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Subject Review

Molecular Cancer Research

Mechanisms of Motility in Metastasizing Cells

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Abstract

Cell migration and invasion are critical parameters in the metastatic dissemination of cancer cells and the formation of metastasis, the major cause of death in cancer patients. Migratory cancer cells undergo dramatic molecular and cellular changes by remodeling their cell-cell and cell-matrix adhesion and their actin cytoskeleton, molecular processes that involve the activity of various signaling networks. Although in the past years, we have substantially expanded our knowledge on the cellular and molecular processes underlying cell migration and invasion in experimental systems, we still lack a clear understanding of how cancer cells disseminate in metastatic cancer patients. Different types of cancer cell migration seem to exist, including single-cell mesenchymal or amoeboid migration and collective cell migration. In most epithelial cancers, loss of the cell-cell adhesion molecule E-cadherin and gain of mesenchymal markers and promigratory signals underlie the conversion of epithelial, differentiated cells to mesenchymal, migratory, and invasive cells, a process referred to as the epithelial-to-mesenchymal transition. Although solitary migrating epithelial cancer cells have mostly undergone epithelial-to-mesenchymal transition (mesenchymal migration), and sometimes even lose their cell-matrix adhesion (amoeboid migration), collective migration of cancer cells in cell sheets, clusters, or streams is also frequently observed. The molecular mechanisms defining the different modes of cancer cell migration remain in most parts to be delineated. *Mol Cancer Res; 8(5); 629–42. ©2010 AACR.*

Despite major efforts in metastasis research, we still lack detailed insights into how cancer cells actually migrate out of primary tumors and invade into neighboring tissue, how they enter (intravasate) into the blood or the lymphatic circulation, how they survive "homelessness" and immune surveillance in the bloodstream, and how they target certain organs to leave (extravasate) the blood circulation and to initiate metastatic outgrowth in specific target organs. Obviously, the migratory and invasive capabilities of a cancer cell present critical parameters in the metastatic cascade. Plenty of molecular pathways define distinct types of migration and invasion in a cancer cell–autonomous manner, including single-cell amoeboid and mesenchymal migration and collective cell migration (1, 2). In many instances, stromal cells, such as blood vessel and lymphatic endothelial cells, cancer-associated fibroblasts, or bone marrow–derived inflammatory cells, act as modulators of cancer cell migration and invasion and as pathfinders in the extracellular matrix (3). Moreover, chemokine gradients within the tumor microenvironment or in the blood and lymphatic system, as well as the establishment of an appropriate "metastatic niche" in future metastatic organs, contribute to the targeted colonization of distant organs (4). In this review, we present various concepts on the signaling pathways and molecular mechanisms underlying the onset of cancer cell migration and invasion during tumor progression and metastasis. The molecular details of actin cytoskeleton remodeling and changes in cell-substrate adhesion during cell migration and invasion have been recently summarized in several excellent reviews (5-11).

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Loss of Cell-Cell Adhesion

To leave the primary tumor and to invade into the surrounding tissue, tumor cells dissolve their cell-cell contacts; adjust their cell-matrix adhesion sites to a more transient, migratory, and invasive mode; and follow a chemoattractive path through the extracellular matrix, often facilitated by secreted proteinases. Similar to developmental processes like gastrulation or neural crest cell migration, differentiated epithelial tumor cells lose their epithelial morphology and migrate to a distant site to form a new structure, in this case secondary tumors. Such temporary and reversible phenomenon is known as epithelial-to-mesenchymal transition (EMT), a process that is currently a major focus in metastasis research (12-14). The actual occurrence of EMT in patients is still debated; however, with more detailed molecular and histopathologic analysis and the discovery of novel markers, there is increasing evidence for EMT in various human cancers (15-17). However, cancer cells can also migrate and invade in the absence of EMT and may use a broad repertoire of cell migration and invasion (18, 19).

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One of the hallmarks of EMT and the concomitant induction of cell migration and invasion is the loss of the epithelial cell-cell adhesion molecule E-cadherin, the major component of epithelial adherens junctions. Concomitant with its loss, expression of the mesenchymal cell-cell adhesion molecule N-cadherin is increased, a process also known as the cadherin switch (20, 21). In fact, bereavement of E-cadherin function is sufficient to induce tumor cell migration and invasion and tumor progression *in vitro* and *in vivo* (22-25). Loss of E-cadherin function occurs during malignant progression in almost all epithelial cancers, serving as a clinical indicator for poor prognosis and metastasis. In many cases, its functional loss is caused by germline and somatic gene mutations, chromosomal aberrations, transcriptional repression, and DNA hypermethylation of the E-cadherin (*cdh1*) gene (22, 26, 27).

A large number of growth factors and their activated signal transduction pathways are known to provoke the loss of E-cadherin function and to induce cancer cell migration and invasion (Fig. 1), including transforming growth factor β (TGFβ; ref. 28), hepatocyte growth factor (HGF; ref. 29), members of the epidermal growth factor (EGF) family (30), insulin-like growth factor (IGF; ref. 31), fibroblast growth factor (FGF; refs. 32, 33), and Notch signaling (34). In addition, hypoxic conditions, frequently existing in a tumor microenvironment, induce cancer cell expression of c-Met, the bona fide receptor of HGF, and CXCR4, the signaling receptor of the chemokine CXCL12 (SDF1), and further stimulate cancer cell migration and dissemination (35, 36).

Among the many molecular alterations, these signaling pathways activate one or several transcriptional repressors of E-cadherin gene expression, such as Snail1 (Snail), Snail2 (Slug), ZEB1 (δEF1), ZEB2 (Sip1), E47, and Twist (ref. 37; Fig. 1). Notably, among the increasing numbers of miRNAs implicated in cancer progression, members of the

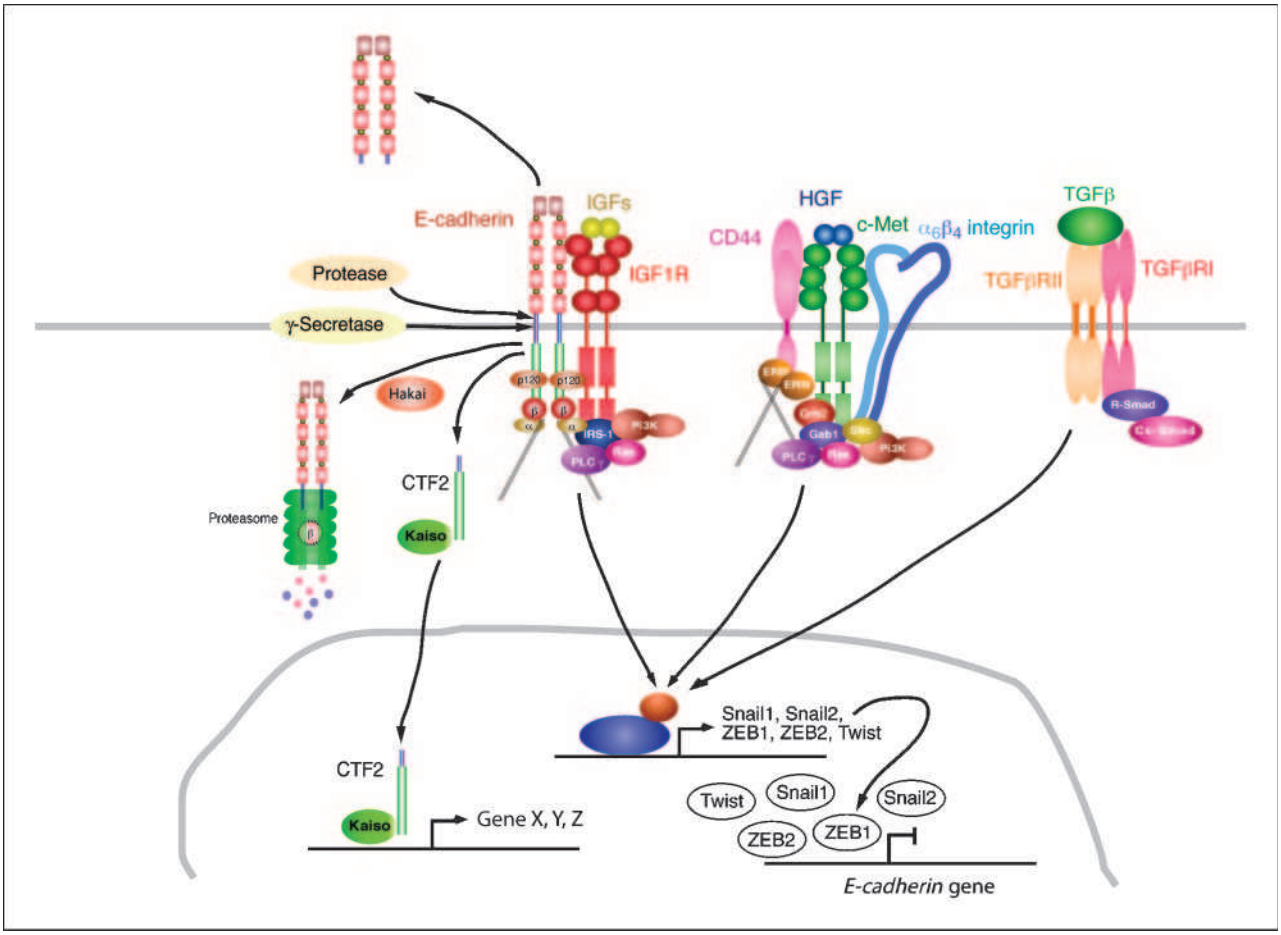

FIGURE 1. Signals upstream of the loss of E-cadherin. Schematic representation of the signaling pathways leading to the upregulation of transcriptional repressors and the repression of E-cadherin gene expression. IGF1 receptor (IGF1R) interacts and phosphorylates E-cadherin and catenins leading to their subsequent internalization, ubiquitylation by the E3 ligase Hakai, and proteasomal degradation. The HGF receptor c-Met assembles a complex consisting of CD44 and $\alpha_4\beta_6$ integrin, which together facilitate signal transduction by c-Met. TGFβ stimulates Smad-mediated signaling by binding and activating its receptors TGFβRI and TGFβRII. All these receptor complexes synergize through downstream effector signaling pathways in inducing the expression of transcriptional repressors, such as Snail1 and Snail2, ZEB1 and ZEB2, and Twist. E-cadherin can also be cleaved by proteases to generate a soluble form of the extracellular domain of E-cadherin. γ-Secretase cleavage of E-cadherin results in the formation of a COOH-terminal fragment (CTF2) that translocates to the nucleus and modulates Kaiso-mediated transcriptional repression (see text for details).

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miR-200 family have been specifically shown to play an important role in TGFβ-induced EMT by regulating the production of ZEB1 and ZEB2 (38-42). Binding of the transcriptional repressors to the E-cadherin gene promoter eventually leads to epigenetic silencing of the gene in a multistage process (43). First, Snail1 recruits a histone deacetylase to the E-cadherin promoter complex, thereby inducing histone deacetylation. Subsequently, polycomb repressor complex 2 (PRC2) is drafted to modify histones by methylation. Snail1 also induces expression of ZEB1, which in turn engages a second, PRC2-independent repressor complex that further inhibits E-cadherin expression. In addition, new interaction partners of Snail1 have been identified, such as the LIM-domain protein Ajuba, which recruits arginine methyltransferase 5 (PRMT5) to support Snail1-mediated transcriptional repression (44). Yet, how the initial silencing of the E-cadherin gene promoter converts into a long-term repression by DNA hypermethylation remains to be resolved (45). During TGFβ-induced EMT of mammary gland cells, a decrease in active histone modifications (H3K9Ac and H3K4me3) and an increase in the repressive histone modification H3K27me3 has been observed concomitant with an increase in DNA methylation of the E-cadherin and the α_4 integrin promoters (46). Notably, TGFβ withdrawal promotes a reversion of EMT and triggers the reexpression of E-cadherin and α_4-integrin in the absence of a loss of promoter DNA hypermethylation, whereas both H3K9Ac and H3K4me3 modifications are restored and H3K27me3 is reduced, suggesting a dominance of histone modification over DNA methylation in epigenetic control of the E-cadherin and α_4-integrin genes. Finally, E-cadherin expression is also directly repressed by miRNA-9, which is found upregulated in breast cancer cells (47).

E-cadherin function can also be impaired by preventing its transport to the plasma membrane through O-glycolysation (48) or by proteolytic cleavage or endocytosis from the plasma membrane (49-52). Proteolytic cleavage of E-cadherin can generate E-cadherin fragments that exert signaling functions. For example, the shedded extracellular domain of E-cadherin has been shown to interfere with epithelial cell-cell adhesion and to induce cell migration (53). Moreover, γ-secretase–mediated cleavage produces a COOH-terminal, cytoplasmic fragment (CTF2) of E-cadherin that is imported into the nucleus, where it modulates the interaction between p120-catenin and Kaiso, a transcriptional repressor, and thus impinges on gene expression (ref. 54; Fig. 1). E-cadherin–mediated cell adhesion complexes can also be disassembled through phosphorylation of E-cadherin or β-catenin by receptor tyrosine kinases or by the non–receptor tyrosine kinase Src, resulting in E-cadherin endocytosis, ubiquitylation by the E3 ubiquitin ligase Hakai, and subsequent proteasomal degradation (refs. 55, 56; Fig. 1B). Moreover, focal adhesion kinase activated by integrin-mediated cell-matrix adhesion may also phosphorylate β-catenin and thus induce its ubiquitylation and degradation and the disassembly of the E-cadherin cell adhesion complex (57).

E-cadherin can be endocytosed through clathrin or caveolin-dependent mechanisms (58-60). For example, EGFR-stimulated invasion of epithelial cells relies on endocytosis of E-cadherin, which involves the activities of the GTPase Arf6 and the Arf6 guanine nucleotide exchange factor (GEF) GEP100 (61). On EGFR activation, GEP100 binds to specific phosphotyrosine residues of EGFR and then activates Arf6 localized in cell-cell adhesions, which in turn induces E-cadherin endocytosis. Notably, the activated GEP100/Arf6 complex is also critical in the regulation of cell-matrix adhesion and cell migration by stimulating the recycling of β_1-integrin (62). Conversely, the Arf6-specific GTPase-activating protein (GAP) Smap1 has been shown to counteract E-cadherin endocytosis (63).

Loss of E-cadherin function leads to a disruption of adherens and tight junctions and the loss of cell polarity, and also liberates proteins from the cytoplasmic cell adhesion complex. Besides its critical role in assembling the E-cadherin–mediated cell adhesion complex, β-catenin (and also γ-catenin) plays an important role in canonical Wnt signaling. Upon loss of E-cadherin function, liberated β-catenin is rapidly phosphorylated by glycogen-synthase kinase 3β (GSK3β) in the adenomatous polyposis coli (APC)/axin/GSK3β complex and subsequently degraded by the ubiquitin-proteasome pathway (ref. 64; Fig. 2A). If the tumor suppressor APC is nonfunctional, as is the case in many cancer cells, or if GSK3β is repressed by an activated Wnt signaling pathway, β-catenin accumulates at high levels in the cytoplasm. Subsequently, it translocates to the nucleus, where it binds to members of the Tcf family of transcription factors and modulates the expression of target genes implicated in cell proliferation, transformation, and tumor progression, such as c-Myc, cyclin D1 and D2, fibronectin, matrix metalloproteinase 7 (MMP-7), Id2, CD44, axin-2, Tcf-1, the cell adhesion molecule L1-CAM, the metastasis gene S100A4, and others (65-67). Another recently discovered target of β-catenin/Tcf signaling is fascin, an actin-bundling protein that is essential for filopodia formation and cancer cell invasion (68). In fact, fascin and L1-CAM are expressed in cells of the invading front of colorectal cancers that also exhibit nuclear β-catenin (69, 70). However, such activation of β-catenin/Wnt signaling by the loss of E-cadherin function seems to depend on cell context and the mutational status of the components of the Wnt signaling pathway, and thus is found active in some but not all cancer types (25, 47, 71).

The juxtamembrane domain of E-cadherin binds to p120-catenin, which is important for the correct membrane localization of E-cadherin (72-75). Similar to β-catenin, upon loss of E-cadherin function, p120-catenin is also liberated from the cytoplasmic cell adhesion complex and accumulates in the cytoplasm (Fig. 2B). There, p120-catenin represses the activity of RhoA and activates Rac and Cdc42 (76-78). All three GTPases are key regulators of actin assembly and play an essential role in the stability of cell-cell adhesion and the induction of cell migration by enforcing actin stress fibers (RhoA) and the formation of migratory membrane protrusions, such as

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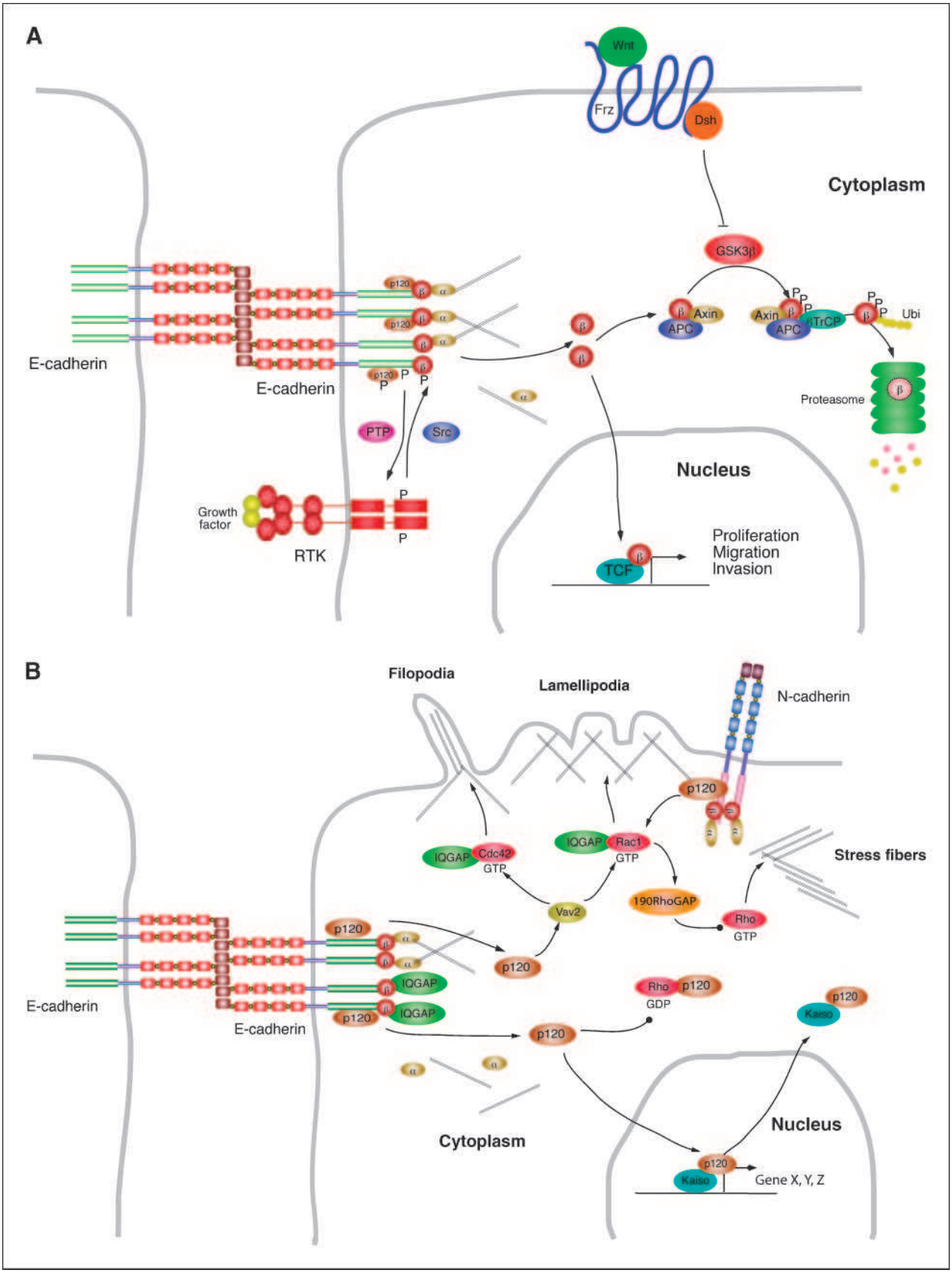

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lamellipodia and filopodia (Rac and Cdc42, respectively; Fig. 2B, see below). p120-catenin also interacts with cortactin and interferes with Arp2/3-dependent cortical actin and stress fiber polymerization (79).

On the other hand, liberated p120-catenin can traffic to the nucleus where it binds the transcriptional repressor Kaiso. In contrast to β-catenin/Tcf–mediated transcription, where β-catenin acts as a transactivator, p120-catenin has no transactivation domain and rather releases Kaiso from its promoter binding sites and thus activates gene expression by derepression (ref. 80; Fig. 2B). Kaiso seems to bind to Tcf/β-catenin target genes and to hypermethylated tumor suppressor genes; however, p120/Kaiso target genes are still poorly defined (81).

The Cadherin Switch and Cell Migration

In most epithelial cancers, the loss of E-cadherin function during tumor progression results in an increased expression of the mesenchymal cadherin, N-cadherin (and sometimes other mesenchymal cadherins), with a drastic change in the adhesive properties of cancer cells, as they lose their affinity for epithelial neighbors and gain affinity for stromal cells. The cadherin switch by itself seems to provoke cell migration and invasion (82, 83) and correlates with poor prognosis (83-85). During the cadherin switch upon loss of E-cadherin function, the transcriptional repressor Twist seems to be critical for the induction of N-cadherin (and also fibronectin) gene expression (86, 87).

Similar to E-cadherin, N-cadherin also exerts both cell-cell adhesion and signaling functions that seem to be mechanistically linked. For example, traction forces generated by retrograde actin flow during neurite extension directly modulate N-cadherin–mediated cell adhesion (88). The mechanical engagement of N-cadherin induces local actin polymerization and ensures the integrity of the cell adhesion complex. On the other hand, N-cadherin interacts with various growth factor receptors and modulates signal transduction (Fig. 3A). For example, by binding both β-catenin in the N-cadherin/β-catenin cytoplasmic cell adhesion complex and platelet-derived growth factor receptor (PDGFR), Na^+/H^+ exchanger regulatory factor-2 has been shown to physically link N-cadherin to PDGFR (89). PDGFR activation, in return, is known to induce actin reorganization, cell proliferation, differentiation, and migration (90, 91). Interestingly, PDGF stimulation of NIH3T3 cells leads to the colocalization of N-cadherin, p120-catenin, and p190RhoGAP in dorsal circular ruffles, structures known to depend on growth factor–mediated Rac activation and RhoA inhibition (92-94). Apparently, p120-catenin together with p190RhoGAP coordinates the antagonistic functions of Rac and RhoA, which plays a critical role in defining the structure of the actin cytoskeleton (refs. 76, 95; Fig. 2B). The active form of RhoA stimulates focal adhesion formation and contractility through assembly of predominantly radially oriented actin stress fibers, whereas Rac activation induces cell spreading, cell migration, and membrane ruffling through actin polymerization at the cell periphery. Moreover, Rac activation inhibits Rho activity, which can also be achieved by p120-catenin overexpression (96).

N-cadherin also interacts with fibroblast growth factor receptors (FGFR; Fig. 3A). The interaction between N-cadherin and FGFR prevents the internalization of FGFR upon FGF binding and results in a sustained mitogen-activated protein kinase (MAPK) pathway activation together with increased cell motility, MMP secretion, and invasiveness (97-99). A similar stimulation of FGFR is also observed with neural cell adhesion molecule (NCAM), an immunoglobulin domain cell adhesion molecule (100). NCAM achieves a similar stimulation of cell migration and invasion by directly binding and activating FGFR through its fibronectin type III domains (ref. 100; Fig. 3B). NCAM-mediated activation of FGFR signaling differs substantially from FGF-induced FGFR signal transduction: NCAM maintains high FGFR signaling by preventing receptor endocytosis and provokes increased cell-substrate adhesion, cell migration, and invasion, whereas FGF predominantly induces a short-lived FGFR signal and mainly promotes cell proliferation (101, 102). Notably, NCAM is one of the first genes upregulated during EMT in a number of cancer types, and, in these cancer cells, it is required for cell adhesion, migration, and invasion (ref. 24; Fig. 3B).

Proteolytic processing of N-cadherin also generates shedded extracellular domain fragments and intracellular fragments with potential signaling functions (Fig. 3A). In neurons, bone morphogenic protein-4 provokes cleavage of N-cadherin by ADAM10 and by PS1/γ secretase to produce a cytoplasmic fragment of N-cadherin, N-Cad/CTF2 (refs. 103, 104; Fig. 3A). N-Cad/CTF2 is able to bind the transcription factor cAMP-responsive element binding

FIGURE 2. Signals downstream of loss of E-cadherin. Schematic view of the signaling pathways and molecular processes mediating cell migration and invasion upon loss of E-cadherin function. A, following the loss of E-cadherin function, for example, by RTK-mediated signaling and phosphorylation of E-cadherin or catenins, catenins are released and may accumulate in the cytoplasm. Cytoplasmic β-catenin is sequestered by the APC/axin/GSK3β complex, phosphorylated by GSK3β, ubiquitylated by the E3 ligase βTrCP, and thereby earmarked for rapid proteosomal degradation. However, upon activation of the Wnt signaling pathway, GSK3β is repressed and β-catenin is no longer phosphorylated. It translocates to the nucleus, where, together with Tcf-family transcription factors, it modulates expression of a number of target genes known to be involved in cell proliferation and tumor progression. B, displacement of p120 catenin, as well as the activity of IQGAP1, results in the modulation of the activity of Rho-family GTPases. Upon loss of E-cadherin function, cytoplasmic p120-catenin acts as a GDI and represses RhoA and thus stress fiber formation, whereas it activates Rac1 and Cdc42 through the GEF Vav2 and thereby induces the formation of lamellipodia and filopodia, respectively. Rac1 and Cdc42, in turn, repress RhoA activity through the GAP 190RhoGAP and inhibit IQGAP1 by binding to it. Together, these activities induce reorganization of the actin cytoskeleton and the migratory behavior of tumor cells (see text for details).

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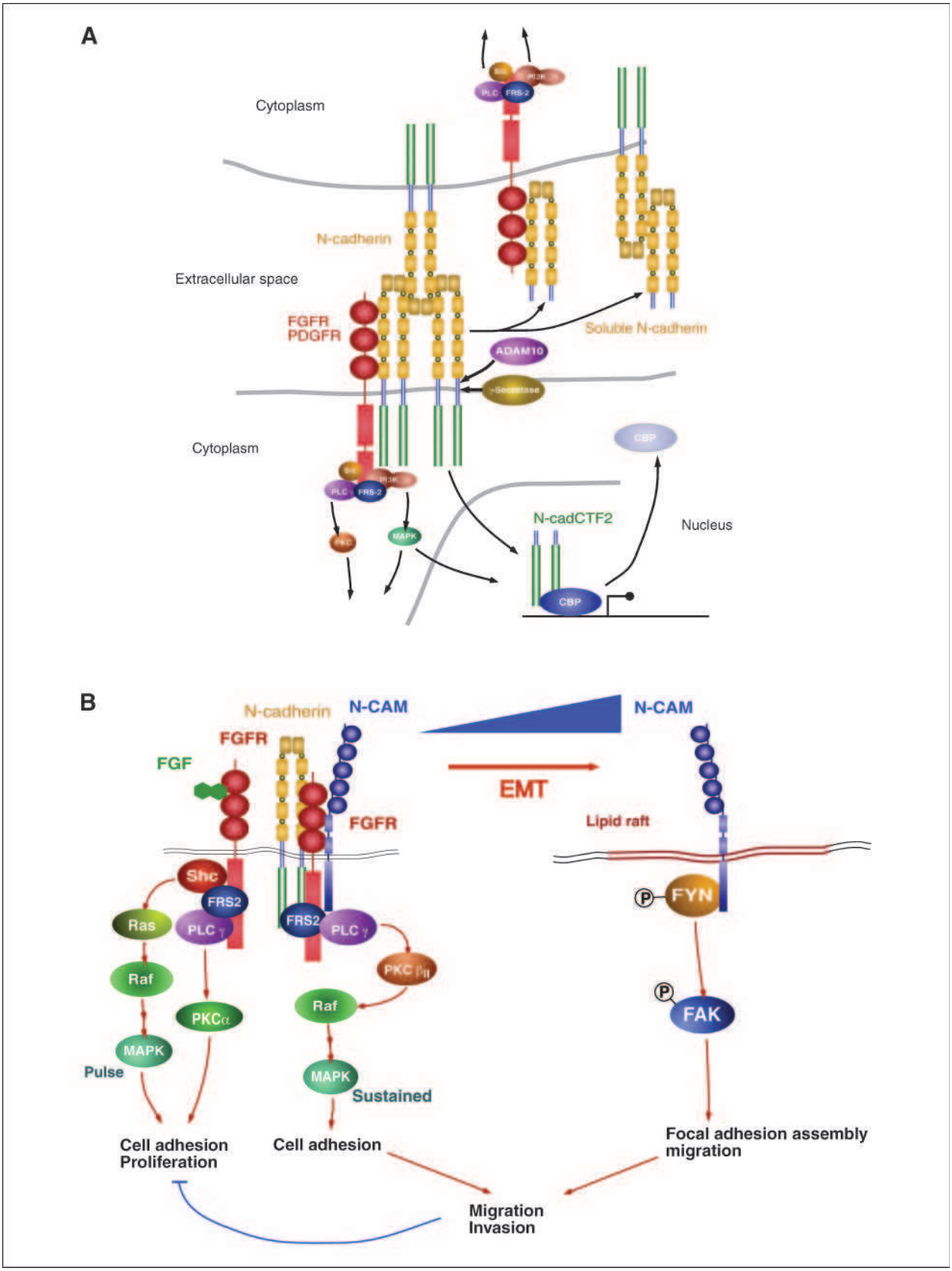

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protein (CREB)-binding protein (CBP) and to induce its proteasomal degradation. The subsequent repression of CPB/CREB–mediated transcriptional control stifles expression of genes important for proliferation and differentiation, such as c-Fos. N-Cad/CTF2 has also been shown to promote migration of neural crest cells by increasing the expression of β-catenin and its target genes (105). However, the actual contribution of N-cadherin processing to tumor invasion and metastasis still needs to be determined.

Single-Cell Migration

Cell migration can be classified into single-cell migration (amoeboid or mesenchymal, solitary, or in indian files) or collective cell migration (in cell sheets, strands, tubes, or clusters; refs. 1, 106). Differences in extracellular protease activities, integrin-mediated cell-matrix adhesion, cadherin-mediated cell-cell adhesion, cell polarity, and cytoskeletal arrangement define the type of cell migration and invasion. Finally, stromal cells may contribute to the different types of migration, such as stromal fibroblast leader cells in collective cell migration and macrophage pioneer cells in single-cell migration. In morphogenic processes during embryonic development, single-cell migration or indian file–like invasion of cells in most cases involves the loss of epithelial polarity and the gain of mesenchymal morphology during EMT. Examples are neural crest cells leaving the neural tube or migrating limb muscle precursor cells.

In cancer, the various types of cell migration can be found with different degrees and combinations. For example, colorectal cancer cells that have lost E-cadherin expression disseminate as solitary, actively migrating cells, and, once arrived at their destination organ, redifferentiate to form secondary tumors with a phenotype comparable with the primary tumor. In contrast, squamous cell carcinomas invade predominantly in a collective type of cell migration (see below). Cancer cells in most parts use morphogenic developmental programs to control their migratory and invasive capabilities. These processes involve a dramatic reorganization of the actin cytoskeleton and the concomitant formation of membrane protrusions required for cell motility in a complex three-dimensional environment, including lamellipodia, filopodia, podosomes, and invadopodia (5, 6, 8, 107).

Members of the Rho GTPase family play a pivotal role in transmitting signals from growth factor and cell adhesion receptors to effector proteins of actin cytoskeleton remodeling (10, 108-110). RhoGTPases are activated upon GTP binding and inactive in their GDP-bound form. RhoGTPase activation is tightly controlled by GEFs, GAPs, and guanine nucelotide dissociation inhibitors (GDI). GEFs activate RhoGTPases by promoting the exchange of Rho-bound GDP by GTP. This is counteracted by GAPs that raise the intrinsic GTPase activity of RhoGTPases and the hydrolysis of bound GTP to GDP. Finally, GDIs bind inactive Rho-GDP and prevent their interaction with RhoGEFs, and thus, activation.

RhoA, Rac1, and Cdc42 are the best studied members of the RhoGTPase family, and their critical role in cell migration and invasion has been repeatedly shown (10, 109-111). In the GTP-bound form, RhoGTPases activate downstream effector proteins. These include for Rac1 the serine/threonine kinases p21-activated kinases (PAK1-3) and myosin light chain kinase; for Cdc42, PAK1-6, WASP, N-WASP, and mDia2; and for RhoA, the ROCK kinases I and II, Citron, and mDia1 and mDia2. It should be noted that RhoGTPases are critical for many biological processes in a cell's life, not only for cell migration and invasion but also for cell proliferation and survival (110, 112, 113). With regard to cell migration and invasion, RhoA induces actin stress fiber formation and regulates cytoskeletal configurations affecting cell-cell or cell-matrix adhesion. Conversely, Rac1 is involved in lamellipodia and membrane ruffle formation, and Cdc42 excites filopodia formation (114, 115). Based on their central function in actin remodeling and their ability to activate MMP, RhoGTPases play an important role in tumor cell invasion and metastasis (116). Novel imaging technologies to visualize the homeostasis of adherens junctions and the activities of RhoGTPases in live cells in a temporal and spatial manner have provided exciting new insights into the tight control of actin cytoskeleton assembly and disassembly during cell migration (117, 118). For example, experiments using fluorescence resonance energy transfer probes for RhoA activity in live cells have revealed that RhoA activity is found not only at the retracting tail of a migrating cell, as has been previously assumed, but also at the leading edge (93).

FIGURE 3. The contribution of N-cadherin and NCAM to cancer cell migration. Schematic depiction of how N-cadherin and NCAM, both cell adhesion molecules upregulated during malignant tumor progression, mediate proinvasive signaling. A, N-cadherin has several functions that may all contribute to tumor invasion and metastasis: First, cell-cell adhesion to N-cadherin–expressing cells of the stroma. Second, binding and activation of FGFR, which results in FGFR signaling and the promotion of cell survival, migration, and invasion. Third, cleavage and shedding of the extracellular domain of N-cadherin by ADAM metallopeptidase domain 10 (ADAM10). Shedded N-cadherin may neutralize N-cadherin–mediated cell-cell adhesion and/or stimulate FGFR signaling on neighboring cells. Fourth, cleavage of N-cadherin by γ-secretase results in the translocation of the COOH-terminal fragment of N-cadherin (CTF2) to the nucleus, where it binds CBP and induces its degradation, thus modulating CBP-mediated gene expression. B, NCAM expression is upregulated during EMT and associates with FGFRs outside of lipid rafts and with c-Fyn in lipid rafts to mediate sustained MAPK signaling resulting in focal adhesion assembly and cell migration. Low levels of NCAM form a complex with FGFR and PLCγ, leading to the activation of the Raf-kinase PKCβII and thus to sustained activation of the MAPK pathway and cell adhesion. Upon loss of E-cadherin function during EMT, NCAM is highly expressed, and a subset localizes to lipid rafts where it associates with $p59^{Fyn}$, leading to focal adhesion kinase (FAK) phosphorylation, focal adhesion assembly, and cell migration. Both NCAM-mediated signaling pathways are required for cell migration and invasion. In contrast, FGF-induced stimulation of FGFR results in PLCγ-mediated activation of PKCα and a short pulse activation of the Ras/Raf/MAPK pathway, resulting in cell adhesion and proliferation. FGF-induced signaling is overruled by NCAM-mediated signaling (adapted from ref. 24).

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The tight regulation of actin cytoskeleton remodeling is not only critical for cell motility but also for other cellular processes, such as endocytosis and intracellular trafficking, and it seems meaningful that a direct link between endocytotic pathways, actin assembly, and cancer cell invasion has been established (119). Receptor tyrosine kinase–mediated motogenic activation of Rac occurs at the endosome where the RacGEF Tiam1 is engaged. Rab5-dependent endocytosis is then required to localize Rac to the plasma membrane and to induce the formation of migratory actin protrusions. Such stepwise activation ensures a spatial regulation of Rac activity by the endosomal-recycling pathway and by the RacGEF Tiam1. Tight control of endocytosis in migrating cells is also required for the rapid recycling of β_1-integrin at pseudopodal membrane protrusions and the retention of a pool of β_1-integrin at the cell front by the GTPase Rab25 (120). Interestingly, mutant p53, as frequently found in malignant cancers, promotes the trafficking of β_1-integrin and of EGFR, resulting into increased β_1-integrin/EGFR signaling and cell invasion and metastasis (121).

As mentioned above, depending on the presence of epithelial or mesenchymal cadherins, the localization and function of p120-catenin and thus the activity of RhoGTPases change dramatically (122). In epithelial cells, p120-catenin localizes at the cell membrane and associates with E-cadherin where it controls the activity of RhoA and Rac1. RhoA activity, which is required for the initial cell-cell contact formation, is downregulated in established, mature cell adhesions. Both activation and inactivation of RhoA require the p120-catenin–dependent recruitment of RhoGEFs, like Vav2, or RhoGAPs, like p190-RhoGAP. The recruitment of p190-RhoGAP results in the activation of Rac1, which leads to the stabilization of E-cadherin junctions by inhibiting the activities of IQ-domain GAP1 (IQGAP1), a Rac1 effector protein, and a mediator of E-cadherin endocytosis (see also below). Moreover, the actin cytoskeleton underlying cell contacts is reorganized and stabilized (123).

Upon loss of E-cadherin in migrating cancer cells, p120-catenin binds to mesenchymal cadherins at the cell membrane, but is also found localized in the cytoplasm (Fig. 2B). Cytoplasmic p120-catenin functions as a RhoA-GDI that binds and represses RhoA activity (124). Simultaneously, p120-catenin bound to mesenchymal cadherins at the cell membrane promotes Rac1 activity and induces the formation of lamellipodia. Thus, both cytoplasmic and membrane-sequestered p120-catenin cooperate to induce cell motility during EMT. Interestingly, Rac1 inhibits RhoA activity by inducing the production of reactive oxygen species, which in turn activate p190RhoGAP by inhibiting low molecular weight protein tyrosine phosphatase (92, 125). Moreover, expression of Snail1 is increased upon reactive oxygen species production by Rac1b, a splice variant of Rac (126). The importance of RhoGTPases in tumor cell migration and malignant tumor progression is also underscored by the observation that Rac1b is highly expressed in malignant breast tissues and that cytoplasmic p120-catenin together with RhoA downregulation correlates with poor prognosis in colorectal cancer (124).

The RacGEF Tiam1 also exerts a critical function in both E-cadherin–mediated cell-cell junction stability and during EMT. Loss of Tiam1 activity is required for the induction of EMT; forced expression of constitutive active forms of Rac1 (RacV12) or Tiam-1 prevents HGF-induced EMT in epithelial cells (127, 128). Conversely, Tiam1 is required for Rac1-mediated integrin-mediated laminin-5 deposition, cell spreading, and cell motility in keratinocytes (129). Interestingly, ablation of Tiam-1 in a mouse model of chemically induced skin carcinogenesis reduces tumor incidence but increases tumor malignancy, thus demonstrating an ambivalent role of Rac1 in tumor formation and tumor progression (130).

RhoC, a close family member of RhoA, has been previously identified as having a strong prometastatic activity (131). Indeed, although dispensable for development and postnatal life, ablation of RhoC function during mouse mammary carcinogenesis has no effect on primary tumor growth, yet decreases tumor cell motility and metastasis (132). Notably, miRNA10b, upregulated during EMT and tumor progression by the transcription factor Twist, has been found to repress the expression of the transcription factor HoxD10, which, in turn, represses RhoC expression (133). Supporting its prometastatic role, expression of RhoC correlates with high metastatic potential in oral squamous cell carcinoma, pancreatic adenocarcinoma, and inflammatory breast cancer (132, 134).

All the molecular pathways and regulatory circuits discussed above may be found in solitary migrating cells. However, based on morphologic and functional differences, single-cell migration has been distinguished into mesenchymal cell migration and amoeboid migration (1, 135). Either one or a mixture of both is found in cancer cells. Mesenchymal cell migration is exerted by spindle-shaped, fibroblast-like cells, such as fibroblasts, endothelial cells, smooth muscle cells, and cancer cells. It is characterized by cellular movements driven by a leading edge with Rac-induced cell protrusions and actin polymerization as cortical cables and stress fibers. These cells remodel the extracellular matrix by proteolysis and exhibit a slow turnover of β_1- and β_3-integrin–mediated focal adhesions and rather slow cell migration. In contrast, amoeboid cell migration is used by rounded cells, such as hematopoietic stem cells, leukocytes, and cancer cells, which in a push-and-squeeze type of migration make their way through the extracellular matrix. Their movement is driven by RhoA/ROCK–mediated bleb-like protrusions with active myosin/actin contractions and with cortical actin, yet a lack of stress fibers. The ECM is remodeled only by mechanical force in the absence of significant proteolytic activity. Non-integrin adhesion mechanisms rather than specific integrin-mediated focal adhesions are used, and thus no focal adhesion turnover is observed, and the cells migrate rather fast (106, 136, 137). However, the extent and type of collagen cross-linking seems to affect the requirement for proteolysis during amoeboid migration *in vitro*, and

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MT1-MMP proteolytic activity seems indispensable for amoeboid migration in naturally cross-linked collagen *in vitro* and *in vivo* (135).

The molecular pathways underlying the differences between mesenchymal and amoeboid cell migration are just being elucidated. For example, a siRNA screen for RhoGEFs and RhoGAPs has revealed specificities in the activation of RhoA and Rac that directly affect mesenchymal and amoeboid migration (138). The RhoGEF Dock3 and the adaptor protein NEDD9 seem to be required for Rac activity and for elongated cell movement. Once activated, Rac represses phosphorylation of myosin light chain 2 through WAVE2, thus repressing amoeboid (Rho-mediated) movement. Conversely, activated RhoA signaling, through ROCK, activates the RacGAP ARHGAP22, thus suppressing Rac activity and with it mesenchymal motility. The amoeboid migration of A375 melanoma cells in three-dimensional matrix also depends on ROCK-mediated actin-myosin contraction (139). Here, an independent siRNA screen has revealed that phosphoinositide-dependent protein kinase 1 (PDK1), as protein and not as kinase activity, is required together with RhoA-GTP to localize ROCK-I to the plasma membrane, which results in phosphorylation of myosin light chain and cell motility. PDK1 directly competes with RhoE for binding ROCK-I, thereby overcoming the negative regulation by RhoE. These experiments link phosphoinositide 3-kinase signaling and RhoA activity through a noncatalytic function of PDK1. Tightly controlled remodeling of the actin cytoskeleton also plays a critical role in defining the mode of cell motility. For example, ablation of cofilin function converts amoeboid migrating cancer cells into mesenchymal-type cells (140). These experimental results exemplify the large plasticity of solitary cancer cells in selecting different types of invasive migration.

Collective Invasion

In contrast to the various types of solitary migration, collectively migrating cells maintain their cell-cell junctions and migrate in sheets, strands, tubes, and clusters, either still in connection with their originating tissue or as separated, independently migrating clusters (2, 141). On a cellular level, there are only few differences to solitary migrating cells. Collectively migrating cells also form membrane protrusions, such as ruffles and pseudopodes; they use cell-matrix adhesion receptors, such as β_1-integrin and β_3-integrin, to form focal adhesions connected to the actin cytoskeleton; they direct proteolytic breakdown of the extracellular matrix to generate a path through the matrix scaffold; and they use the actin-myosin contractile apparatus for local contraction and cell movement. Yet, in contrast to their solitary migrating counterparts, collectively migrating cells do not retract their cellular tails but rather exert pulling forces on neighboring cells that are connected by adhesion junctions. Thereby, cells keep in most, but not all, cases their position in the collective structures, such as in two-dimensional sheets and in three-dimensional solid strands.

Collective migration is used during many developmental morphogenic processes, in which cells move in groups, with tight or loose association between each other and with protrusions that distinguish actively and directionally migrating cells from their neighboring followers. Such combination of cell migration and cell adhesion requires a fine-tuned crosstalk between cell-cell adhesion and cell contractility (142, 143). Examples are the morphogenic movement of cells of the inner blastocyst, converging extension of the vertebrate embryo, and closure of the dorsal surface and trachea morphogenesis in the *Drosophila* embryo. In zebrafish, the cells forming the prospective lateral midline represent a migrating group of cells in the absence of any EMT. Also, mammary branching morphogenesis, vascular sprouts during angiogenesis, and keratinocytes migrating across the wound matrix are typical examples of collective cell migration in sheets, tubes, or cell clusters (2). Depending on the species, the process of gastrulation exhibits a mixture of collective cell migration and cells that have undergone EMT, also indicating that the borders between single-cell migration and collective migration are murky.

Collective cell migration has been best studied in migrating border cells of the *Drosophila* oocyte. These cells migrate as cohorts between nurse cells toward the oocyte. This migration is dependent on myosinVI and E-cadherin, wherein myosinVI stabilizes E-cadherin adhesion complexes (144). Yet, excessive adhesion, for example, caused by a mutation in the gene *Hindsight*, prevents collective migration. The mammalian homologue of *Hindsight, RREB*, is also required for mammary epithelial tubular morphogenesis. It is essential for regulating the dynamic changes in cell-cell adhesion required during collective migration (145). Border cell cluster integrity and adhesion plasticity is regulated by Jun-kinase–mediated signal transduction, which also affects the polarity factor Bazooka and the cytoskeletal adaptor Paxillin, thus functionally connecting cell adhesion, cell polarity, and cell migration (143, 146).

In cancer, collective cell migration and invasion is found in distinct cancer types, including high and intermediate differentiated types of lobular breast cancer, epithelial prostate cancer, large cell lung cancer, melanoma, rhabdomyosarcoma, and most prominently in squamous cell carcinoma. Invading cells of these cancers form cone- or finger-like fronts and intravasate and disseminate as cell clusters, a form of metastatic dissemination that seems to be highly efficient in embolizing lymphatic or blood vessels and in surviving circulation (147).

To penetrate the extracellular matrix as a cell collective, the leading cells generate an invasion path. High-resolution multimodal microscopy has shown that the guiding cells use β_1-integrin–mediated focal adhesions and local expression of MT1-MMP at their leading edges to cleave collagen fibers and orient them in a way that generates tube-like microtracks into which the collective mass migration of follower cells can occur (137, 148). Thus, similar to single-cell migration, invasive migration and proteolytic

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matrix remodeling are interdependent processes that also control collective cell migration.

Insights into the molecular regulation of collective cell migration have also been obtained by investigating the expression of the cell surface glycoprotein podoplanin (T1α-2, aggrus, gp36), a 38 kDa, type-I sialo-mucine–like transmembrane glycoprotein, expressed in a variety of cell types, including kidney podocytes, alveolar type I cells, lymphatic endothelial cells, platelets, and several other cell types (149). Podoplanin is also expressed in a number of different cancer types, including squamous cell carcinoma of the oral cavity, larynx, lung, cervix, esophagus, and skin, in dysgerminomas of the ovary, in granulosa cell tumors, in testicular germ cell tumors, in mesothelioma, in a subset of lobular breast cancers, and in tumors of the central nervous system (19, 149, 150). Most of these cancer types, in particular squamous cell carcinoma, are well known to exhibit cone- or finger-like collective invasion into neighboring tissue. Notably, in most of these cancer types, podoplanin expression is mainly confined to the outer cell layer of the invading tumor front.

Forced expression of podoplanin in human keratinocytes and in MCF7 breast cancer cells induces cell migration and invasion with a significant decrease of cellular stress fibers and a concomitant formation of filopodia-like membrane protrusions, even in the presence of E-cadherin expression (19, 151). Podoplanin-expressing A431 squamous cell carcinoma cells exhibit higher tumorigenicity and, similar to the expression in squamous cell carcinomas of patients, exhibit collective invasion and express podoplanin in the outer cell layer of the invading front when transplanted into mice (152). Moreover, transgenic expression of podoplanin in tumor cells of the Rip1Tag2 mouse model of pancreatic β-cell carcinogenesis leads to the formation of invasive carcinomas in the absence of a cadherin switch and EMT (19). Thus, podoplanin seems to shift the invasion pattern from single-cell invasion involving EMT to collective invasion in the absence of EMT.

In contrast, expression of podoplanin in Madin-Darby canine kidney cells induces cell motility and invasion in the presence of full EMT with a loss of E-cadherin expression and the gain of N-cadherin expression (153). This activity is dependent on the short COOH-terminal domain through which podoplanin physically associates with ezrin and moesin but not radixin. This interaction results in an elevation of RhoA GTPase activity, and both ezrin and RhoA function are required for podoplanin-induced EMT. In addition, overexpression of podoplanin in Madin-Darby canine kidney cells and also MCF7 cells leads to a marked increase in phosphorylation of ezrin in a RhoA-dependent manner (19, 153). Thus, ERM proteins may link podoplanin expression to the observed rearrangement of the actin cytoskeleton. Recently, p120-catenin has also been implicated in the functional connection between collective cell migration and the maintenance of E-cadherin–mediated adherens junctions (154). Ablation of p120-catenin in A431 squamous cell carcinoma cells results in the loss of three-dimensional, collective invasion induced by EGF, which depends on the membrane localization of p120-catenin and on intact E- or P-cadherin–mediated cell-cell adhesion. However, the detailed mechanism of how podoplanin mediates collective cell migration remains to be elucidated.

Besides using cell-autonomous mechanisms, collectively migrating cancer cells may also rely on the support of stromal cells to pave their way through the extracellular matrix. Indeed, leader cells are identifiable in culture experiments, with distinct morphology, highly polarized cell shape, and the formation of lamellipodia (155). These leader cells are connected by E-cadherin–mediated junctions to their neighboring cells, and when dragging their neighbors, holes forming in the migrating cell mass are filled by cell proliferation. Moreover, in coculturing experiments between carcinoma cells and stromal fibroblasts, the fibroblasts lead the migrating carcinoma cells and prepare migratory tracks by proteolytic digestion and by force-mediated remodeling of the matrix (156). This process involves Rho-mediated myosin light chain activity in fibroblasts and the activities of Cdc42 and myotonic dystrophy kinase–related CDC42-binding protein kinase in carcinoma cells. Such stromal support seems required for epithelial cells that are retaining their epithelial organization. In contrast, collectively migrating highly invasive carcinoma cells (that have undergone EMT) or mesenchymal sarcoma cells are able to digest their way through the matrix without any support by stromal cells (137).

These clinical and experimental observations underscore the importance of collective cell migration in malignant tumor progression. However, the regulatory pathways underlying collective cell migration have just begun to be elucidated and its clinical manifestations, prognostic value, and actual contribution to metastasis remain to be assessed.

Conclusions/Perspectives

Cancer cell migration and invasion are certainly critical processes in the metastatic cascade. They can be induced and executed by various signaling pathways and regulatory networks. Many of these pathways seem to overlap with developmental processes and are being abused by cancer cells and the tumor microenvironment. Yet, although we have made substantial progress in the understanding of the molecular mechanisms underlying cancer cell migration and invasion in experimental systems, we still lack sufficient insights into the actual processes at work in cancer patients. This divergence between clinicopathologic and experimental observations is mainly based on the lack of appropriate surrogate markers and the lack of complex *in vivo* models that appropriately recapitulate human stochastic carcinogenesis. However, it is likely that the ongoing cell biological research on cell migration will provide the urgently needed tools for the development of improved diagnosis and prognosis and eventually for the design of innovative therapies.

Only few therapeutic approaches that are currently in development or in clinical trials specifically target cancer

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cell migration, such as interfering with integrin functions of invasive cancer cells. However, by interfering with important signaling pathways that are known to modulate cell proliferation, survival, and differentiation, they may also affect cell migration and invasion. Examples are inhibitors against the activities of different receptor tyrosine kinases, such as EGFR, IGF1R, c-Met/HGF receptor, PDGFR, and FGFR, as well as various antiangiogenesis regimen or even combinations thereof. Altogether, such multifaceted inhibitory approaches may provide efficient therapeutic measures that repress not only primary tumor outgrowth but also metastasis formation by interfering with cancer cell migration and invasion. However, the cellular and molecular variations to cancer cell migration discussed above raise the caveat that this endeavor will not be easy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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