Matrix metalloproteinases target protease-activated receptors on the tumor cell surface

Matrix metalloproteinases, or MMPs, have been implicated in tumor invasion and metastasis by virtue of their ability to degrade the extracellular matrix (ECM) barrier. However, MMPs are also capable of cleaving non-ECM molecules. The protease-activated receptors (PARs) are the latest MMP targets. The thrombin receptor PAR1 has now been shown to be cleaved and activated on the tumor cell surface by stromal-derived MMP1. The resulting PAR1 activates intracellular G proteins to turn on the migratory and invasive program in tumor cells. This MMP-PAR axis may represent a novel signaling pathway communicating between tumor and stromal cells during tumor progression.

The MMP family is comprised of 25 structurally related zinc-dependent proteinases either secreted into extracellular milieu or anchored on the cell surface to cleave extracellular matrix (ECM) molecules such as collagens, elastins, proteoglycans, and glycoproteins (Overall and Lopez-Otin, 2002; Pei, 1999). While MMPs have been implicated in diseases such as arthritis, asthma, atherosclerosis, and emphysema, it is their role in tumor invasion and metastasis that has attracted most of the attention. As ECM-degrading enzymes, the MMPs have been associated with tumor invasion and metastasis ever since the discovery of the first MMP activity in tumor cell-conditioned media. Experimental evidence, both in vitro and in vivo, has been overwhelming in proving the causative role of MMPs in tumor progression (Coussens et al., 2002). The major debate now is centered on how MMPs actually mediate tumor progression. The classic view that MMPs clear the path for invading tumor cells remains dominant. An emerging paradigm is that MMPs serve as regulators of the tumor microenvironment, such that tumor cells gain a proliferative, migratory, and invasive phenotype by cleaving not only ECM components, but also proteinase inhibitors, adhesion molecules, growth factor binding proteins, and a growing number of cell surface receptor molecules (Egeblad and Werb, 2002).

The protease-activated receptors, or PARs, are a family of 4 G protein coupled receptors (GPCRs) sharing the same mechanism of activation by proteolysis. Each PAR carries its own ligand, which is masked N-terminally at resting state (Coughlin, 1999; Ossovskaya and Bunnett, 2004). Many serine proteases, including thrombin, FXa, FVIIa, trypsin, tryptase, cathepsin G, MT-SP1, and plasmin, have been identified as potential activators for one or more of the PARs. These proteases can cleave the scissor bond at R^{41}-S, R^{34}-S, K^{38}-T, or R^{47}-G in PAR1-4, respectively, to expose the tethered ligand, which then binds to the second extracellular loop on the same receptor and activates it intramolecularly (see Figure 1). The activated PARs initiate signal transduction across the membrane to activate intracellular G proteins, which regulate pathways for cell morphology, secretion, cell proliferation, migration, and adhesion in cells such as platelets, cardiomyocytes, and smooth muscle cells under normal physiological conditions (Ossovskaya and Bunnett, 2004). Since many of the proteases are released and activated during tissue damage, the PARs could serve as sensors for injury and generate appropriate responses, like inflammation and wound repair.

Solid tumors can be considered in many ways as chronic wounds that never heal (Dvorak, 1986). PARs, especially PAR1 and PAR2, have also been shown to be upregulated in malignant tumors in breast and prostate (Ossovskaya and Bunnett, 2004). PAR1 in particular has been shown to be oncogenic in transforming NIH3T3 cells (Whitehead et al., 1995), and promotes tumor cell invasion in breast cancer cells (Even-Ram et al., 1998). Since the tumor microenvironment, like the wounding site, is rich in proteases, one would have assumed that thrombin or other serine proteinases released by tumor, activated stromal, infiltrating immune, or endothelial cells were responsible for the activation of PAR1 on the cancer cell surface.

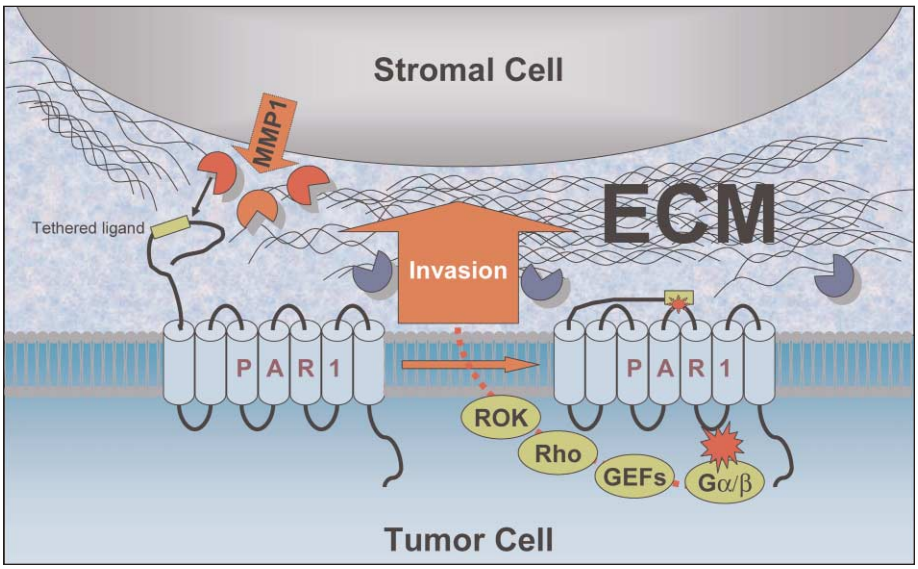

Figure 1. MMP1 activates PAR1 to mediate tumor cell invasion

Stromal cells synthesize and secrete MMP1 to the extracellular milieu, where it is activated. Active MMP1 cleaves type I collagen in the ECM. MMP1 also binds to and cleaves the extracellular N terminus of PAR1 to release a tethered ligand. Upon binding to the second extracellular loop, the ligand activates the intracellular G proteins (Gα/β) across the membrane and initiates a cascade of events which lead to the invasive phenotype for cancer cells.

A MMP-PAR axis

In a recent issue of *Cell*, Boire et al. reported that it is a matrix metalloproteinase, MMP1, rather than any of the serine proteinases, that targets PAR1 on breast cancer cells to confer a promigratory and proinvasive phenotype (Boire et al., 2005) (see Figure 1). First, they showed that MCF-7, a breast cancer cell line known to be deficient in PAR1 and noninvasive in vitro and nontumorigenic in nude mice (Even-Ram et al., 1998), can be converted into being invasive and tumorigenic upon the transfection of a functional PAR1, but not the nonactivating mutant F43A. Secondly, they demonstrated that siRNA knockdown of PAR1 in MDA-MB-231 cells rendered them noninvasive and unable to migrate. Through proteinase inhibitor profile studies, they ruled out any role for the known PAR activators, i.e., the serine proteinases, especially thrombin. Surprisingly, inhibitors known to inhibit MMPs blocked both migration and invasion toward the fibroblast conditioned media, suggesting that one of the MMPs may be the activator for PAR1. Eventually, they identified MMP-1, not MMP-2, -3, -7, or -9, as the activating proteinase for PAR1 in their experimental system. Mechanistically, they demonstrated that MMP-1 directly triggers PAR1-mediated Ca^{2+} signals in MCF7 cells, and both MMP1 inhibitors and PAR1 antagonists inhibited cell migration in vitro and tumor growth in nude mice. Although MMP-1 may turn out to be the only MMP that can cleave and activate PAR1, this elegant work certainly establishes likely MMP/PAR axis signaling between tumor and stromal cells (Figure 1).

Into the future

The MMP1/PAR1 story will inspire many lines of investigations. It would be of great interest to determine the actual cleavage site on PAR1 by MMP-1. As a founding member of the MMP family, MMP1 has been thoroughly interrogated by a variety of techniques to define its specificity. A cleavage at the scissile bond Arg-Ser, as suggested in PAR1, has not been identified for MMP-1 so far, even in a comprehensive search using a peptide library (Turk et al., 2001). It is possible that the interaction between MMP-1 and PAR1 is unique to generate a novel specificity. Secondly, the active state of MMP1 in the media conditioned by stromal fibroblasts should be analyzed in detail. It is quite surprising that culture media with 5%–10% fetal bovine sera contain any freely active MMP1. One might have assumed that there would be enough proteinase inhibitors provided by the sera to quench any active proteinases. Therefore, it is possible that MMP1 becomes active upon interacting with the tumor cell surface, or even when in contact with PAR1. It would be exciting to determine how MMP-1 interacts with PAR1 on the cell surface. PAR1 may turn out to be the long-sought-after receptor for MMP-1. Finally, the list of MMPs that may potentially cleave and activate PAR1, or other PARs, should be exhausted.

Retrospectively, one may wonder why the MMP field missed PARs as obvious targets. First, the cleavage site at R^{41}-S, R^{34}-S, K^{38}-T, or R^{47}-G in PAR1-4 would have precluded the consideration of any MMP as activators, simply because neither R nor K is known to be favored by the MMPs immediately amino-terminal to their cleavage sites. Second, blockade of tumor cell migration and invasion by MMP inhibitors has traditionally been interpreted to be consequence of blocking the cleavage of ECM components bound by the migrating or invading cells (Coussens et al., 2002). Finally, early biochemical screening for MMP substrates paid little attention to targets on the cell surface, perhaps due to inherent difficulties in obtaining and studying membrane proteins (Pei, 1999). Nevertheless, the discovery of the MMP-PAR pathway should encourage a systemic search for MMP substrates on the tumor cell surface, and perhaps even mark a new beginning for MMP biology—cell surface proteolysis. In the meantime, some of the MMP literature on tumor cell migration and invasion may be reinterpreted in light of the MMP-PAR axis. Some of the observed results may not be due to the suppression of ECM degradation, but rather the inhibition of MMP-mediated PAR1 signaling.

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