Cell

Leading Edge
Review

Cancer Invasion and the Microenvironment: Plasticity and Reciprocity

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Cancer invasion is a cell- and tissue-driven process for which the physical, cellular, and molecular determinants adapt and react throughout the progression of the disease. Cancer invasion is initiated and maintained by signaling pathways that control cytoskeletal dynamics in tumor cells and the turnover of cell-matrix and cell-cell junctions, followed by cell migration into the adjacent tissue. Here, we describe the cell-matrix and cell-cell adhesion, protease, and cytokine systems that underlie tissue invasion by cancer cells. We explain how the reciprocal reprogramming of both the tumor cells and the surrounding tissue structures not only guides invasion, but also generates diverse modes of dissemination. The resulting ''plasticity'' contributes to the generation of diverse cancer invasion routes and programs, enhanced tumor heterogeneity, and ultimately sustained metastatic dissemination.

Introduction

Cancer invasion and metastasis are landmark events that transform a locally growing tumor into a systemic, metastatic, and live-threatening disease. The initial steps of local invasion include the activation of signaling pathways that control cytoskeletal dynamics in tumor cells and the turnover of cell-matrix and cell-cell junctions, followed by active tumor cell migration into the adjacent tissue (Chambers et al., 2002; Friedl and Wolf, 2003; Sahai, 2007). Metastasis then occurs when invading tumor cells engage with blood and lymph vessels, penetrate basement membranes and endothelial walls, and disseminate through the vessel lumen to colonize distant organs (Fidler, 2003). Like cells in primary tumors, cells in metastases also proliferate, invade, and enter blood vessels, leading to secondary metastasis (Kienast et al., 2010; Armstrong et al., 2011; Hou et al., 2011).

In the past few decades, cell and tumor biologists have identified the mechanisms of cell migration in normal and malignant cells, including the regulation of cell adhesion and cytoskeletal dynamics (Lauffenburger and Horwitz, 1996; Ridley et al., 2003; Sanz-Moreno and Marshall, 2010). However, attempts to define the rate-limiting mechanisms that govern invasive and metastatic cancer cell migration, such as a dominant signaling pathway, receptor-ligand interaction, or protease-substrate interaction, have largely failed. Instead, cancer cell invasion is now regarded as a heterogeneous and adaptive process. Indeed, it is this ''plasticity'' in cell adhesion, cytoskeletal dynamics, and mechanotransduction that perpetuates migration and dissemination under diverse structural, molecular, and even adverse microenvironmental conditions (Friedl and Wolf, 2010; Sahai, 2007; Sanz-Moreno and Marshall, 2010).

Plasticity of invasion, together with other hallmarks of neoplasia, including cancer cell growth, survival, and genomic instability, lead to morphological, signaling, and genetic differences between primary and metastatic lesions within the same patient (intrapatient heterogeneity), within the same lesion (intratumoral heterogeneity), and across time (Choi et al., 2011; Honeth et al., 2008; Lopes et al., 2009; Shapiro et al., 2011; Stoecklein et al., 2008; Wang et al., 2009).

Such heterogeneous tumor progression is mirrored by an ''activation'' response of stromal cells nearby the growing tumor, including fibroblasts, endothelial cells, and macrophages. Once ''activated,'' these cells reorganize the structure and composition of the connective tissue by depositing extracellular matrix components (ECM), cytokines, and growth factors (Egeblad et al., 2010; He et al., 2011; Picchio et al., 2008; Shapiro et al., 2011). By remodeling the tissue structure, releasing growth factors, and imposing metabolic stress, the reactive tumor stroma, in turn, influences cancer cell functions, often enhancing tumor growth and invasion and aggravating cancer resistance during metabolic challenge and therapy (Alexander and Friedl, 2012; Giese et al., 2003; Sansing et al., 2011; Yao et al., 2011). Thus, in a reciprocal manner, tumor cells influence the stroma and vice versa, jointly driving cancer progression (Nelson et al., 2008; Xu et al., 2009). Here, we summarize the adhesion, protease, and cytokine systems that underlie tissue invasion by cancer cells. We discuss how the reactive tumor

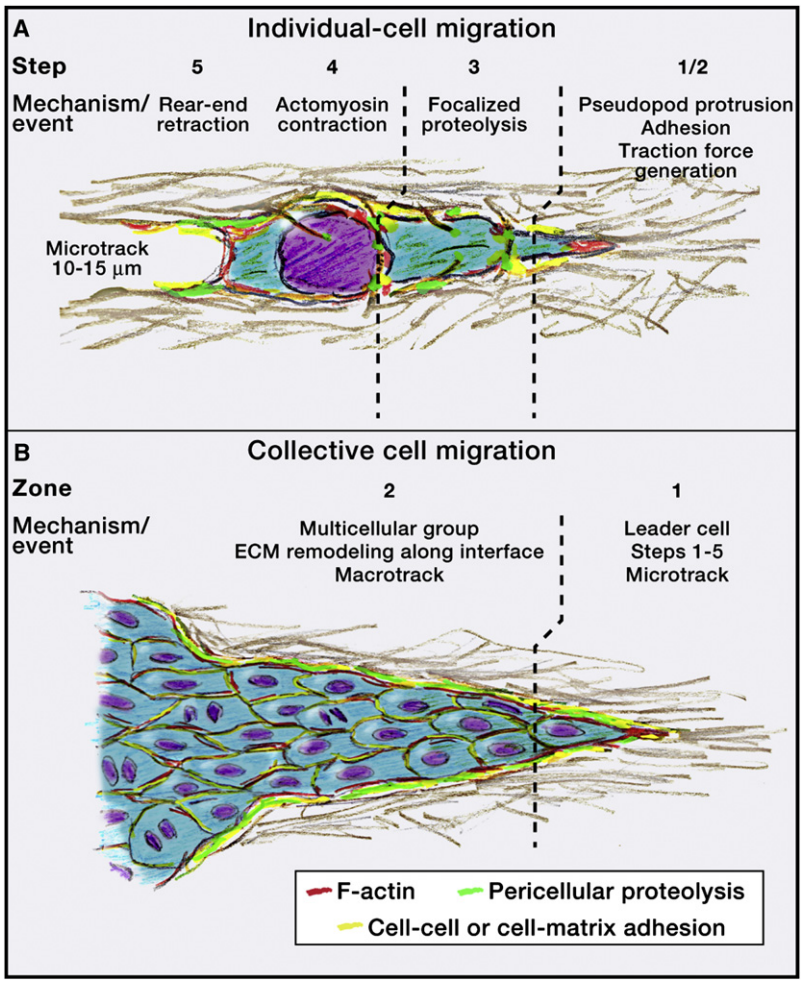

Figure 1. Cell Migration: A Multistep Process

In general, cells can migrate individually or collectively as multicellular groups.

(A) Single-cell migration involves five molecular steps that change the cell shape, its position, and the tissue structure through which it migrates.

(B) Collectively migrating cells form two major zones: zone 1, in which a "leader cell" generates a proteolytic microtrack at the front of the migrating group, and zone 2, in which the subsequent cells then widen this microtrack to form a larger macrotrack. (Figure modified from Friedl and Wolf, 2008.)

microenvironment steers plasticity and perpetuates invasion and metastatic dissemination.

Mechanisms of Cell Migration

Cancer invasion is a cyclic process in which the cell changes shape, produces morphological asymmetry, and then translocates the cell body. Depending on the cell type and tissue environment, cells can migrate in two major ways: individually, when cell-cell junctions are absent, or collectively as multicellular groups, when cell-cell adhesions are retained (Friedl and Wolf, 2010) (Figure 1). The underlying process in both types of migration is the dynamics of the cytoskeleton coupling with cell surface receptors that engage with surrounding tissue structures; thus, the cytoskeleton serves as the cell's engine, and the cell surface receptors act as its transmission (Ridley et al., 2003). Cancer cells recapitulate the types and mechanisms of migration used by normal, nontumor cells. They activate the same machineries for changing shape, generating force, and remodeling ECM (Friedl, 2004) as normal cells, but neoplastic cells lack physiological "stop signals" immobilizing and anchoring the cells (Cox et al., 2001), which arguably perpetuates neoplastic cell migration.

Invasive single-cell migration results from five interdependent molecular steps that change the cell shape, its position, and the tissue structure through which it migrates (Friedl and Wolf, 2009; Lauffenburger and Horwitz, 1996; Sheetz et al., 1999) (Figure 1A). In step 1, the cytoskeleton polarizes by actin polymerization and forms a leading protrusion at the opposite end of a "pre-uropod" region, which marks the constitutive rear end of the cell (Estecha et al., 2009; Poincloux et al., 2011). In step 2, the leading edge protrusion engages with extracellular substrates, followed by recruitment and adhesion of cell surface receptors that form focalized clusters and couple extracelluar adhesion to intracellular mechanosignaling and force generation (Friedl et al., 1997). In step 3, several micrometer rearward of the leading edge, cell surface proteases become engaged with extracellular scaffold proteins and execute locally controlled proteolysis (Friedl and Wolf, 2009). This proteolysis modifies the molecular and mechanical tissue properties and allows space for the advancing cell body (Friedl et al., 1997). In step 4, the small GTPase Rho activates myosin II, and contraction mediated by actomyosin generates tension inside the cell. In step 5, this contraction is followed by the gradual turnover of adhesion bonds at the trailing edge, which slides forward while the leading edge protrudes further.

In most cells, the leading edge protrusion is controlled by the small GTPase Rac or Cdc42, which generate pseudopodia or filopodia that engage with ECM substrate (Sanz-Moreno and Marshall, 2010). In some cell types with low Rac activity or in poorly adhesive environments, a variation of steps 1 and 2 occurs (i.e., the pseudopod protrusion and adhesion steps) in which the leading edge generates leading bleb-like or even bleb- and pseudopod-free smooth membrane propulsions. These propulsions are stabilized by cortical F-actin and intercalate between extracellular tissue structures (Lorentzen et al., 2011; Poincloux et al., 2011). Here, the force is generated near the rear pole in an actomyosin- and integrin-dependent manner (Poincloux et al., 2011).

The physicochemical steps in single-cell migration are coordinated within the same cell body and executed in a synchronous, often pulsatile manner, which allows the cell body to protrude and

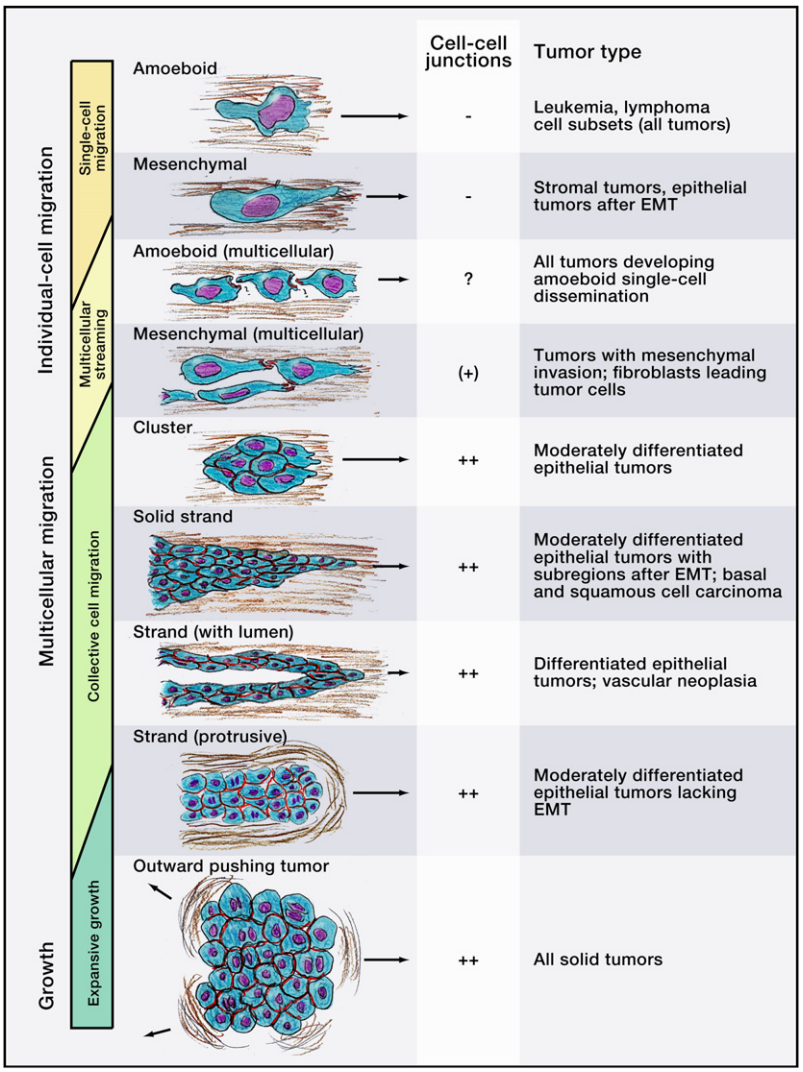

Figure 2. Modes of Cell Movement Implicated in Cancer Invasion and Metastasis

Single-cell and collective cell migration can be further partitioned based on the specific cell-cell junctions, the contractility of cytoskeleton, and the turnover of cell attachments to extracellular matrix (ECM). These modes of migration can be further unstable and change upon alterations of cell-cell interactions, cell-ECM adhesion, or cytoskeletal contractility, resulting in intermediate phenotypes.

generate traction in an oscillatory manner (Ridley et al., 2003). If multiple cells originate from the same location, such as a tumor, the "leader cell" forms a proteolytic microtrack of locally removed ECM barriers (zone 1). The following cells then widen or excavate this microtrack by mechanical force and proteolysis to form a larger macrotrack (zone 2) (Ilina et al., 2011; Wolf et al., 2007) (Figure 1B). In collective migration, protrusion and retraction are coordinated in a "supracellular manner," in which cytoskeletal protrusion and contractility are mechanically mediated through cell-cell junctions (Hidalgo-Carcedo et al., 2011; Tambe et al., 2011), allowing the cell group to behave as "mega-cell" (Figure 1B).

Patterns and Diversity of Cancer Cell Invasion

The five-step model of cell migration is active in many types of cell movement for both normal and neoplastic single cells. Operationally, individual cell and multicellular migration follow the paradigm of active cell migration, whereas multicellular growth leads to passive cell movement by pushing (Figure 2).

Rounded/Amoeboid Migration

Cells migrating with low adhesion force or high actomyosin-mediated contractility adopt morphologically spherical shapes. This is referred to here as amoeboid migration because the *Dictyostelium discoideum* amoeba migrates by this mechanism (Friedl et al., 2001). Amoeboid movement, which uses Rac-dependent filopodia, has small or diffusely organized adhesion sites that generate weak to negligible adhesion force toward the substrate (Lämmermann and Sixt, 2009). The second form of amoeboid movement, which uses Rho-dominated blebbing, lacks defined adhesions and mediates cell translocation by propulsion using either blebs or smooth membrane protrusion at the leading edge and lateral intercalation (Lorentzen et al., 2011; Paluch et al., 2006; Poincloux et al., 2011). Amoeboid cells tend to migrate in the absence of proteolytic ECM breakdown by adapting their shape to and squeezing through tissue gaps and trails (Wolf et al., 2003b). The origin of amoeboid tumors is often hematopoietic or neuroectodermal, including leukemias, lymphomas, and small cell lung carcinoma, but amoeboid movements are also detected as cell subsets in most other tumor types (Madsen and Sahai, 2010; Wolf et al., 2003b).

Mesenchymal Migration

When cytoskeletal protrusions and adhesion capabilities are strongly developed, invading cells adopt spindle-shaped, elongated morphology with focalized cell-matrix adhesions containing multimolecular integrin clusters and proteolytic activity toward ECM substrates (Wolf et al., 2007). Focalized proteases on the cell's surface generate small microtracks through which subsequent cells can follow (Friedl and Wolf, 2009) (Figures 1A and 2). Mesenchymally migrating tumor cells originate from tumors of the connective tissue, including soft tissue sarcomas. They also originate from all other tumor types after the tumor cells dedifferentiate and lose cell-cell junctions (Brabletz et al., 2001; Friedl and Wolf, 2009; Sanz-Moreno et al., 2008).

Multicellular Streaming

When individual cells move one after each other using the same path within the tissue, it is referred to as "multicellular streaming." This occurs mainly when individual cells become

chemotactically attracted by a particular source or jointly follow microtracks that are often present in peripheral connective tissue (Kulesa and Gammill, 2010). In neoplasia, multicellular streaming is often seen as chain- or swarm-like (i.e., diffuse) tissue infiltration of many tumor cells in hematologic and solid tumors (Kedrin et al., 2008).

Collective Invasion

Collective invasion requires cell-cell adhesion and multicellular coordination to occur simultaneously with migration, which results in multicellular groups and strands originating at the interface between tumor and stroma (Friedl et al., 1995; Ilina and Friedl, 2009). Collective invasion may adopt different morphologies, which depend on the cell type, the number of jointly moving cells, and the tissue structure being invaded. For instance, groups of cells can form small clusters, solid strands, or files; if epithelial polarity is retained during migration, these structures can even form an inner lumen (Friedl and Gilmour, 2009). In most cases of collective cancer invasion, one or several leader cells with mesenchymal characteristics form the tip of multicellular strands and generate forward traction and pericellular proteolysis toward the tissue structure (Gaggioli et al., 2007; Khalil and Friedl, 2010). In a second type of collective invasion, a blunt bud-like tip protrudes along tissue space consisting of multiple cells that variably change position, lacking defined leader cells (Ewald et al., 2008); this type of invasion occurs preferentially in soft tissues and cells of strong epithelial polarity. Collective migration is prevalent in morphogenesis during development and recapitulated in most epithelial and mesenchymal tumor types (Friedl and Gilmour, 2009; Friedl et al., 1995).

Expansive Growth

Some surrounding tissues impose little to no physical confinement on proliferating tumor cells and thus do not hinder the expansion of a cancerous lesion. When tumor cells grow into these tissues, the increase in volume leads to multicellular outward pushing with intact cell-cell junctions and no signs of active migration (Iguchi et al., 2008; Ishizaki et al., 2001). Eventually, this expansive growth without migration results in spherical lesions within a "capsule" of ECM, formed by aligned collagen fibers in circular orientation (Ishizaki et al., 2001). Expansive growth may displace cells by volume expansion and pushing when migration activity is absent or, if coupled with migration, contributes to and enhances collective invasion (Ilina et al., 2011) (B. Weigelin and P.F., unpublished data).

Although these migration modes can be classified as morphologic and mechanistic entities for experimental and conceptual purposes, cells often display features from multiple modes in three-dimensional (3D) tissues. This includes intermediate or transition states in which cells may change their molecular profiles and switch migration mode (e.g., from proteolytic to non-proteolytic migration or single-cell to collective migration) (Friedl and Wolf, 2010; Wolf et al., 2003a). For example, when individual cells become attracted by the same chemotactic source, they may first undergo multicellular streaming with short-lived cell-cell junctions that briefly form and resolve again; when cell-cell adhesion molecules are then upregulated, the cells may join each other and convert to a collective migration mode (Kulesa and Gammill, 2010). Thus, diverse molecular programs jointly determine the morphology and mechanism by which normal and neoplastic cells move through tissues.

Physical and Molecular Determinants of Invasion

The molecular mechanisms underlying each migration mode depend on a set of connected mechanical and signaling pathways, which vary in their coordination and strength depending on the particular migration mode. Such variations include the organization of the cytoskeleton; the capability to remodel tissue structures; and the type, strength, and turnover of cell-substrate adhesions, cell-cell adhesions, and intercellular communication. Stromal signals modulate all of these pathways through chemokines, cytokines, and growth factors (Friedl and Wolf, 2010).

Different types of adhesion systems contribute directly or indirectly to mechanocoupling between the actin cytoskeleton and extracellular ECM (Figure 3A) or cell surface scaffolds (Figure 3B). Together, these adhesion systems govern migration.

ECM Receptors

Integrins are heterodimeric surface receptors composed of α and β chains. Together, these chains mediate adhesion and mechanotransduction to extracellular ligands, including $\alpha2\beta1$ integrin predominantly binding to fibrillar collagen; $\alpha V\beta3$, $\alpha V\beta1$, and $\alpha5\beta1$ interacting with fibronectin; and $\alpha3\beta1$ and $\alpha6\beta1$ engaging with laminin (Hynes, 2002). After associating with ligands, the cytoplasmic tails of integrins connect to cytoskeletal adaptor proteins, including talin, paxillin, and kindlin and the mechanosensing modulators vinculin and p130Cas (Geiger et al., 2009; Grashoff et al., 2010). Adaptor and mechanosensing modulator proteins engage with the actin cytoskeleton and trigger signaling to protein kinases, including the focal adhesion kinase (FAK) and Src (Geiger et al., 2009; Hodivala-Dilke et al., 1999; Hynes, 2002). Downstream integrin effectors further include the small GTPases Rac and Rho, which reinforce cell protrusion and rear contraction (Ridley et al., 2003). In addition to contact to ECM substrate, integrin engagement with extracellular ligands is also activated by inside-out signaling through Rac, the Ras-related GTPase Rap1, and talin (Lee et al., 2009; Ridley et al., 2003).

CD44 and its alternatively spliced variants bind to hyaluronic acid (i.e., a high-molecular weight glycosaminoglycan abundantly present in all connective tissues) and, with low affinity, to heparan sulfate, collagen, and fibronectin (Zöller, 2011). CD44 connects to the actin cytoskeleton by the adaptor proteins ezrin, radixin, and moesin (ERM) and ankyrin and mediates intracellular signaling through Src kinase and small Rho GTPases, including RhoA (Zöller, 2011). CD44 and its splice variants also bind to chemokines and growth factors, and they enhance signaling through *cis* interactions with growth factor receptors, including the hepatocyte growth factor receptor (c-Met), fibroblast growth factor (FGFR-1), the epidermal growth factor receptors (EGFR), and its variants ERBB2–4. Thus, CD44 delivers joint ECM and growth factor signaling to invading cells (Couchman, 2010; Zöller, 2011). CD44 also serves as a coreceptor for other adhesion receptors, including integrins and podoplanin (Zöller, 2011). Podoplanin is a cell surface mucin that connects to the actin cytoskeleton through ezrin. Podoplanin signals to enhance RhoA activity, which strongly increases cell invasion (Martín-Villar et al., 2006; Wicki et al., 2006). Given its extensive crosstalk

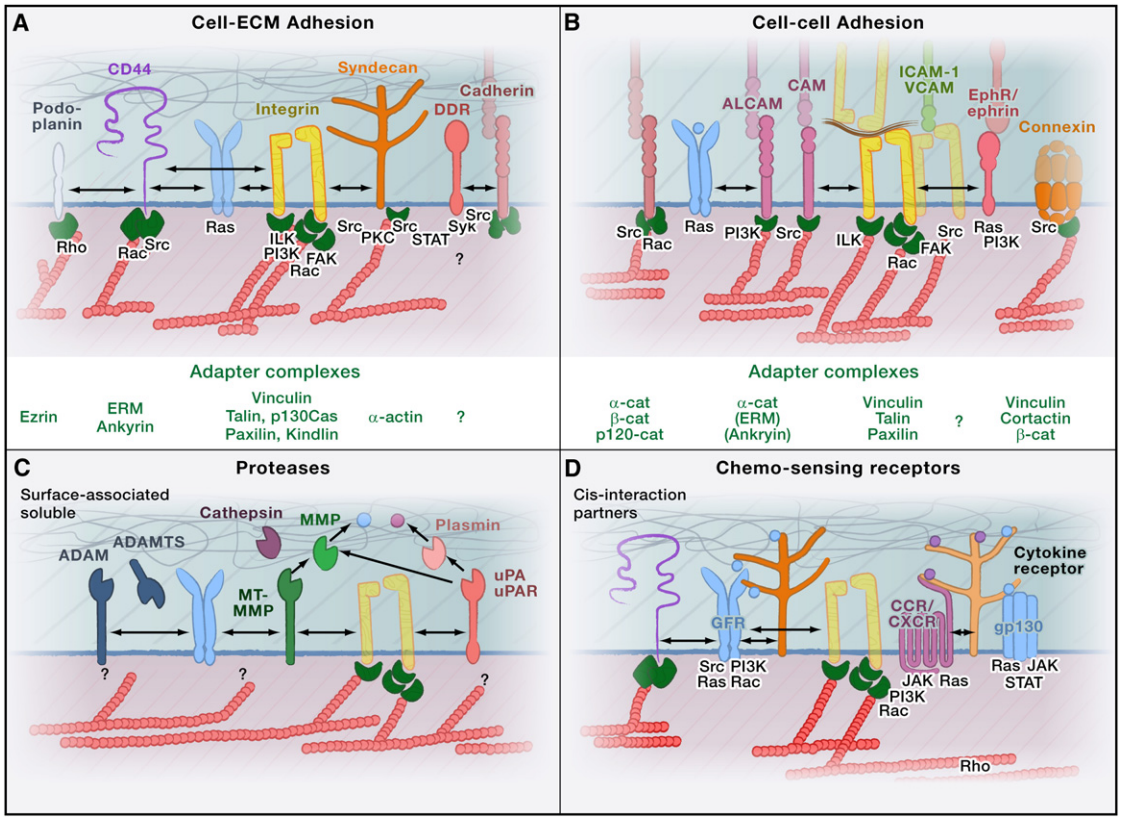

Figure 3. Molecular Determinants of Cell Migration

Simplified view of molecules mediating adhesion and migration signaling.

(A) Cell surface receptors and adaptors that mediate the dynamic interface between the actin cytoskeleton and promigratory signaling and the extracellular matrix (ECM).

(B) Cell surface proteins that mediate and regulate interactions between cells. Similar adhesion mechanisms may mediate homotypic cell-cell cohesion during collective invasion and transient and more dynamic heterophilic interaction to resident tissue cells encountered during tissue invasion.

(C) Protease systems upregulated in cancer progression, invasion, and metastasis.

(D) Receptors for chemokines, cytokines, and growth factors, which sense soluble, ECM-, or proteoglycan-bound factors and interaction partners. Green symbols represent selected intracellular adapters to the actin cytoskeleton, as specified below the drawing (A and B); shaded labels represent major signaling molecules regulating actin organization and cell migration.

with other receptor/ligand systems, it is unclear whether CD44 serves as bona fide adhesion receptor that mediates adhesion and mechanotransduction in the absence of other pathways or whether its primary role is to provide cosignaling (Maaser et al., 1999).

Similar to CD44, membrane-bound proteoglycans, such as syndecans, glypicans, and neuropillin, interact through their sugar moieties weakly with ECM components, including hyaluronic acid, fibronectin, collagen, or elastin. These interactions enhance adhesion in cooperation with integrins, and together with integrins and growth factor receptors, the proteoglycans deliver signals via PKC and Src (Couchman, 2010; Theocharis et al., 2010). Syndecans-2 and -4 engage with ezrin or α-actinin, respectively, and they couple to the actin cytoskeleton. However, their direct contributions to adhesion and migration are still unclear (Couchman, 2010).

The discoidin domain receptors DDR1 and DDR2 interact selectively with fibrillar collagen and transmit signaling via STAT5, NFκB, and p38 MAPK/ERK or the Src-related kinases Syk, Shc, and Src, respectively (Neuhaus et al., 2011; Vogel et al., 2006). DDRs support E- and N-cadherin-mediated cell-cell adhesion (Eswaramoorthy et al., 2010; Shintani et al., 2008), and they increase proteolytic cell functions via matrix metalloproteinases (MMPs), including MMP-1, MMP-2, MMP-9, and MMP-10 (Ruiz and Jarai, 2011). The signaling activity of DDRs after they bind to the ECM is well established; however, whether DDRs connect to the actin cytoskeleton and directly contribute to mechanotransduction is unknown.

Integrins are thus the main adhesion and mechanotransduction system for interstitial migration, with modulatory input and crosstalk to alternative cell-ECM and growth factor signaling systems through CD44, cell surface proteoglycans, and DDRs.

Cell-Cell Adhesion Receptors

Receptors that transmit cell-cell adhesion forces toward the actin cytoskeleton provide cooperation between tumor cells during collective invasion (Giampieri et al., 2009; Ilina and Friedl, 2009). These receptors also support single-cell and collective movement along the surfaces of other tissue-resident cells encountered during the migration process (Figure 3B).

Members of the cadherin family of adhesion receptors mediate homotypic interactions between cells of the same type and heterotypic interactions between different cell types. These interactions include stable cell-cell adhesion through adherens junctions (Harris and Tepass, 2010), dynamic adhesion via the transient co-engagement of small GTPases Rac1 and RhoA, and dynamic junctional remodeling by cytoskeletal dynamics (Kardash et al., 2011). In both stable and dynamic cell-cell adhesion, cadherins engage with cytoskeletal adaptor and signaling proteins, including α-catenin, β-catenin, and p120-catenin, which connect to the actin and microtubule cytoskeleton (Berx and van Roy, 2009; Harris and Tepass, 2010; Reynolds, 2010). Depending on the type of tumor, different sets of cadherins are expressed and involved in cell-cell interaction, including E-, N-, and P-cadherins, cadherin-11, and cadherin-13 (Berx and van Roy, 2009). In polarized resting epithelium, E-cadherin suppresses migration signaling by inhibiting Rac1 (Kitt and Nelson, 2011) and further maintains cell-cell cohesion, polarity between the basal and luminal layer of an epithelium, and epithelial stability. In contrast, in activated and neoplastic epithelium, E-cadherin and other cadherins jointly coordinate collective movements (Friedl and Gilmour, 2009). In activated epithelial cells, cosignaling of E-cadherin and integrins, together with downstream Src activation, enhances actin dynamics and actomyosin contractility, leading to both single-cell and collective migration (Geisbrecht and Montell, 2002; Kardash et al., 2010; Martinez-Rico et al., 2010). When co-engaged with DDR1, E-cadherin signaling limits actomyosin contractility along cell-cell junctions, which stabilizes cell-cell junctions and supports collective invasion (Hidalgo-Carcedo et al., 2011). Compared to E-cadherin, N-cadherin and cadherin-7 mediate weaker adhesion strengths (Chu et al., 2006) and are associated with further increased motility in cancer. This enhanced motility is most likely due to N-cadherin and cadherin-7's co-engagement with growth factor receptors, including FGFR or PDGFR, which enhances downstream signaling through MAPK and PI3K (Berx and van Roy, 2009). Thus, cadherins show duality in delivering both migration-inhibiting and migration-promoting signaling in a context-dependent manner (Martinez-Rico et al., 2010).

The immunoglobulin superfamily of cell adhesion molecules (CAM) mediates homophilic cell-cell interactions in neoplastic cells through the direct or indirect coupling to the actin cytoskeleton via actin-binding adaptor proteins α-actinin, ankyrin, and ezrin (Gavert et al., 2010; Maness and Schachner, 2007). This family of adhesion molecules includes L1CAM, EpCAM, NCAMs, or ALCAM.

L1CAM is upregulated in the leading front of collectively invading epithelial tumors that display a stabilized mesenchymal phenotype with high invasion capability (Bergmann et al., 2010; Hung et al., 2010). This is consistent with a role for L1CAM in leader-cell function and partial EMT during collective invasion (Gavert et al., 2011). Likewise, ALCAM is upregulated in cell-cell junctions of collectively invading epithelial cancer associated with increased metastasis (van den Brand et al., 2010).

Similar to leukocytes, tumor cells develop heterophilic cell-cell interactions with endothelial cells and platelets that express ICAM-1, VCAM-1, or PECAM-1. These interactions occur through β1 and candidate integrins β2, αVβ3, and α4β7 expressed by the tumor cells and mediate intravascular migration and adhesion arrest of circulating tumor cells (Hynes, 2002; Stoletov et al., 2010). Integrins may further engage with ECM proteins tethered and immobilized on encountered cell surfaces (e.g., fibronectin and laminin) and mediate cell-cell adhesion between tumor cells (Casey et al., 2001).

Besides mechanocoupling, CAMs enhance the signaling of integrins and growth factor receptors (e.g., EGFR and FGFR) through ERK, ILK, or Src (Kiefel et al., 2011; Zecchini et al., 2011). Their contributions to homotypic interaction between tumor cells and heterotypic interactions between tumor and stromal cells make CAMs versatile mechanotransduction and signaling devices in both single-cell and collective invasion.

Several other receptor families contribute to cell-cell contacts and multicellular coordination. These include connexins that form gap junctions (Li et al., 2008), as well as ephrins and Eph receptors. Ephrins and Eph receptors weaken homotypic and heterotypic binding by engaging with alternative sets of ephrins expressed by neighboring tumor and stromal cells (Astin et al., 2010), thereby contributing to tumor cell guidance and migration in a tissue context-dependent manner.

Overall, tumor cells engage in a variety of overlapping and synergistic cell-matrix and cell-cell adhesion systems that balance cell-cell cohesion within the tumor and cohesion toward stromal interfaces.

Protease Systems

In both tumor and stromal cells, multiple protease systems are upregulated with overlapping substrate specificities. These systems include MMPs, ADAMs, cathepsins, the serine protease urokinase plasminogen activator (uPA), and its receptor uPAR (Mason and Joyce, 2011; Rizki et al., 2008) (Figure 3C). Upregulated proteases contribute to tumor invasion and progression through at least three distinct mechanisms (Egeblad and Werb, 2002; Wolf and Friedl, 2011).

First, cell surface proteases, notably membrane-type (MT) MMPs and ADAMs (a disintegrin and metalloproteinases), execute contact-dependent proteolysis of structural ECM proteins, including fibrillar and nonfibrillar collagens, fibronectin, and laminins, as well as ECM-tethered, matricellular proteins (e.g., tenascin and glypican) (Sabeh et al., 2004, 2009; Wolf et al., 2007). Proteolytic ECM degradation has a dual function: (1) it generates biologically active epitopes of ECM components with adhesion- or migration-promoting effects (Kenny et al., 2008), and (2) it structurally remodels tissue to form de novo gaps and trails bordered by multifiber ECM bundles (Gaggioli et al., 2007; Sabeh et al., 2009; Wolf et al., 2007).

Second, proteases that are secreted and tethered to the cell surface, notably MMPs and ADAMs, enzymatically process other proteases and cell surface receptors, including adhesion and growth factor receptors (Overall and Blobel, 2007). This controls the activation and turnover of these receptors and thus accounts for adaptive changes of receptor availability on both tumor and stromal cells and interstitial protease content.

Finally, secreted proteases, particularly MMPs and plasmin, regulate the repertoire of available extracellular growth factors by enzymatic activation, inactivation, or degradation (Dean et al., 2008; Mu et al., 2002; Sounni et al., 2010). MMPs and ADAMs

can release ECM-bound factors, which then form diffusing gradients toward neighbor cells (Shiao and Coussens, 2010).

Thus, the proinvasive tumor microenvironment dominated by proteases consists of both structural ECM remodeling supported by pericellular proteolysis and deregulated proteolytic processing of chemokines, growth factors, and their receptors, which impacts both tumor and stromal cells.

Chemokines, Growth Factors, and Their Receptors

The transition from a fixed, tissue-anchored state to a mobile state is often induced by extracellular chemokines, cytokines, and growth factors released by tumor cells themselves or activated stromal cells. These factors engage redundant and nonredundant intracellular signaling networks in both tumor and stromal cells (Figure 3D). Invasion-promoting chemokines include CXCL12, CXCL10, CCL21, or CCL25. They mediate and perpetuate invasive migration of tumor cells in the primary tumor and likely during metastatic dissemination (Allinen et al., 2004; Zlotnik et al., 2011). Migration-promoting signals induced by chemokines and their receptors CXCR4, CXCR3, and CCR9 are mainly mediated by JAK/PI3K/JNK, PI3K, Src-family kinase Syk, and the small GTPases Rac1, RhoA, and Rap1 (El Haibi et al., 2010; Lee et al., 2009; Tybulewicz and Henderson, 2009). Besides their control on the cell cycle and cell survival, many growth factors, including HGF, EGF, FGF, and TGFβ, share signaling through ERK, JNK, Src, mTOR, and PI3K pathways toward Rac and Cdc42 activation and enhanced cytoskeletal dynamics (Massagué, 2008; Shapiro et al., 2011; Trusolino et al., 2010). Because of their pleiotropic effects, promigratory conditioning of the tumor-associated tissue increases: (1) the invasion and dissemination of tumor cells; (2) the motility and activity of stromal cells, including fibroblasts and macrophages; (3) the recruitment and transendothelial migration of circulating leukocytes and precursor cells into the tumor stroma; and (4) the mobilization of bone marrow-derived cells into the circulation through systemic growth factor effects in other organs, including the bone marrow (Orimo et al., 2005; Padua and Massagué, 2009; Roussos et al., 2011; Zlotnik et al., 2011).

Thus, multiple overlapping adhesion and signaling networks cooperate toward molecular and structural reorganization of contacted tissues and support tumor cell invasion and metastatic dissemination.

Heterogeneity of Invasion Routes

In vivo, cancer invasion and metastatic dissemination depend upon two interconnected complementary cell escape strategies. The first and simplest strategy is the movement of cells along pre-existing tissue structures in which the available space matches or exceeds the volume of the cell or cell group. The second strategy results from proteolytic breakdown of tissue structures to generate de novo space required for invasion (Wolf et al., 2009; Wolf and Friedl, 2011).

Guidance Structures in Tissues

Recently, two-dimensional (2D) and 3D microscopy have mapped the structural organization of tissues during cell invasion, and intravital microscopy has been used to examine experimental tumors in vivo (Pittet and Weissleder, 2011 [this issue of *Cell*]). These approaches, combined with histopathological analysis of human tumors, strongly suggest that both single-cell and multicellular tumor invasion are guided and supported by pre-existing structures and interfaces present in every tissue (Alexander et al., 2008; Condeelis and Segall, 2003; Grytsenko et al., 2011; Schedin and Keely, 2011) (Figure 4). Conceptually, tissue structures that guide invasion can be categorized as "2D" and "3D" depending on whether cells adhere to a substrate on one or several sides. 2D surfaces, with even or irregular conformation, form nearly barrier-free track-like gaps and trails that typically contain interstitial fluid and glycosaminoglycans. In vivo, most 2D surfaces are encountered in a 3D context, such as a second opposing surface, or a nearby 3D scaffold; therefore, with the notable exception of adherence to the wall of a larger vessel, cell invasion is, in most cases, constitutively three dimensional.

Inner-body surfaces are always covered with an epithelium or endothelium layer, and thus, interacting cells depend upon cell-cell rather than cell-matrix interactions. 2D cell surfaces include: the peritoneum covering all internal organs; the pleura covering the lungs and thorax wall; the ventricles of the brain; and inner surfaces of larger blood and lymph vessels (Figure 4A). Viewed from a cell mechanics angle, cell surfaces allow for highly effective, almost barrier-free dissemination of tumor cells. This is observed during peritoneal or pleural carcinosis in which tumor and other cells readily spread centimeters, likely by both active migration and migration-independent passive drift (Zecchini et al., 2011).

When viewed at microscopic resolution, connective tissue is not a uniform, homogeneous meshwork of ECM, but rather, it is composed of nonrandom structures, including discontinuities formed by surface-like gaps and tracks. The anatomic function of these gaps and tracks is likely transportation of tissue fluids, tissue elasticity, and mechanical sliding of tissue components relative to each other. 3D tracks with bordering 2D interfaces are formed by larger anatomic structures covered by a basement membrane, including small blood vessels, myofibers, nerve tracks, and adipocytes (Figure 4B). Similar longitudinal tracks are formed by bundled 3D collagen fibers (Figure 4C). These "inner surfaces" likely correspond anatomically to narrow clefts ("shrinkage artifacts") that are abundantly present in virtually every tissue after fixation and, when reconstructed three dimensionally, display a 3D track system (O. Ilina and P.F., unpublished data) along and between fibrillar interstitial tissue structures. In cancer lesions and tumor xenografts monitored by 3D intravital microscopy, these interfaces are often used by invading cells with little sign of structural alteration or degeneration (Alexander et al., 2008; Condeelis and Segall, 2003).

Other tissue-specific guidance structures preferentially used by metastasizing cells are bone cavities, which are covered by a monolayer of lining cells, and the perivascular tracks in brain vessels formed between glial cells and the basement membrane of vascular smooth muscle cells (Figure 4D). Another special case of barrier-free dissemination is the lumen of small vessels, which provide a tube-like track for rapid intravascular dissemination of cancer cells through capillaries in peripheral tissue and liver sinusoids (Tsuji et al., 2006) (Figure 4A).

Lastly, 3D scaffolds composed of randomly organized fibrin and collagen fibrils provide a combination of 1D (the string-like linear fiber) and 3D scaffold with pores of complex geometry

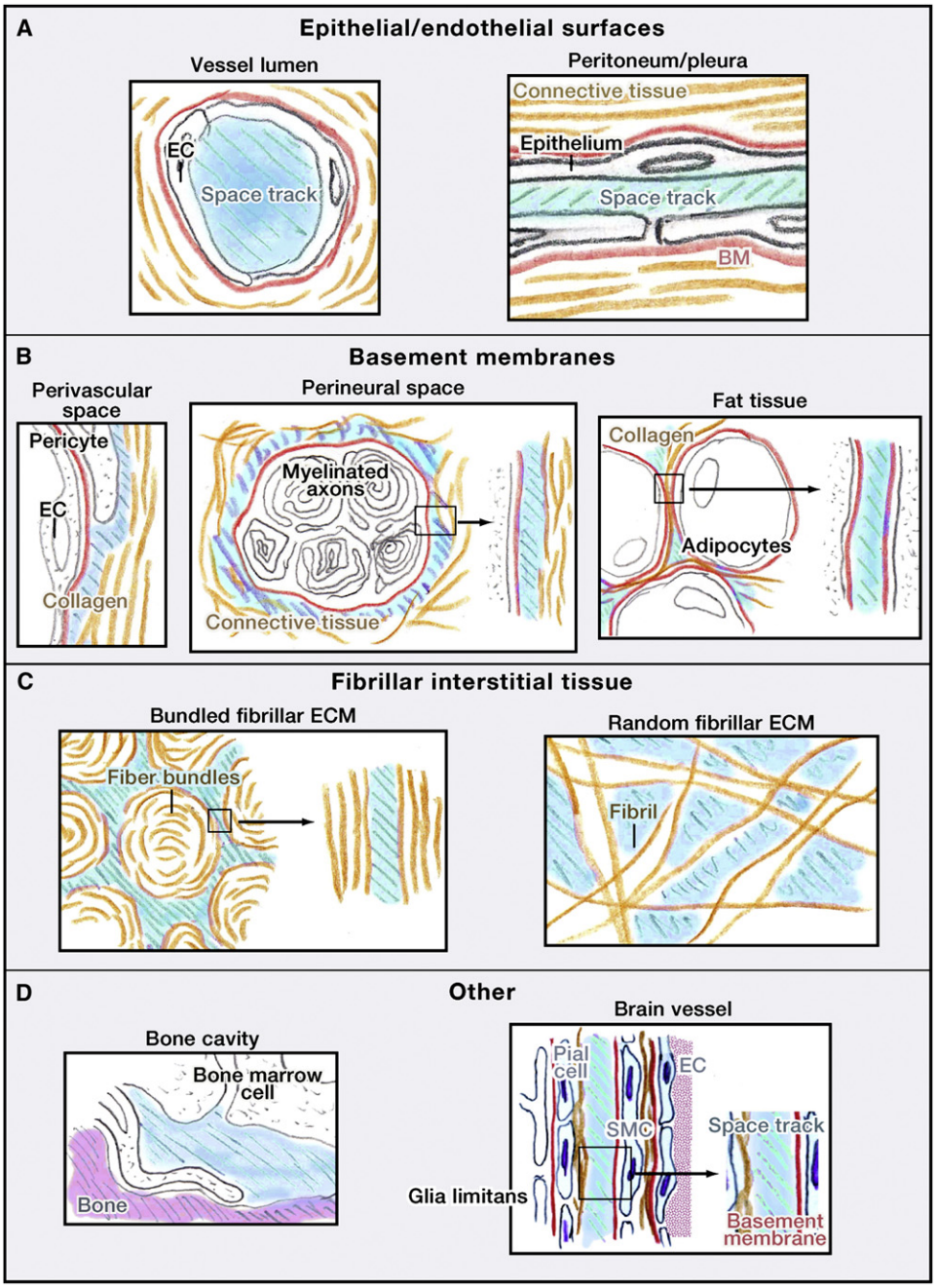

Figure 4. Anatomic Tissue Structures Guiding Cancer Invasion
(A) Epithelial and endothelial surfaces devoid of ECM.
(B) Basement membranes interfacing with the ECM between cells and tissues.
(C) Collagen-rich interstitial scaffolds of compact or loose structure and organization.
(D) Complex interfaces composed of both cell surfaces and ECM scaffolds. Solid multimeric scaffold structures interface with tissue pores and track-like gaps (cyan).

(Doyle et al., 2009; Wolf et al., 2009) (Figure 4C). Such ECM networks are predominant in loose connective tissue, such as the dermis of young mice, provisional tissue such as a fibrin clot after tissue wounding, and as largely ECM-free tracks (formed by astrocytes and neuronal fibers) the white matter of the brain (Grytsenko et al., 2011; Wolf et al., 2009). These networks are similar to in vitro-reconstituted 3D collagen matrices or basement membrane equivalents frequently used for cell invasion research.

Invading cells are thus required to accommodate diverse geometries and molecular ligand systems for adhesion and migration in vivo. Whereas in vitro-reconstituted ECM models,

due to their cell-independent polymerization process, mimic random ECM structures, complex cell and tissue engineering is needed to recapitulate the multicomponent complexity of 2D interface-based track geometries of guiding scaffolds in vivo (Ilina et al., 2011).

Molecular Guidance Cues

Physical space is likely translated into directed cell polarity and cytoskeletal dynamics through receptor-mediated molecular recognition of the adjacent scaffold structure. Invading cancer cells often simultaneously integrate signals from: (1) ECM molecules, including collagens, laminins, fibronectin, and elastin; (2) cell surfaces, including cadherins, CAMs, and proteoglycans; and (3) gradients of promigratory factors, i.e., chemotactic (soluble factors) and haptotactic gradients (ECM-bound factors). Molecular sensing through adhesion and chemotactic receptors directs tumor cell migration to mediate chemotaxis and haptotaxis (i.e., directional moltility along a gradient of cell adhesion sites or substrate-bound chemoattractants) jointly with physical contact guidance, which is often tissue context dependent. Besides the physical scaffold structure, the ECM guidance of invading cells is mediated by covalently and noncovalently associated accessory components deposited by stromal cells.

Guidance by ECM

The molecular and physical characteristics of the ECM strongly contribute to cell adhesion, migration, and cell fate decisions with consequences for cancer cell invasion and dissemination. The quantitatively most abundant and important component of connective tissue is collagen type I, which serves as structural frame for cells and other scaffold proteins (Grytsenko et al., 2011; Wolf et al., 2009). In the activated tumor stroma, the density of collagen fibers is often increased (i.e., desmoplasia), and hyaluronan, proteoglycans, and glycoproteins (e.g., fibrin, fibronectin, and vitronectin) become upregulated. Together these molecules decorate and ''functionalize'' the collagen scaffold. In vitro, mesenchymal fibroblasts and cancer cells migrate along 2D or through 3D collagen using $\alpha2\beta1$, $\alpha1\beta1$, or $\alpha11\beta1$ integrins and $\alpha5\beta1$, $\alpha V\beta3$, and $\alpha V\beta5$ for migration along or through fibrin or fibronectin scaffolds (Even-Ram and Yamada, 2005; Maaser et al., 1999).

Integrins also mediate the migration of normal and cancer cells along structural components of basement membranes by engaging with collagen type IV ($\alpha1\beta1$, $\alpha2\beta1$), laminins ($\alpha3\beta1$, $\alpha6\beta1$), fibrillin ($\alpha5\beta1$, $\alpha V\beta3$, $\alpha V\beta6$), perlecan, and versican ($\beta1$) (Hynes, 2002). Consequently, perivascular invasion of glioma cells and perineural invasion of pancreatic cancer cells are linked to the function of laminin-binding $\beta1$ integrins (Piao et al., 2009; Ryschich et al., 2009), but the mechanisms of other basement membrane-dependent routes, including peri- and intramuscular guidance and adipose tissue invasion, are unknown.

Most ECM proteins undergo enzymatic postprocessing by cell-derived proteases (Figure 3C) or crosslinking proteins. Fibrillar collagens become glycosylated and crosslinked by lysyl oxydases (LOX) and lysyl hydroxylases, which increases collagen stiffness and resistance to assault by pH changes and proteolytic degradation (Levental et al., 2009; Paszek et al., 2005). Normal and neoplastic cells sense differences in physical ECM properties and migrate preferentially toward regions of increased stiffness, termed durotaxis (Lo et al., 2000). Stiff substrate enhances and reinforces the clustering of integrins and the secondary formation of focal adhesions and cytoskeletal linkages through the adaptor proteins p130Cas and vinculin (Grashoff et al., 2010; Sawada et al., 2006). This augments both cell contractility and mesenchymal functions (Levental et al., 2009).

Consequently, the upregulation of peri-tumor collagen production favors cancer cell invasion and metastasis in breast and other cancer models (Goetz et al., 2011; Levental et al., 2009; Paszek et al., 2005). Besides modulating cell invasion, the physical tissue properties determine the fate of normal cells with consequences for cell growth and differentiation (Discher et al., 2005). As a central downstream signaling pathway that connects mechanotransduction to gene expression, cell proliferation in response to substrate stiffness is regulated by Yap1, a transcription factor downstream of the hippo pathway (Dupont et al., 2011). Yap1 engagement supports epithelial stem cell growth and hyperproliferation, which is counteracted by signaling through α-catenin (Schlegelmilch et al., 2011). Thus, Yap1 represents an important mechanosensitive candidate effector for neoplastic progression in cells with deregulated cadherin/catenin axis.

Guidance along Cell Membranes

Besides cell-ECM interactions mediating cell migration, an understudied but emerging mechanism is the guidance by cell-cell junctions. Besides the epithelium covering inner-body cavities and endothelium forming the lumen of blood and lymph vessels, intermittent and likely discontinuous cell scaffolds are abundant in most tissues, including fibroblast networks, macrophages, and epithelial structures (Figure 4). In developing zebrafish, primordial amoeboid germ cells migrating individually through a cell-rich tissue scaffold employ E-cadherin and Rho-mediated actomyosin contraction for migration (Kardash et al., 2011). The small group of germ cells of the developing *Drosophila* ovary, called border cells, provides an example of collective migration mediated by E-cadherin. These cells are connected by E-cadherin, guided by EGF, and depend upon E-cadherin for migration (Geisbrecht and Montell, 2002). Cell-cell junctions thus represent an alternative mechanotransduction mechanism for migration, when cell-matrix adhesions are downregulated or absent. However, though effective in morphogenesis, the role of cadherin-based cell-cell interactions in tumor cell invasion is still unclear.

Secreted Guidance Molecules

Many chemokines and growth factors contain one or several ECM-binding domains, which immobilize the factors in tissues, thereby forming a stable promigratory scaffold. Chemokines and growth factors contain binding sites to heparan sulfate side chains (Lortat-Jacob et al., 2002) that are present in interstitial and cell surface proteoglycans and heparin (Hynes, 2009).

After functionalization, a scaffold contains both adhesion sites (for integrins and other receptors) and immobilized migration-inducing signal (via CCR or GFR) on the same geometric structure, such as a fibril or basement membrane, to support cell protrusions and adhesion in close vicinity along the same substrate. In 3D invasion models of branching morphogenesis, which support collective sprouting of epithelial ducts of the mammary or salivary gland, immobilization of FGF10 to heparan

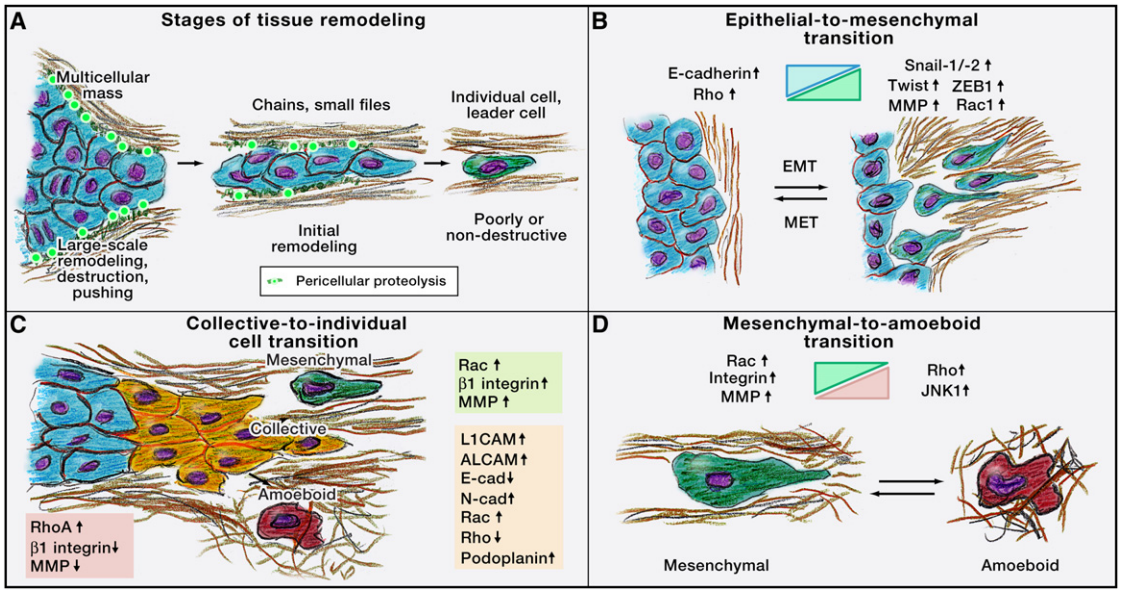

Figure 5. Plasticity of Cell-Matrix Interaction, Invasion, and Tissue Remodeling

(A) Migrating cells transition from an initial nondestructive dissemination to migration that involves small- and large-scale tissue remodeling. The pre-existing space available to invading cells governs the caliber of individual and multicellular invasion and becomes iteratively widened by pericellular proteolysis.

(B) Epithelial-to-mesenchymal transition of a stable epithelium after downregulation of cell-cell junctions and facilitated single-cell detachment.

(C and D) Invasion programs display plasticity, or adaptability, including transition from collective cell migration to individual cell migration (C) and mesenchymal-to-amoeboid transition (D). Key regulators of these transitions altered in expression or function are indicated.

sulfate maximizes duct elongation and growth by engaging FGFR2 signaling; in contrast, a diffusion-only variant of FGF10 lacks the proinvasive function and supports only growth (Makarenkova et al., 2009). Likewise, the chemokine CXCL12/SDF-1 and EGF interact with interstitial heparan sulfate and form stable gradients that guide migrating tumor cells and passenger leukocytes (Allinen et al., 2004; Netelenbos et al., 2002; Wyckoff et al., 2004).

TGFβ is a master inducer of mesenchymal invasion and stem cell functions in cancer cells. It is immobilized to the ECM via fibronectin and fibrillin by latent TGFβ-binding proteins and becomes released through limited proteolysis mediated by MMPs or furin that are activated and released by activated stromal cells (Mu et al., 2002). Alternatively, integrins bind to latent TGFβ-binding protein complexes and pull to induce a conformational change that is required to make ECM-bound TGFβ accessible for its receptors (Wipff et al., 2007). Osteopontin is a secreted cytokine-like proteoglycan that binds to CD44 and integrins. Osteopontin is upregulated in many tumor types and strongly supports invasive cell guidance (Bellahcène et al., 2008).

In addition to these examples, gene expression profiling and proteomics have revealed abundant sets of soluble factors and ECM proteins upregulated in the microenvironment of tumors, indicative of complex signaling pathways induced in both tumor and stromal cells (Allinen et al., 2004; Wang et al., 2004). Besides their local peri-tumor function, many deposited factors diffuse into blood and lymphatic fluid, which unfolds systemic, hormone-like functions. CXCL12/SDF-1 and osteopontin are important examples of these soluble factors. Tumor-derived CXCL12/SDF-1 mobilizes bone marrow stem cells that become recruited into the tumor and contribute to the formation of tumor blood vessels (Orimo et al., 2005), and osteopontin activates bone marrow-derived cells that integrate into the tumor and accelerate tumor outgrowth (McAllister et al., 2008).

Thus, multiple and partially overlapping mechanisms contribute to the mechanical and molecular guidance of tumor cells, but their crosstalk and hierarchy still remain unknown.

Plasticity of Invasion and Metastasis Programs

Together, the different modes of cancer cell invasion, the receptors and cytoskeletal regulators available for cell-cell and cell-matrix adhesion, the divergent degree of ECM remodeling capability, and the range of invasion-guiding molecular and physical tissue environments provide a multiscale framework of combinatorial possibilities or states that allow cancer invasion to be a plastic and adaptive process (Friedl, 2004; Friedl and Wolf, 2010). Consequently, with altered tissue composition and conditioning by released factors, tumor cells undergo changes in signaling and function that lead to secondary effects in the invaded tissue and, in turn, the tumor cells themselves.

Plasticity of Tissue Structures

Interstitial cancer cell invasion occurs in different phases that can be labeled operationally as an initial, nondestructive guidance phase, followed by a phase of tissue remodeling. In a stepwise manner, invasive migration leads to the production of pores, tunnels, and lagunae, which guide and can be populated by mobile tumor cells (Alexander et al., 2008; Condeelis and Segall, 2003) (S.A., unpublished data) (Figure 5A). With upregulated MMPs, most notably MT1-MMP/MMP14, pericellular proteolysis executed by tumor cells themselves or fibroblasts

generates micro- and macrotracks bordered by condensed ordered collagen bundles, which strongly support both single-cell and collective invasion (Friedl et al., 1997; Gaggioli et al., 2007; Goetz et al., 2011; Wolf et al., 2007) (Figure 5A). These de novo tracks guide tumor cells and, with pressure exerted by the invading cells, become gradually widened until the tissue space consumed by invading cell masses matches the regression of the ECM (Gaggioli et al., 2007; Ilina et al., 2011; Wolf and Friedl, 2011). Such trails, often filled by tumor cells, are abundant in most interstitial collagen-rich tissues, including desmoplastic stroma (Levental et al., 2009; Paszek et al., 2005). Thus, despite its increased absolute collagen density, the signals, gaps, and trails present in desmoplastic stroma enhance cancer invasion and progression rather than acting as barrier.

By direct and indirect mechanisms, desmoplastic tissue remodeling is a strong mediator of neoplastic progression, invasion, and metastasis (Egeblad et al., 2010). As key mediators of desmoplasia, resident fibroblasts and immigrated fibroblast precursor cells receive activation signals through growth factors, including TGFβ, IL-1, and PDGF. They then develop into cancer-associated myofibroblasts (CAF) that deposit, remodel, and contract fibrillar collagen (De Wever et al., 2008; Egeblad et al., 2010). As a physiological process of the connective tissue reorganization, the postpartum involution of the mammary gland leads to the deposition of fibrillar collagen and collagen-induced release of proinflammatory COX-2 (Lyons et al., 2011). This reorganization is sufficient to impose growth, invasion as multicellular strands and metastasis programs in otherwise benign or less aggressive breast tumor lesions (Lyons et al., 2011). A similar progression of breast cancer is triggered by activated fibroblasts that reorganize and condense the breast stroma to aligned, bundled collagen tracks that condition breast cancer invasion and metastasis (Goetz et al., 2011).

In bone metastases, bone resorption is executed by osteoclasts in which RANKL (receptor activator of NF-κB ligand) is activated by a TGF-β– and MMP13-dependent mechanism; this allows the growing tumor to expand into de novo space, which eventually results in local bone destruction (Nannuru et al., 2010; Nannuru and Singh, 2010). In all cases, pre-existing tissue space is first filled by invading cells without apparent degradation, and then, with increasing cell density and the upregulation of MMPs and other proteases, the tissue is degraded and reorganized. As an outcome, cancer invasion leads to secondary loss of tissue integrity and function, including tissue necrosis, ulceration, and vessel rupture. Accordingly, the structures detected by histology represent statically looking snapshots of an otherwise dynamic and plastic process by which the growing and invading tumor replaces and eventually destroys interstitial tissue.

Plasticity of Cell-Cell Junctions: The Epithelial-to-Mesenchymal Transition

A central molecular program enhancing tumor cell invasion in response to environmental triggers is the epithelial-to-mesenchymal transition (EMT). EMT initiates or augments invasive functions by enhancing Rac-dependent mesenchymal migration. It also contributes to cell growth, cell survival, and the reemergence of stem cell characteristics (Thiery et al., 2009) (Figure 5B).

During EMT, upstream signals through growth factors of the tumor stroma, including Wnt, TGFβ, FGF, and EGF, lead to the activation of transcriptional repressors, including ZEB1, Twist, and Snail1 and 2, which directly and indirectly inhibit E-cadherin transcription (Spaderna et al., 2008; Yang et al., 2004). For example, in breast cancer cells undergoing EMT in response to MMP-3 (which cleaves cell surface E-cadherin and thus weakens cell-cell junctions), the onset of migration depends on Rac activation and cell-derived production of reactive oxygen species (ROS), which in turn upregulate Snail (Radisky et al., 2005). With E-cadherin expression diminished, adherens junctions and the signaling thereof are weakened or replaced by less stringent cell-cell adhesions through N-cadherin or L1CAM (Gavert et al., 2007; Yano et al., 2004). This results in the disturbance of apicobasal polarity and cell anchoring to the basement membrane, which, in turn, allows the cells to acquire a mobile mesenchymal phenotype (Thiery et al., 2009). The EMT program further favors a stem cell-like phenotype that invades, disseminates, and is able to establish distant metastases (Mani et al., 2008).

The induction of EMT with downregulation of E-cadherin expression is likely tunable, dependent on whether complete or partial EMT signaling is present. As consequence, EMT can be complete with loss of E-cadherin and the typical EMT signaling and protein expression profile. However, EMT may be partial with different levels of E-cadherin expression retained, and even EMT-like dissemination without EMT-associated gene expression patterns may develop (Christiansen and Rajasekaran, 2006; Gavert et al., 2011; Pàez-Ribes et al., 2009; Wicki et al., 2006). In epithelial cancer lesions, EMT is detected in a few often cohesive cells located at the leading edge, as well as small cohesive groups and individual cells scattered and moving independently without connection to the main tumor (Brabletz et al., 2001; Gavert et al., 2007). Thus, besides representing a program for complete loss of cell-cell junctions, EMT further may contribute to collective cell functions, including collective invasion. This is consistent with the prominent collective invasion of primary mesenchymal tumors and melanoma (Alexander et al., 2008; Hegerfeldt et al., 2002).

EMT is also thought to represent a program transiently controlled by the microenvironment, which locally downregulates epithelial characteristics and facilitates cell escape from the primary tumor. However, with local upstream signaling lost, cells undergo mesenchymal-to-epithelial reversion after metastatic seeding in the secondary organ (Spaderna et al., 2006; Thiery et al., 2009). Thus, EMT-dependent invasion and metastasis programs are strongly responsive to microenvironmental changes and adaptive in their signaling program and associated invasion dynamics.

Plasticity of Cell-Matrix Interactions and Cytoskeletal Dynamics

The executive mechanotransducing mechanisms of cell migration are plastic and allow the rapid adaptation to environmental changes and challenges; these adaptations often result in transitions between different modes of migration (Friedl and Wolf, 2010; Sanz-Moreno and Marshall, 2010). Such plasticity likely originates in response to tissue microregions and responses to therapeutic challenge. The natural regulation of gene expression

and signal states in tumor cells by the microenvironment thus accounts for the often heterogeneous invasion pattern in progressing tumor lesions. In addition, diversity of persisting invasion is caused by rewiring of signaling networks and differential cell survival during tissue damage and therapy (Alexander and Friedl, 2012).

In collectively invading tumors, cell-cell coordination and signaling are mediated by either E-cadherin expressed at levels that do not confound the migration process or alternative cadherins, including N- or VE-cadherin (Yano et al., 2004) (Figure 5C). Mechanisms of collective invasion with expressed E-cadherin in cell-cell junctions include the upregulation of: L1CAM, which strongly promotes migration (Gavert et al., 2011; Shtutman et al., 2006); the guanin nucleotide exchange factor Tiam-1, which activates Rac1 but maintains adherens and tight junctions (Mertens et al., 2005; Walch et al., 2008); or podoplanin, which increases RhoA activity in the presence of E-cadherin-based adhesions (Wicki et al., 2006).

Similar to the EMT program, the transition from collective cell migration to individual cell migration (i.e., the collection-to-individual transition) is triggered by local Rac1 engagement, allowing for ectopic tip cell behavior, substrate engagement, and eventually, cell detachment (which is facilitated by down-regulation of cadherin-based cell-cell adhesion) (Figure 5C). Environmental stimuli can favor single-cell detachment from tumors, partly through EMT and partly in the absence of EMT (Bertout et al., 2008; Pennacchietti et al., 2003). These stimuli include TGFβ, EGF, and other growth factors, but also metabolic stress, such as acidification of the stroma causing a shift in tumor metabolism (i.e., the Warburg effect) and hypoxia with reactive HIF-1α signaling (Bertout et al., 2008; Pennacchietti et al., 2003).

Alternatively, amoeboid dissemination may originate from collective invasion when cell-cell junctions are abandoned and release the cells toward a single-cell migration program of low integrin-mediated adhesion and high Rho-mediated cortical actomyosin contractility (Hegerfeldt et al., 2002; Sanz-Moreno et al., 2008). In breast cancer lesions, EGF secreted predominantly by activated macrophages activates and guides tumor cells that have detached from the epithelial main mass by amoeboid dissemination (Wyckoff et al., 2004). Thus, the type of migration maintained after detachment from the multicellular state depends upon the governance of adhesion strength, cytoskeletal protrusions and contractility, and the competence to remodel the ECM.

Mesenchymal invasion may undergo secondary conversion to amoeboid, rounded migration by diverse mechanisms, such as a decrease in Rac activity and concomitant activation of Rho-mediated actomyosin contractility (Sahai and Marshall, 2003). The therapeutic inhibition of MMPs can also trigger amoeboid migration because pericellular proteolysis by MMPs prompts conversion to nonproteolytic amoeboid cell deformation to bypass narrow ECM barriers (Wolf et al., 2003a) (Figure 5D). Likewise, amoeboid movement in otherwise mesenchymal cells is induced by inhibiting chemokine-meditated Rac activation (Gérard et al., 2007), activating Rho by inhibiting p190RhoGAP (Nimnual et al., 2003), or engaging EphA2 (an indirect Rho activator) (Parri et al., 2009). Lastly, gene mutations that impact integrin availability or the Rac/Rho balance may lead to plasticity of invasion and metastasis. Mutation or loss of p53 leads to enhanced integrin turnover and recycling, which converts cells to the amoeboid dissemination mode and strongly enhances invasion/metastasis (Gadea et al., 2007; Muller et al., 2009).

Thus, in context, with gain or loss of cell-cell junctions and adaptive signaling control through Rac and Rho, cancer invasion programs are plastic and responsive to microenvironmental signals and molecular interference, which secures migration under challenging conditions (Madsen and Sahai, 2010). These basic conversion mechanisms have been established for in vitro conditions using cell lines, and their relevance for tumor lesions in vivo await confirmation by using 3D histopathology combined with intravital imaging (Pittet and Weissleder, 2011). Likewise, how invasion plasticity is connected with or distinct from EMT programs remains to be shown in vitro and in vivo. This will identify EMT-dependent and -independent routes and niches of natural and therapy-induced plasticity of invasion and their contribution to metastatic dissemination (Christiansen and Rajasekaran, 2006).

Plasticity and Reciprocity—A Model

The mechanisms of spatiotemporal plasticity (i.e., to change phenotype and function) and reciprocity (i.e., to do this by processing signals received from the environment) are fundamental to the step-wise changes in both tumor cells and the microenvironment, a process that receives further drift with cells moving from one environment to another. The concept of dynamic reciprocity for cells engaging with and thereby altering the ECM was originally coined by Paul Bornstein for cell-matrix interactions in wound healing (Bornstein et al., 1982) and was further developed by Mina Bissell and coworkers for epithelial morphogenesis (Bissell et al., 1982) and cancer (Nelson et al., 2008; Xu et al., 2009). Accordingly, plasticity and reciprocity account for the morphologic and functional inter- and intralesion heterogeneity driven by complementary mechanisms, including genomic instability as well as epigenetic, signaling, and functional adaption to cope with altering environmental conditions (Figure 6A). Such "fate-changing" events that trigger significant adaptation in tumor cells occur in response to metabolic changes in the microenvironment, including hypoxia and severe metabolic stress (Bertout et al., 2008), as well as chronic growth factor stimulation and inflammation (Allinen et al., 2004; Polyak et al., 2009) (Figure 6B).

A critical common mediator of plasticity and reciprocity is the change of cell position. Cell invasion provides access to different physical and molecular structures, including the local tumor stroma and secondary organs after metastatic colonization, and thus refines signaling input (Figures 6B and 6C). Consequently, cancer invasion and metastasis are both cause and consequence of plasticity and reciprocity. Over time, the changes driving adaptive reprogramming of tumor cells and the reactive tumor stroma thus lead to a kinetic, ever-changing coevolution of the tumor with its environment (Hanahan and Weinberg, 2011; Polyak et al., 2009). Related tissue remodeling processes, such as morphogenesis and wound healing, follow well-defined programs with rate-limiting steps and end points (e.g., for limb or organ formation or closure of a tissue defect). In contrast, cancer is more flexible in time, space,

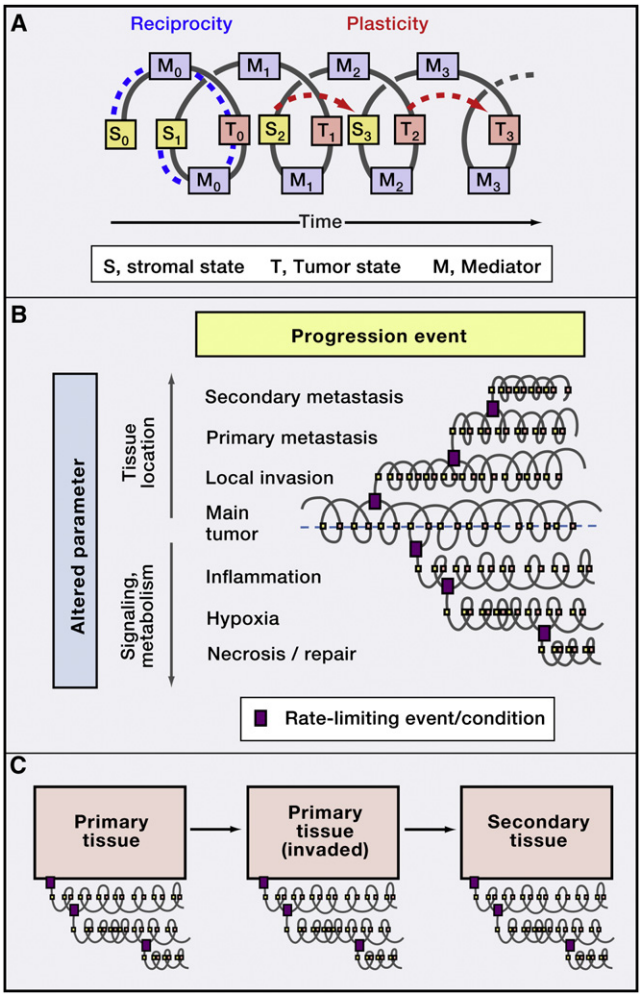

Figure 6. Reciprocity, Plasticity, and Evolution of Tumor Cell Invasion and Metastasis

(A) Reciprocal crosstalk between tumor cells and the stroma (i.e., stromal cells together with ECM and released factors) results in evolutionary plasticity of both tumor cells and the tissue environment. "Reciprocity" results from the bidirectional communication between stromal (S) and tumor (T) compartments, which is transmitted by mediators (M) released by both compartments in a reciprocal manner. Stromal alterations include cell-derived physicochemical changes of the microenvironment, such as deposited ECM components, ECM degradation and remodeling, change of ECM stiffness and porosity, and released cytokines and growth factors. Plasticity of the cell phenotype and function consists of: changes in the activation, migration, and differentiation state of the cell; metabolic switches; and epigenetic alterations that may further prompt secondary genomic instability. Consequently, with each cycle of interactive engagement with the stroma, the cell state diverges from its origin, leading to progression of the tumor or the metastasis (indicated as spiral).

(B) Branching and altered direction of reciprocal plasticity in the course of cancer progression. Direction-changing dichotomy is reached by a change in the position of the tumor cell, which results in a different tissue location and change in environmental input (upper spirals); likewise, step-wise bifurcation of reciprocal evolution may be induced by changes of the local tissue conditions, including altered composition of infiltrate cells during inflammatory and metabolic stress, insufficient perfusion resulting in hypoxia, and tissue repair programs induced by spontaneous or therapy-induced (tumor) necrosis (lower spirals).

(C) Second- and third-order reciprocity. Reciprocal plasticity can evolve during metastatic progression to generate second- and third-order reciprocity. In the course of metastasis, tissue-specific reciprocity and microenvironmental inputs synergize to drive local plasticity of tumor cells and the tumor stroma, resulting in the evolution of tumor subregions with diverse progression and adaptation capabilities. As a consequence, reciprocity and plasticity may impose parallel or divergent evolution of cell clones and populations.

signaling programs, and genetics and thus represents a perpetuating process without a clear end point, illustrated here as open-ended spirals (Figure 6).

Concluding Remarks

Well-defined experimental conditions in vitro have allowed the precise delineation of receptor-ligand interactions and their basic involvement in invasive migration, but their complexity and synergistic availability in vivo make it challenging to identify the dominant and compensation mechanisms that maintain and rescue metastatic dissemination (Friedl and Wolf, 2003; Madsen and Sahai, 2010; Sanz-Moreno and Marshall, 2010). Although the intracellular machinery that generates force via actomyosin is well-defined, the range of molecular and physical adhesion and transmission modes at the cell and tissue level support the adaptation of cell migration. This adaptation is similar to and likely intertwined with the compensation and plasticity of focal adhesion and other signaling networks during cell invasion (Zaidel-Bar et al., 2007). Consequently, because most pathways of adhesion, proteases, and chemokine/growth factors exhibit ample overlap and redundancy, the natural or therapeutically induced loss of one mechanistic pathway may lead to a drift in signaling and mechanotransducing effector networks. This may trigger alternative mechanisms of invasion and dissemination instead of inhibiting function.

Recent studies identified two unexpected examples for alternative migration modes in 3D environments: the interstitial migration of leukocytes independently of integrin (Friedl and Weigelin, 2008; Lämmermann et al., 2008; Lämmermann and Sixt, 2009) and the propulsive cell migration of normal and neoplastic cells by blebs (Fackler and Grosse, 2008; Lorentzen et al., 2011; Poincloux et al., 2011). The contribution of these types of cell movement to metastatic cancer invasion in vivo awaits determination. In addition to active actomyosin-driven migration, other mechanisms of cell transport include passive drift along tissue structures and cell pushing by expansive growth. Both of these alternative mechanisms still have not been formally integrated into the spectrum of cell translocation principles.

The invaded tissue is often regarded as a homogeneous and passive scaffold that the tumor cells modify in a unidirectional manner, and indeed, this notion has been strengthened by most 2D and 3D in vitro models (Friedl et al., 1997; Wolf et al., 2009). The concept of plasticity and reciprocity of cancer invasion, however, describes invasion as a reciprocal process governed by multiple sets of overlapping, redundant, and potentially, ever-changing active and passive mechanisms of molecular mechanotransduction. This adaptability renders cell invasion and metastasis as a robust perpetuating process, the targeting of which—if ever possible—will require understanding the hierarchy of stringent control points. To this end, systems biology and mathematical modeling approaches are required to classify rate-limiting nodes and modifiers of molecular mechanotransduction for each migration mode and tissue-context.

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