natl Acad Sci USA* 2003, 100:10281–10286
30. Lewis JM, Truong TN, Schwartz MA: Integrins regulate the apoptotic response to DNA damage through modulation of p53. *Proc Natl Acad Sci USA* 2002, 99:3627–3632
31. Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ: Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* 2005, 436:123–127
32. Yan B, Wang H, Peng Y, Hu Y, Wang H, Zhang X, Chen Q, Bedford JS, Dewhirst MW, Li CY: A unique role of the DNA fragmentation factor in maintaining genomic stability. *Proc Natl Acad Sci USA* 2006, 103:1504–1509