

Cell Migration through Extracellular Matrix: Membrane-Type Metalloproteinases Make the Way

Vito Quaranta

Department of Cell Biology, The Scripps Research Institute, La Jolla, California 92037

Every so often, a paper comes along that brings clarity to an issue. Clarity is not necessarily a final resolution, but rather a conceptual framework for productively addressing that issue. Such a paper could be based on a breakthrough discovery, an intriguing observation, a flash of intuition, or a systematic analysis. An excellent example of the latter is in this issue of *The Journal of Cell Biology* (Hotary et al., 2000). With a deceptively simple experimental layout, Hotary et al. (2000) explore the relative role of soluble versus membrane-anchored matrix metalloproteinases (MMP) in tissue morphogenesis. In their *in vitro* morphogenesis and matrix invasion models, the conclusion is clear cut: membrane-anchored (MT-MMPs), not soluble MMPs, effectively regulate cell migration through extracellular matrix and affect self-organization of cells into tubular structures (Fig. 1).

Cell migration through the matrix is a key component of morphogenesis, i.e., how tissue or organs attain their shape. It is truly an invasive process, whereby cells move into and possibly colonize new territory, and that is why one can speak of cell invasion and morphogenesis in the same breath, and test them with the same assay (Hotary et al., 2000). According to their differentiated type, the migratory cells may give rise to new structures within the matrix they invaded, e.g., tubules, alveoli, or acini, shaping tissues and organs. It is evident, then, why we would like to know the molecular details of migration through the matrix: what motivates cells to migrate, how they do it, how is the process controlled.

There is general agreement that MMPs are important in the execution of migration through the matrix and of invasion, based on abundant data correlating invasive phenomena with the presence of MMPs (Stetler-Stevenson et al., 1993; Werb, 1997). In recent years, mainly via isolation of gelatinolytic activities and homology cloning, the burgeoning protein family of MMPs has come to include, in man, close to 20 members (Table I). Several of these degrade collagens (Table I), the most abundant components of the extracellular matrix, though fine substrate specificity is still at issue (Koshikawa et al., 2000). The majority of MMPs are secreted proteins generally requiring activation for enzymatic activity. A few are true transmembrane pro-

teins, the membrane-type metalloproteinases or MT-MMPs, which are expressed at the cell surface in activated form.

Does the MMP structural diversity reflect functional redundancy or specialization? With their systematic expression of proteinases in select cell types, followed by challenge of specified complex extracellular matrices, Hotary et al. (2000) addressed this fundamental question and came away with an unexpected mechanistic insight (Fig. 1). Key to this accomplishment was their effort to merge modern trends from the fields of proteinase biochemistry, cell migration, tumor invasion, and morphogenesis. Such multidisciplinary approaches often electrify fields and, as in most cell biology problems today, are badly needed in MMP research at this junction. For historical reasons, and because MMPs have been for the most part characterized in biochemistry laboratories, the emphasis of the field has been on enzymatic activities, mechanisms of activation, kinetics and substrate specificity (Nagase and Woessner, 1999). The combined output of several outstanding groups has produced an extraordinary in depth understanding of MMP structure–function relationships, of intricate activation mechanisms and proteinase interactions with natural or man made inhibitors, and provided a solid foundation for MMP enzyme biochemistry (Docherty et al., 1992; Strongin et al., 1993; Morgunova et al., 1999). In contrast, the cell biology of MMPs has lagged behind. Targeted gene disruption by homologous recombination has produced MMP knockout mouse strains with phenotypes ranging from the mild to the dramatic (Itoh et al., 1998; Vu et al., 1998; Holmbeck et al., 1999). Attempts to explain these phenotypes in molecular terms have further raised our discomfort for the currently poor understanding of MMPs in terms of their cell biology. The Hotary et al. (2000) paper is an important step towards bridging the gap between enzyme biochemistry and whole organism analyses of MMP phenotypes. Their findings already begin to make some sense of the fact that soluble MMP knockout mice present themselves with mild developmental phenotypes, whereas MT1-MMP knockout mice display severe abnormalities in bone formation, angiogenesis and collagen turnover, leading to dwarfism, dysmorphic skull, and precocious death (Holmbeck et al., 1999; Zhou et al., 2000). Thus, the next wave in this arena should be analyzing the effects of MMPs on cell behavior in complex model systems, allowing us to dissect the *in vivo* functions of MMPs in greater detail.

Address correspondence to Vito Quaranta, Department of Cell Biology, SBR-12, The Scripps Research Institute, 10550 North Torrey Pines Rd., La Jolla, CA 92037. Tel.: (858) 784-8793. Fax: (858) 784-2246. E-mail: quaranta@scripps.edu

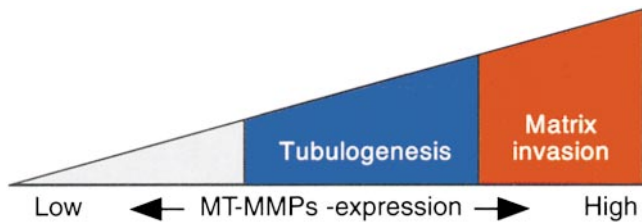


Figure 1. Schematic representation of a proposed correlation between expression of membrane-anchored MMPs (MT-MMPs), invasion, and tubulogenesis (drawing by N. Koshikawa, based on results published in this issue by Hotary et al., 2000).

Migration through the matrix may be a property also of neoplastic cells, even though they may have originated from nonmigratory cells. Contrary to morphogenesis, the results of neoplastic cell migration are often disastrous: tumor invasion and metastasis set in. Important questions then arise: do neoplastic cells in fact migrate through the matrix and ultimately invade tissues by the same mechanisms as normal cells? And, how do they acquire ability to migrate? A result of Hotary et al. (2000) offers an opportunity for reflection on possible answers. In their system, the genesis of tubular structures requires invasion of the matrix, and MT-MMPs appear critical for this. However, express too much of them, and the morphogenetic program is lost. Rather, nondescript matrix invasion takes place (Fig. 1). This result suggests that intriguingly simple rules may determine whether matrix invasion will give rise to organized structure. Admittedly, this hypothetical conclusion may stretch data interpretation a little too far, but it could nonetheless stimulate appropriate experimentation for testing its validity. The attractiveness of this hypothesis is that it may offer some mechanistic underpinning to the process of cancer invasion.

The stakes in MMP research are high because of their involvement in human pathology, e.g., cancer invasion, metastasis, or tissue degenerative diseases, (Matrisian,

1992; Stetler-Stevenson et al., 1993). There have been substantial investments in identifying drug targets based on our knowledge of soluble MMPs (Nelson et al., 2000). The payoff, though, is still below expectations. Inhibitors of MMPs are being taken all the way to Phase III clinical trials, e.g., for cancer treatment. Not all results are in yet, but thus far outcomes are less than spectacular (Yip et al., 1999). In hindsight, was too much being asked of the soluble MMPs? Perhaps. In fairness, though, soluble MMPs used to be the only game in town and, being secreted, their expression, handling, and characterization is easier than membrane-bound proteins. The membrane-anchored forms of MMPs, of which MT1-MMP is the best known, are late arrivals. Furthermore, the fact that MT1-MMP physiologically activates MMP2 (a soluble collagenase), might have distracted investigators from looking at it as an MMP in its own right (Sato et al., 1994). For instance, substrates for MT1-MMP are not well understood. There had been signs in the field that some fresh looks were necessary, and the Hotary et al. (2000) paper may crystallize this mood, signaling a shift in focus towards membrane-anchored MMPs and providing impetus for new experimental frameworks.

A major challenge facing us remains: How do MMPs operate? The matrix surrounding the cells, of which collagens tend to be a dominant component, is often thought of as a physical barrier constraining movement. MMPs, several of which show collagenolytic activity, can degrade the matrix, creating openings. A long-standing view maintains that matrix degradation should be enough to form such openings, but not too much so as to reduce traction. Such a view is easily accepted because it is rooted in human experience in the macroscopic world: ever got your car stuck in mud (SUV owners need not reply)? On the other hand, traction may take on a whole different meaning on the scale at which cells operate. Efforts to quantify the mechanical properties of extracellular matrices should help define whether or not a substrate is permissive for migration, and one should be prepared for surprises. After all, overexpression of MT1-MMP disrupts tubulogenesis,

Table I. The Matrix Metalloproteinase Family

	MMP number	Common name	Substrate
Secreted	1	Interstitial collagenase	Collagens
	2	Gelatinase A	Gelatin, collagens, laminin-5
	3	Stromelysin 1	Collagens, laminin-1, fibronectin
	7	Matrilysin	Gelatin, fibronectin, laminin-1
	8	Neutrophil collagenase	Collagens
	9	Gelatinase B	Gelatin, collagens
	10	Stromelysin 2	Collagens, laminin-1, fibronectin
	11	Stromelysin 3	Alpha-1-antitrypsin
	12	Macrophage elastase	Elastin
	13	Collagenase 3	Collagens
	18	Collagenase 4	?
	19	None	?
	20	Enamelysin	?
Membrane anchored	14	MT1-MMP	Pro-MMP2, gelatin, collagens, laminin-5
	15	MT2-MMP	Pro-MMP2, gelatin
	16	MT3-MMP	Pro-MMP2, collagens
	17	MT4-MMP	TNF- α

but enhances invasion (Fig. 1). Thus, proteinases may affect cell detachment, cell-cell adhesion, receptor-matrix interactions, or, perhaps, the way cells perceive surrounding matrix (Giannelli et al., 1997; Koshikawa et al., 2000; Pozzi et al., 2000), in addition to removing mechanical barriers.

An intriguing result of Hotary et al. (2000) is that altering a topogenic signal had no effect on the ability of MT1-MMP to disrupt tubulogenesis, suggesting that its delivery by intracellular transport mechanisms to precise locations on the cell surface does not matter much. One cannot discount the possibility that MMPs wandering across the plasma membrane are recruited to hot spots of activity by polarized receptors (Brooks et al., 1996). More radically, though, we might have to revisit the well-rooted concept that, for effectiveness, proteinases must be concentrated at the leading edge of invading cells. Again, this concept derives its popularity more from an anthropomorphic view of how a moving cell should be engineered, than from hard data. An alternative view could be that proteolysis of the close pericellular matrix, whether or not focused at a hot spot, sets in motion morphogenetic programs. Maintaining an open mind (Werb, 1997) and looking for informative model systems should be a high priority.

Having made all of these considerations, it is still surprising that, in the Hotary experiments, none of the seven soluble MMPs had any effect on tubulogenesis in collagen gels, particularly since most of these MMPs are well characterized collagenases (Table I). Could it be that soluble MMPs, stimulated by SF/HGF, were already at a maximum in that system, so that no further disruptive effects were detectable upon overexpression? This is possible but unlikely, because the disruptive effects on tubulogenesis of MT1-MMP overexpression required membrane anchoring, seemingly regardless of expression levels. Could it be that soluble MMPs are generally not included in morphogenetic programs because of their lack of spatial specificity? Time will tell, though the available knockouts of two major collagenases, MMP2 and MMP9, would already suggest that their participation in morphogenesis is not essential. Thus, MMP2 deficient mice display minor growth retardation, but appear to develop normally otherwise (Itoh et al., 1998). In MMP9 deficient mice, vascularization of growth plates causes skeletal defects eventually overcome after birth by compensation (Vu et al., 1998).

In summary, to identify which MMPs are important in matrix invasion, Hotary et al. (2000) took the direct route: express them one at a time in informative, albeit complex, cell model system, and see what happens. The difficulty of this approach lays not in its conception, but rather in committing to its elaborate execution. Hence, the considerable lag from the time reagents first became available to the time one laboratory produced the experimental data. In retrospect, what was required was the blending of several distinct skills in one place, and an effort thorough enough to allow for meaningful side by side comparisons. The study reported in this issue (Hotary et al., 2000) did just that, and the reward is an insight that, though glimpsed at by others, remained unproven: membrane-anchored

MMPs, the MT-MMPs, play a primary role in cellular invasion of collagenous matrices and, at the right levels of expression, may promote tubulogenesis. Final answer? Yes, in this system, based on the thoroughness of the Hotary study and the permutations they tested. For generalization or textbooks status, this conclusion is definitely one to be reckoned with and to be falsified, in a popperian sense, using other in vitro, and more importantly in vivo systems. Along this road, no doubt, the cell biology of MMPs holds in reserve many surprises for us.

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