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EphA2 bears plasticity to tumor invasion

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During metastatic tumor progression, cancer cells are exposed to different microenvironments. The microenvironment with which they negotiate within primary tumor is unlike the one they encounter when invading into stroma or vasculature, and different also from what they will be exposed to as they spread into secondary sites. Therefore, one property that cancer cells acquire is plasticity. The cells modulate their behavior by integrating intracellular signaling with cell surface receptor interactions, as well as with the physical confines and cues of the extracellular matrix (ECM).

Depending on such signals, cancer invasion can occur by either collective cell groups or individual cells. Collective invasion and epithelial-to-mesenchymal transition are important mechanisms for invasion across basement membranes and interstitial tissues via ECM proteolysis. Cancer cells can also switch to more amoeboid-type invasion by generating actomyosin contractility and squeezing through gaps in ECM. These invasive processes are regulated by multiple signaling pathways, including those mediated by receptor tyrosine kinases (RTK). Besides growth factor receptors, RTKs involved in cell–cell and cell–ECM signaling, such as Eph receptors and collagen receptor DDR1, regulate cell migration and segregation in response to immediate cell microenvironments.

In a recent study, we found that EphA2 and membrane-type 1 matrix metalloproteinase (MT1-MMP, MMP14) are co-expressed in invasive breast carcinoma cells, where EphA2 cleavage by MT1-MMP induces a switch from collective to rounded, single-cell invasion.[1] Among the membrane proteases involved in regulation of diverse cell functions, a disintegrin and metalloproteases (ADAM) are most widely associated with signaling via ectodomain shedding and activity-regulating cleavages of receptors and other cell-surface proteins or growth factor precursors (**Fig. 1**). In contrast, MT-MMPs are typically considered as downstream effectors of the signaling pathways driving cancer and stromal cell invasion by ECM degradation.[2] Ectodomain shedding by MT-MMPs has more recently emerged as a regulatory mechanism of signal transduction, as well as of the adhesion, growth, and invasion of normal and malignant cells. Our results have revealed unique RTK–MT1–MMP interactions in tumor invasion and vascular remodeling.[1,3-6] Interestingly, the same RTK or cofactor cleavages and regulation can also involve ADAMs or other membrane-bound proteases. Therefore, systematic insights on potential crosstalk and synergistic or competetive pericellular protease functions will be of interest. The importance of new information on proteolytic networks is further highlighted by results suggesting that, aside from the protease activation cascades, pericellular protease interactions are involved in protease inhibition.[2,7]

In a kinome-screen for cancer-specific MT1–MMP regulators, we have identified two RTKs, EphA2 and FGFR4, as regulators of tumor invasion.[1,5] The cancer progression-associated FGFR4 variant (G388R polymorphism) works in a complex with MT1-MMP to induce EMT and collective invasion via crosstalk between FRS2-Src pathway and ECM degradation.[6] This interaction, as well as an MT1-MMP-FGFR2-ADAM-9 interaction in mouse calvarial osteogenesis and MT1-MMP-PDGFRβ axis in vascular smooth muscle cells, modulates signaling by cleavages of cofactors rather than RTKs themselves by MT1-MMP.[3,4,7] In contrast, a direct MT1-MMP cleavage of EphA2 triggers homotypic breast cancer cell repulsion and dissemination through RhoA activation and more amoeboid-type invasion.[1] Likewise, MT1-MMP-mediated ectodomain shedding of DDR1 was recently described to regulate collagen-induced receptor signaling.[8] Therefore, MT-MMPs are increasingly appreciated as regulators of cell-microenvironment communications. However, further studies are required to define significance and robustness of these proteolytic processes relative to, e.g., ECM-degradative or non-proteolytic signaling activities of MT-MMPs for cell invasion, differentiation, and growth.

Since the ephrinA ligand-induced signaling through EphA2 is tumor- and migration-supressive, we considered the possibility that the EphA2 cleavage promotes invasion by inactivating these supressive signals. While this might still occur in some conditions, in invasive EphA2-expressing breast carcinoma cells, ephrinA ligand is transcriptionally suppressed. Based on our results, we propose that in these cells, physical EphA2–MT1–MMP interaction and limited EphA2 processing upon cell–cell contact instead regulate signal compartmentalization, cytoskeletal migratory responses, and invasion outcome (**Fig. 1**). In cancer cell signaling, Src kinase is a common mediator of both upstream and downstream RTK signals regulating cell adhesive, mitogenic, and migratory responses, including Rho-mediated cytoskeletal rearrangements. In breast cancer cells, where Src is activated by EphA2, this activity is essential for MT1-MMP cleavage-dependent internalization of the signaling complexes in conjunction with increased RhoA

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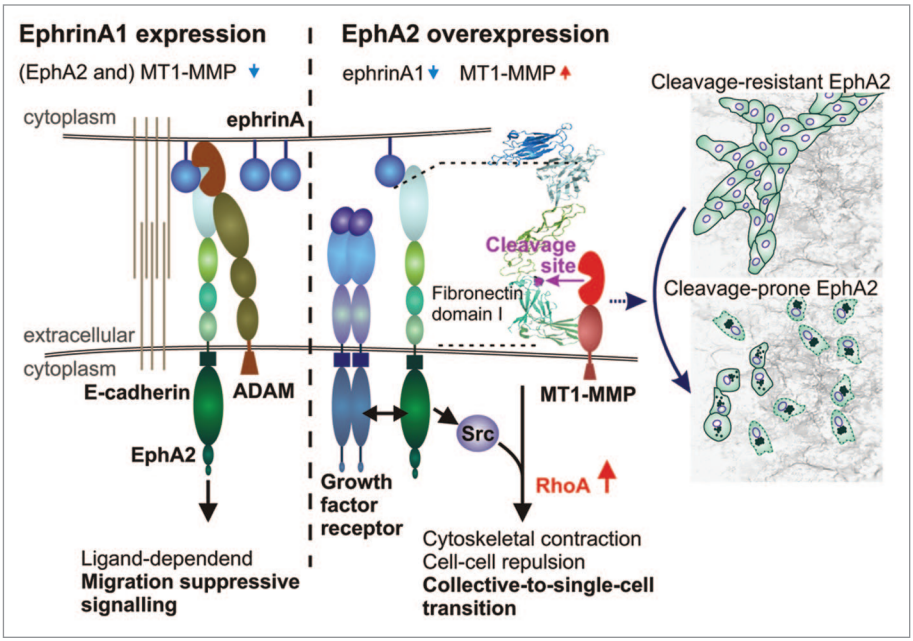

Figure 1. Model of EphA2–membrane protease interactions. In non-invasive ephrin-expressing cells, ephrinA1–EphA2 interaction at cell junctions leads to endocytosis and degradation of the activated receptor, coupled with migration-suppressive responses. Potential ligand-induced Eph–ADAM interaction and cleavage of the membrane-bound ligand in trans from adjacent cell is also depicted. In invasive EphA2-overexpressing cells, increased EphA2-Src signaling is coupled with MT1-MMP upregulation. On the cell surface, cleavage of active EphA2 by MT1-MMP in cis triggers Src activity-dependent intracellular translocation of EphA2, subsequent RhoA-activation, cytoskeletal contractility, and cell–cell repulsion, leading to a switch from collective to single-cell invasion.

activation.[1] The function of Src on ligand-independent EphA2 signaling through growth factor receptor crosstalk remains unclear. However, as Src is a key mediator of the molecular events and cellular outcomes connected to multiple pathways, including the FGFR4-MT1-MMP axis, this kinase serves as a potential integrator of multiple RTK signals.

Extensive evidence supports the remarkable plasticity of tumor cell invasion during metastasis or anticancer drug responses and escape mechanisms. Consistently, EphA2 has also been studied as a therapeutic target. Notably, while the EphA2-MT1-MMP axis operates in basal-type breast carcinoma cells and invasive breast carcinomas, invasive growth, collective invasion, and single-cell dissemination can have different outcomes along tumor progression. Indeed, somatic mutations at the MT1-MMP cleavage site in EphA2 have been found in lung cancer. We showed that this mutation inhibits the cleavage, thus inducing invasive growth and collective cell invasion.[1] Therefore, to fully understand the pathological significance of these mechanisms, we need to investigate how different cell-intrinsic factors or microenvironmental cues affect EphA2 cleavages and invasion modes in distinct tumor contexts.

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