Physiological Mechanisms of Tumor-Cell Invasion and Migration

Recent advances in understanding the complex biology of the microenvironment that underlies tumor invasion and migration have revealed novel and promising therapeutic targets. Pharmacological blockade of intra- and extracellular signaling events that regulate migration and survival of multiple cell types may disrupt the host-tumor conspiracy that allows escape from normal developmental regulation.

Numerous molecular hurdles stand between a newly invasive tumor cell and its distant new home in another organ. The rigor of the journey is reflected by the finding that <0.05% of circulating tumor cells are actually able to become stable metastases (34). The metastatic sequence is understood to involve detachment of cells within a primary tumor, local migration and invasion of stromal tissue, intravasation and transit through blood vessels, capillary bed arrest and extravasation, further local crawling and invasion, attachment, formation of micrometastases, survival, perhaps dormancy, and eventually further proliferation (FIGURE 1) (6, 64).

To overcome this remarkable set of challenges, the invasive cancer cell must borrow a molecular apparatus, or infrastructure, that normally enables important physiological functions, such as morphogenesis (38), neurogenesis (66), and angiogenesis (14). These examples of invasion are under exquisite control and facilitate the development and homeostasis of an organism. In marked contrast, metastatic tumor cells prey upon these molecular systems, often leading to organismal demise (37). Numerous interactions underlie this complicated progression as cancer cells conspire with their local environment to grow, survive, and metastasize (FIGURE 2). Each of the steps involved in tumor metastasis requires numerous specific molecular interactions contributed by the tumor cell and the surrounding extracellular matrix (ECM) and stromal cells (35). These interactions are mediated by contact between the cell and the ECM, by direct cell-cell contact, and by secreted factors.

Tumor Cell Migration Through Tissue Microenvironments

Three-dimensional (3-D) molecular contacts, contributed by the basement membrane, ECM, and adjacent cells, provide an architectural context within which a normal cell functions. Adhesive structures are physical connections between the cytoskeleton (CSK) and the ECM (an extracellular-matrix connection) or between the CSK and the CSK of other cells (a cell-cell connection). There are several adhesion receptor families, including selectins, syndecans, the immunoglobulin cell adhesion molecules, cadherins, and integrins. The best-studied adhesion receptors, and of particular interest in migration, are the...
integrins. These are heterodimeric transmembrane protein structures that bind specific extracellular sites on the ECM and specific intracellular CSK adapter proteins (21). As integrins form connections with the extracellular environment, they provide structural support. They also mediate intracellular signaling through the activation of focal adhesion kinase (22). It is not surprising that the native ECM of a normal cell is a requisite for survival. Indeed, removal of a cell from its supportive matrix can cause that cell to die, also called “anoikis” (18). Recent work has demonstrated that expression of TrkB protein, a neurotrophic receptor, enables a cell to survive, avoiding anoikis, when it has been removed from its native matrix (11). This protein enables the tumor cell to retool its signaling machinery, stimulating the phosphatidylinositol-3-OH and AKT/PKB kinases, leading to inhibition of caspases, key mediators of anoikis.

For a tumor cell to metastasize, it must pass through surrounding stromal elements as it migrates toward a lymph or blood vessel. One important class of molecules within the invasion front is that of the matrix metalloproteinase (MMP). The localized and tightly regulated enzymatic remodeling of stromal tissue by MMPs is an interesting example of collaboration among cell types: MMPs can be secreted by neighboring cells and can become localized and activated on the surface of migrating tumor or endothelial cells (3, 5, 38, 45, 63). Proteolytic activity facilitates migration in several ways. Physical barriers of dense matrix are removed; latent proteases, growth factors, and chemotactic agents (and their receptors) can be activated; integrin-binding sites on the ECM can become exposed and available to transmit migratory and survival signals (4, 9, 42); and bioactive fragments of the ECM itself are produced. These are zinc-binding enzymes that are secreted as proenzymes and are activated in the ECM through the cleavage of an 80-amino-acid sequence located in the amino-terminal domain (15, 40, 54). Groupings within the MMPs have emerged, yielding the stromelysins (which cleave fibronectin, proteoglycans, and nonhelical regions of type IV collagen), the interstitial collagenases (which degrade triple-helical regions of fibrillar collagens types I, II, III, VII, VIII, and X), and the gelatinases (which degrade denatured collagen; native collagen types IV, V, VII, IX, and X; fibronectin; and elastin) (50). Control of MMP function is maintained by the endogenous inhibitors of the MMPs, called the tissue inhibitors of metalloproteinases.

Once ECM barriers at the invasion front have been cleared through proteolytic degradation, the metastatic cancer cell must generate an invasive machinery (32).
As a tumor cell transforms into a migratory cell, the molecular machinery that processes cellular signals for proliferation and survival responses also regulates the formation of migratory behaviors. 

The importance of both adhesion and de-adhesion (24, 27, 30). Rho family GTPases act as central regulators of cytoskeletal dynamics, and the levels of their activated (GTP-bound) states are regulated by a balance between activation by exchange factors (called GEFs) that facilitate replacement of GDP by GTP and inactivation by GTPase-activating proteins (called GAPs) that attenuate signals by stimulating hydrolysis of bound GTP. In cultured fibroblasts (23), activated RhoA stimulates the formation of actin stress fibers and mature elongated focal adhesions, effects that depend on contractile force within the CSK (7). This effect is mediated in part through the Rho effector, Rho kinase, which stimulates sustained CSK contraction by inhibiting myosin light-chain phosphatase (19, 28). Activated Cdc42 stimulates actin polymerization and the formation of thin membrane protrusions called filopodia, and activated Rac stimulates the processes of membrane ruffling and cell spreading that result in formation of actin-rich lamellipodial protrusions. Rho family GTPase activities regulate other types of dynamic adhesive structures, including cadherin-based adherens junctions between cells, and Rac and Rho activities are antagonistic in some contexts (1, 13). The relative activities of the various Rho family GTPases, through their effects on CSK dynamics at specific subcellular locations, mediate transitions between epithelial and mesenchymal phenotypes and integrate environmental information to produce a coordinated migration response.

Integrin-mediated attachment of these cell protrusions to the ECM can occur in a preferred direction, following gradients of ECM binding sites and resulting in directed spreading, called haptotaxis. Alternatively, or in combination with ECM cues, concentration gradients of soluble motogens can morphologically polarize a cell and stimulate directed migration, called chemotaxis. In combination, these activities can result in contact-guided migration along oriented tissue fibers. Tumor invasion occurs in the 3-D ECM, and migration mechanisms used by cells in that environment may not be apparent in 2-D systems. Cell migration, in experimental 3-D systems of various composition, retains the principle elements of haptotactic migration, including clustering of integrins at adhesion sites and detachment of cells by either release or internalization of integrins. There are differences, however, that are not fully understood and that may be significant, because cells must use additional strategies to overcome matrix resistance (16). Cells migrating in 3-D matrices lack stress fibers, focal adhesions, and large lamellipodia but display cylindrical pseudopodia with microspikes and small lamellipodial structures. It is possible that the same contractile forces that inhibit migration by focal adhesion formation on planar surfaces may be promigratory in a 3-D environment where the adhesion sites are more dispersed. Some mammalian cells, including embryonic stem cells, T and B cells, neutrophils, monocytes, and some tumor cells, are capable of an amoeboid type of migration that is characterized by a lack of focal contacts and integrin clusters and by the presence of pseudopod protrusion and retraction that is, in contrast to integrin-guided protrusions, independent of attachment.

The above discussion is based on the behavior of individual cells, but histological observations indicate the presence of invasive clusters of cells in epithelial tumors as well as in blood and lymph vessels. In 3-D collagen matrices, the leading edge of such clusters is comprised of highly motile “pathfinder” cells, whereas the cells in the trailing edge are passive, suggesting that tumor cell clusters may be organized as coherent tissue that contains cells with specialized (e.g., invasive) functions (17, 36, 51).
Environmental complexity

As described, migration and invasion of cells in tumor stroma result from cooperation among pro- trusive, adhesive, contractile, and proteolytic mechanisms and occurs under the combined influence that tumor and stromal cells have on the content and architecture of the ECM as well as on the autocrine and paracrine production and activation of proteinases and chemotactic factors. This complex microenvironment contains migration-stimulating factors such as scatter factor/hepatocyte growth factor produced by fibroblasts, vascular endothelial growth factor and basic fibroblast growth factor (bFGF) produced by tumor cells, MMPs and urokinase-type plasminogen activator produced by fibroblasts and endothelial cells, transforming growth factor-β (TGF-β) produced by tumor cells or released from the ECM by proteinase activity, epithelial growth factor and platelet-derived growth factor produced by tumor cells, cytokines produced by inflammatory cells, and chemokines from inflammatory and stromal cells (35).

Underscoring the role of the tumor microenvironment in invasive and metastatic behavior, stromal matrix and cells induce motility as well. Motility factors within the ECM include vitronectin (2), fibronectin and laminin (39), type I collagen (60), type IV collagen (29), and thrombospondin (2). Motility factors secreted by stromal cells include histamine (61), insulin-like growth factor-I (55), and interleukin (IL)-8 (62). These motility factors have also been described as homing factors, because tumor cells are attracted to the organs that produce them. For example, chemokines secreted by various tissues may be chemotactic for circulating tumor cells, such as metastatic breast cancer, that bear the matching chemokine receptor (44). Motility factors intrinsic to tumor cells are the autocrine motility factors such as hepatocyte growth factor (20), insulin-like growth factor-II (12), and autotaxin (ATX) (56).

The involvement of ATX in tumor metastasis demonstrates a complexity within tumor-host microenvironments, resulting in technological challenges for early cancer detection and therapeutic design. ATX is a widely expressed secreted enzyme that was initially discovered as a melanoma cell autocrine motility factor. ATX stimulates the migration of a variety of cells, including cancer cells, fibroblasts, and vascular smooth muscle cells. ATX promotes aggressive behavior in some tumors through enhancement of invasive, migratory, and angiogenic potentials (FIGURE 4). ATX is overexpressed in various cancers, and its expression can be regulated by several growth and biological factors, including retinoic acid, interferon-γ, IL-1, IL-4, and TGF-β (41, 43).

Recent discoveries that ATX can catalyze the production of two highly potent bioactive lysophospholipids, lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) from the precursors lysophosphatidylcholine and sphingosylphosphorylcholine, respectively, have provided insight into ATX activity (52) (FIGURE 5). LPA and S1P are potent mediators of diverse biological functions. For example, through specific G protein-coupled membrane receptors, extracellular LPA and S1P invoke signaling cascades involving adenyly cyclase, phosphatidylinositol 3-kinase, Ras-MAPK, phospholipase C, Akt/PKB, c-Src tyrosine kinase, the small GTPases Rac and Rho, phospholipase D, and p125 focal adhesion kinase (25). These signaling pathways control numerous events important for normal cellular function, tumor invasion, and metastasis. LPA can modulate cell proliferation, survival, migration, and invasion through multiple pathways. For example, LPA can mediate cytoskeletal organization through focal adhesion formation and integrin activation, N-cadherin-mediated cell clustering, wound healing through platelet aggregation, vascular remodeling, and upregulation of MMPs, as well as neurite retraction (41). S1P stimulates survival signaling and promotes angiogenesis by stimulating endothelial cell motility and tube formation. S1P also inhibits some tumor and leukocyte cell migration (57).

Expression of ATX in Ras-transformed fibroblasts augments their in vitro invasive potential as well as the size, number, and vascularity of tumors formed in mice (16). Inclusion of ATX, or ATX-secreting cells, elicits an angiogenic response in Matrigel plugs inserted subcutaneously in mice (46), including the formation of blood vessels that contain
local expression of ATX and its substrates provides additional regulatory and signaling complexity in the tumor microenvironment. ATX may also be an extracellular target for pharmacological therapy that awaits the design of specific enzyme inhibitors.

Defining Molecular Markers of Metastatic Potential

In a cloak-and-dagger scenario, the metastatic tumor cell capitalizes on physiological mechanisms to create and sustain a pathophysiological state. The growing understanding of the varied molecular terrain that a metastatic tumor cell traverses presents a rich source of potential prognostic indicators, or biomarkers, as well as therapeutic targets. The dependence of tumor cells on the sur-

FIGURE 5. Migratory behavior of tumor cells
The commitment toward migratory behavior involves contributions from extracellular, plasma membrane, and intracellular molecular structures. ATX provides one example of how the same molecule can induce different cellular behaviors depending on the cellular phenotype of the signal-receiving cell.

Both endothelial cells and pericytes. ATX stimulates a tubulogenic response in endothelial cells but does not stimulate a strong migration response in chemotaxis assays. The angiogenic activity of ATX may affect nonendothelial cell targets, including pericytes that can stabilize new vessels. Vascular smooth muscle cells, as well as endothelial cells stimulated with the angiogenic factor bFGF secrete ATX, raising the possibility that three different cell types contribute to the angiogenic response by secreting ATX into a mutually attractive environment. The biological effects of ATX depend on its enzymatic activity (31), and in vitro migration experiments indicate that divergent migration responses (stimulatory or inhibitory) to ATX/LPA and S1P can be regulated by lysophospholipid receptor-type expression and the availability of alternative substrates for ATX (8, 47). The

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rounding ECM and stromal cells highlights the importance of studying the molecular profiles of cells within their native microenvironments. One recent study using protein microarray technology (48) compared the molecular profiles generated between prostatic carcinoma tissue microdissected by laser capture microdissection and cell lines of the same tumor type. The differences between the two profiles demonstrate the need to develop tools that allow the pathologist and basic scientist to have access to primary molecular material from cancer specimens. Moreover, as the steps of metastasis are reviewed, the role of posttranslationally modified proteins in tumor invasion and metastasis, such as cleavage and phosphorylation, is becoming more apparent.

With protein microarrays, the presence of known protein isoforms are probed in tumor cells and/or surrounding stromal cells. Protein microarray formats allow protein lysates from pure cell populations, procured using laser capture microdissection, to be arrayed on a substrate for subsequent probing using antibodies (33). This type of array format has been used to characterize molecular signaling pathway alterations that occur within varying stages of prostate cancer (49). In another, more recent study using protein microarray technology (53), primary and metastatic tumors from the same patients with ovarian cancer have been examined. The proteomic signaling pathways are markedly divergent between the primary tumor cells and the metastatic tumor cells. Intriguing questions arise from such findings. Namely, is the divergent cellular behavior due to the effects of differing microenvironments that surround the primary and metastatic tumor cells? Or rather, is the altered proteomic signaling profile attributable to the development of a divergent clonal population of metastatic tumor cells that emerges under selective pressures? Molecular profiling of each stage of a tumor's life cycle will provide insight into these questions. Furthermore, these kinds of proteomic studies will reveal potential targets for therapeutic intervention.

References


