Tube Travel: The Role of Proteases in Individual and Collective Cancer Cell Invasion

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Abstract
Recent advances in high-resolution multimodal microscopy reveal how MT1-matrix metalloproteinase (MMP)/MMP-14 and other cell surface proteases degrade and remodel the extracellular matrix (ECM) to drive the dissemination of cancer cells into normal adjacent tissue. By cleaving collagen fibers and repatterning them into parallel bundles, individual cells reorient the ECM to permit movement in tube-like microtracks. Cells along the edge of these tubes can excavate ECM outward, generating macrotracks through which collective mass movement of cancer cells can occur. These findings develop our understanding of invasive processes in cancer and how to attack them by interfering with MMP-14 activity. [Cancer Res 2008;68(18):7247-9]

Background
The proteolytic breakdown of proteins of the extracellular matrix (ECM) has long been recognized as a hallmark of invading primary cancer lesions (1). Several classes of proteases contribute to ECM breakdown and remodeling, most of which are up-regulated in the course of metastatic cancer progression in different types of cancers (reviewed in refs. 1–3). During invasive migration, cancer cells use secreted, surface-localized and intracellular matrix metalloproteinases (MMP), serine proteases, and cathepsins to proteolytically cleave and remove different types of ECM substrates at their interface, including collagens, laminins, vitronectin, and fibronectin (3–5). On two-dimensional substrates, focalized actin-rich contact structures including lamellipodia, podosomes, or protruding invadopodia cleave ECM underneath the cell (5, 6).

In contrast to two-dimensional migration, proteolytic contact structures during migration through three-dimensional interstitial ECM remain poorly defined. For cell invasion into three-dimensional tissues, such as collagen-rich interstitial tissue, (peritoneal) basal membranes, and the chorion allantoic membrane, MT1-MMP/MMP-14 was identified as the rate-limiting collagenase and interference with MMP-14 prevents efficient cell movement (7, 8). Thus, proteolytic ECM remodeling is considered both prerequisite and consequence of invasive cell migration, yet its spatiotemporal steps and relevance for different motility programs remains unclear (9).

Experimental studies mostly focus on individual cancer cell behavior, such as single cell polarity and movement. By contrast, tissue invasion in cancer lesions in vivo often results from varied mechanisms, including the invasion of single cells next to collective invasion of cell chains, sheets, and clusters (Fig. 1A; refs. 10, 11). Collective cell migration is well-established in morphogenesis, allowing cells to move together while maintaining cell-cell junctions and, apparently, recapitulated during neoplastic tissue invasion (12). Because invasive solid multicellular strands and masses form a joint-type interface to the ECM, whereas intratumoral regions of cell-cell contact lack interstitial tissue components, protease location, and function toward the adjacent tissue most likely differ from that of individually moving cells (9).

Traditionally, basic protease functions such as substrate specificity, turnover rates, and activity regulation are being classified by biochemical analysis of enzyme-substrate interactions in test solutions. Their integration into complex cell functions, however, requires additional cell-based enzyme activity detection in a tissue context. Recent progress in multimodal submicron-resolved confocal microscopy now allows to identify the location of protease activity in live cells and the consequences for the cleaved tissue structures. Protease dynamics and function are detected by using green fluorescent protein–tagged protease expressed at low level together with fluorescence emission after dequenching of fluorophore-labeled ECM polymer, such as type I and IV collagen (Fig. 1B; refs. 3, 4, 13, 14). ECM fiber location, aggregation state, and loss of structural integrity are further visualized by backscatter microscopy and staining for cleavage-site specific neoepitopes (4, 13). Together, these approaches provide a high-resolution map of temporal and structural changes of matrix digestion during invasive cancer cell migration.

Key Findings
Pericellular proteolysis and tissue micropatterning by invasive single cells. Within three-dimensional collagen matrix as model ECM, most cancer cells spontaneously polarize, generate integrin-dependent cell-matrix interactions, adopt varied spindle-shaped morphologies, and undergo a proteolytic migration mode that leads to the formation of small structural defects along the migration track (13). This motility program is termed "mesenchymal" migration, in reminiscence of activated fibroblasts (2). The narrow leading edge protrudes, attaches to the ECM, and generates anterior traction force, whereas individual collagen fibers become cleaved several micrometer (5–20 μm) posterior to the leading edge and the cell body gradually expands in width (Fig. 1C; ref. 14). Thus, anterior force generation and proteolytic ECM degradation are spatially separated, which prevents premature loss of substrate where the cell pulls on ECM fibers. Rather than being fully dissolved, partially cleaved loose fiber ends remain bound to the cell surface and become realigned as the cell moves forward, which results in parallel fiber orientation bordering a small matrix defect (Fig. 1C).

Thus, single-cell invasion through poorly organized matrix causes ECM micropatterning and microtracks of least resistance (14).
In tissue that provides sufficient spacing (in the range of several micrometers), the inhibition of pericellular proteolysis leads to a cellular and molecular adaptation program, which secures cell movement by shape change and squeezing through matrix gaps, although structural tissue remodeling is abrogated (2, 15). This plasticity of cell migration corresponds to a mesenchymal-amoeboid transition, in reminiscence of constitutively protease-independent trafficking of amoeboid leukocytes through fibrillar ECM (16). Whereas in low-density ECM nonproteolytic migration is fully compensated, migration through high-density tissues is delayed or even ablated, dependent on the mechanical resistance and pore size (7, 8, 14). Thus, cell migration and pericellular proteolysis can be uncoupled from each other by forcing the cells to squeeze through tissue gaps and spaces.

**Tissue macropatterning and transition toward collective invasion.** To date, the best approximation of solid tumor behavior in three-dimensional tissue culture in vitro are multicellular spheroids implanted into three-dimensional ECM, such as collagen, fibrin, or Matrigel. In fibrosarcoma and breast cancer spheroid cultures, a spontaneous transition from initial individual mesenchymal invasion toward multicellular strands occurs along small microtracks generated by so-called "forerunner cells" which may be cancer cells themselves (Fig. 1B) or activated fibroblasts derived from the non-neoplastic tumor stroma (17). Microtracks are sequentially filled by following cells in a cell-cell contact-dependent manner and become further widened, resulting in strand-like collective invasion along a laterally regressing ECM layer (Fig. 1D). ECM macropatterning is executed by multiple cells that collectively fill a small pre-existing tissue gap while remaining connected. By focalizing proteolytic activity toward the cell-ECM interface, a near-continuous ECM layer is generated and further cleaved. Yellow, proteases and MT1-MMP; green, degraded collagen; blue, β1 integrin; red, Filamentous-actin. DAPI, 4,6-diamidino-2-phenylindole.

**Figure 1.** Individual and collective tissue invasions are mediated by two distinct types of pericellular proteolysis. A, different invasion programs in primary melanoma invading the mid-dermis in vivo, including scattered individual cells (arrowheads), multicellular solid stands (Str), nests (N) representing cross-sectioned strands, and single cell chains (IF, "Indian files"). H&E staining, image modified from Friedl and colleagues (11). B, transition from individual to collective invasion from three-dimensional spheroid cultured within a three-dimensional collagen lattice. Single cells (arrowheads) generate small proteolytic tracks (detected by cleavage-site specific COL2 3/4C antibody) that become further remodeled and widened by multicellular strands (Str). This pattern is generated by HT1080 fibrosarcoma cells (image) and MDA-MB-231 breast carcinoma cells (14). C, single-moving proteolytic cells cause ECM micropatterning. After protrusion of an anterior pseudopod (step I) and traction force generation (step II), focal cleavage of individual ECM fibers is executed slightly backward to leading adhesion sites (step III). Transport of loose fiber ends (step IV) results in a small microtrack detected upon forward gliding of the cell rear (step V). D, ECM macropatterning is executed by multiple cells that collectively fill a small pre-existing tissue gap while remaining connected. By focalizing proteolytic activity toward the cell-ECM interface, a near-continuous ECM layer is generated and further cleaved. Yellow, proteases and MT1-MMP; green, degraded collagen; blue, β1 integrin; red, Filamentous-actin. DAPI, 4,6-diamidino-2-phenylindole.
collective mass (Fig. 1B and D). This mechanism by which a cell-rich compartment expands by gradually digesting large tissue interfaces leads to tissue macropatterning (14).

**Roles for MT1-MMP in cancer cell invasion.** Although several proteolytic enzymes including MMP-1, MMP-2, MMP-13, MMP-14, and cathepsins B, K, and L show activity against fibrillar type I and III collagens, the major structural components providing tissue integrity, inhibition, and gene disruption studies strongly suggest that MMP-14 is the rate-limiting enzyme in controlling collagen turnover (7, 8). In different invasion models, function loss of MMP-14 is poorly compensated by other enzymes (7, 8), implicating MMP-14 as potential target in anti-invasive therapy. Consequently, broad-spectrum MMP inhibitors or RNA knockdown of MMP-14 abrogate collagen micropatterning and macropatterning to equal extent (14, 17). Thus, collective invasion requires to gain space via ECM micropatterning and macropatterning, whereas single cell movement can occur independently of protease function (14). These findings suggest that protease inhibition may arrest collective invasion processes but, as potentially unwanted “side effect”, collective-to-amoeboid transition might enhance single-cell dissemination into the surrounding.

**Implications**

Although MMPs have been subject to intense preclinical and clinical cancer targeting studies, and although many studies hint toward a role of several protease classes in cancer progression, no clear picture is yet established (18, 19). Particularly, it is incompletely understood why in some lesions, MMP inhibition might interfere with and in others promote progression (18). Part of such discrepancies might reside in the thus far neglectance of different dissemination programs that either depend or do not depend upon protease function (9). The dissemination of single cancer cells through connective tissues and into lymphatic vessels or tumor-associated blood vessels with defective basement membrane may result from nonproteolytic amoeboid squeezing; therefore, metastatic single-cell dissemination seems as unlikely target function for therapeutic MMP inhibition regimens. Conversely, neoplastic mass invasion and tissue macropatterning might represent interesting target processes for MMP inhibition to prevent or delay destructive tissue invasion into sensitive structures and organs. Based on their collective invasion patterns in histologic sections, clinically relevant candidate lesions for MMP interference might include otherwise therapy-resistant oral squamous cell carcinoma infiltrating the neck and collagen-rich tracheal structures, basal cell carcinoma eroding the orbita or the skull, or relapsing fibrosarcoma at different location (12). Such refined indication for protease inhibition therapy clearly justifies to revisit the role of pericellular ECM breakdown and the contribution of individual proteases to different types of tissue invasion in preclinical and clinical settings.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

Received 2/29/2008; revised 4/4/2008; accepted 5/1/2008.

**Grant support:** Deutsche Forschungsgemeinschaft (FR 1155/7-2) and the Deutsche Krebshilfe (Az. 100950).

We thank Margit Ott and Monika Kuhn for technical support.

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