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EMT, the cytoskeleton, and cancer cell invasion

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Abstract The metastatic process, i.e. the dissemination of cancer cells throughout the body to seed secondary tumors at distant sites, requires cancer cells to leave the primary tumor and to acquire migratory and invasive capabilities. In a process of epithelial-mesenchymal transition (EMT), besides changing their adhesive repertoire, cancer cells employ developmental processes to gain migratory and invasive properties that involve a dramatic reorganization of the actin cytoskeleton and the concomitant formation of membrane protrusions required for invasive growth. The molecular processes underlying such cellular changes are still only poorly understood, and the various migratory organelles, including lamellipodia, filopodia, invadopodia and podosomes, still require a better functional and molecular characterization. Notably, direct experimental evidence linking the formation of migratory membrane protrusions and the process of EMT and tumor metastasis is still lacking. In this review, we have summarized recent novel insights into the molecular processes and players underlying EMT on one side and the formation of invasive membrane protrusions on the other side.

Keywords Actin cytoskeleton · Cancer · Cell adhesion · EMT · Metastasis · Tumorigenesis

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1 Epithelial-mesenchymal transition (EMT) and metastasis

Metastasis, the spread of tumor cells from a primary tumor to a secondary site within the human body remains one of the most life-threatening pathological events. In the last years, major efforts have been taken to understand the molecular mechanism underlying the distinct steps of metastasis, which are (i) detachment of tumor cells from the primary tumor, (ii) invasion into surrounding tissue, (iii) intravasation into blood or lymphatic vessels, (iv) dissemination in the blood stream or the lymphatic system and, finally, (v) extravasation and outgrowth at a secondary site. Each of these steps requires a distinct molecular program in which the modulation of the adhesive and migratory and, thus, the cytoskeletal properties of the disseminating tumor cells play essential roles.

To detach from the primary tumor and to invade into the surrounding tissue, tumor cells have to break down cell-cell contacts, remodel cell-matrix adhesion sites, and follow a chemoattractive path through the extracellular matrix, mined by secreted proteinases. These processes are commonly observed in various non-pathological conditions, such as in developmental processes like gastrulation or neural crest cell migration, where differentiated, epithelial cells dedifferentiate, move to a distant site, and then re-differentiate to form a new structure. This temporary and reversible phenomenon is known as the epithelial-mesenchymal transition (EMT), a process that is currently in the limelight of investigating the onset of cancer cell migration, invasion and metastatic dissemination [1, 2]. During EMT, non-motile, polarized epithelial cells, embedded via cell-cell junctions in a cell collective, dissolve their cell-cell junctions and convert into individual, non-polarized, motile and invasive mesenchymal cells. Thereby, the molecular repertoire of a cell experiences

dramatic changes. For example, the function and expression of the epithelial cell-cell adhesion molecule E-cadherin is lost, whereas the expression of the mesenchymal cell-cell adhesion molecule N-cadherin is induced, a process also known as the cadherin switch. EMT can be prompted by various intrinsic signals (e.g. gene mutations) as well extrinsic signals (e.g. growth factor signaling). Among the growth factors known to induce EMT are transforming growth factor β (TGFβ)[3], hepatocyte growth factor (HGF) [4], members of the epidermal growth factor (EGF) family [5], insulin-like growth factor (IGF)[6], and fibroblast growth factor (FGF)[7, 8]. Recently, also Notch signaling has been implicated in EMT in human breast cancer cells by activating the transcription factor Snail2 (Slug), a potent repressor of E-cadherin gene expression [9]. Changes in the composition of the extracellular matrix (ECM) are also able to induce EMT, as shown for collagen I and hyaluronan [10, 11].

With the diversity of signals inducing EMT the complexity of the interactive downstream effector pathways increases. Among the candidates which are engaged by TGFβ-induced EMT are the small GTPases RhoA and Rac1 [12, 13], Ras [14], phosphoinositol–3 kinase (PI3K) [15], Mitogen-activated protein kinase (MAPK) [13], integrin-linked kinase (ILK) [16], and the Jagged1/Notch signaling pathway [17]. With increasing interest in microRNAs, miR-200 and miR-205 have been recently shown to play an important role in TGFβ-induced EMT by modulating the function of ZEB1 (δEF1) and ZEB2 (Sip1), transcriptional repressors of E-cadherin gene expression [18]. Such complexity of interactive signaling upstream and downstream of the induction of EMT also explains why EMT is not a simple matter of changes in a cell's adhesive capabilities or its cytoskeletal organization, it rather represents a fundamental reprogramming of almost every aspect of a cell's biology. Still, the different signaling cascades underlying EMT can be grouped into biological programs and, apparently, tumor cells undergoing EMT hijack programs that are central for developmental processes. The actual occurrence of EMT in patients is still highly debated, yet with more detailed molecular and histopathological analysis and the advent of novel markers there is increasing evidence identifying EMT in various human cancers [19, 20]. Still, many aggressive, invading tumors do not exhibit a molecular signature of EMT, suggesting that EMT may not be involved in every type of single cell invasion and that some tumors may undergo a partial or incomplete EMT [21]. In fact, cancer cells can invade in the absence of EMT and have a broad repertoire for invasion, including amoeboid or collective cell invasion [22, 23].

This review highlights recent novel insights into EMT research with a specific focus on the remodeling of the actin cytoskeleton and the formation of invasive structures during EMT and tumor cell invasion.

2 Epithelial cell-cell adhesion

The formation of a stable, polarized epithelium requires tight cell-cell and cell-matrix connections. E-cadherin is the major component of epithelial adherens junctions (AJ) which mediate, along with tight junctions, intercellular adhesion. AJ are located basal to the apical tight junctions (TJ) and form a belt-like structure which tie neighboring cells together (zonula adherens). E-cadherin is the prototype family member of classical cadherins, single-span transmembrane glycoproteins that interact in a calcium-dependent, homophilic manner with E-cadherins on neighboring cells. E-cadherin-mediated cell-cell adhesion complexes are anchored to the actin cytoskeleton via its cytoplasmic domain and β-catenin and α-catenin (Fig. 1 a). Thus, the formation of E-cadherin-mediated cell-cell adhesion fundamentally modulates the organization of cytoskeleton. This classical view of a direct connection between the E-cadherin/β-catenin/α-catenin complex and the actin network has been challenged by recent studies demonstrating that a reconstructed cadherin-catenin complex fails to bind actin filaments *in vitro* [24], and that E-cadherin, localized in electron-dense microdomains called spot adherens junctions (SAJs), binds to actin in an α-catenin-independent manner [25]. Here, the authors describe a model in which two distinct actin populations are involved in the zonula adherens architecture (Fig. 1 a). One population represents stable, non-dynamic patches of highly organized actin to which the E-cadherin/β-catenin complex is attached in an α-catenin-independent manner (SAJs). The second population of actin is an underlying, dynamic actin framework to which the SAJs are linked and correctly positioned by α-catenin. One protein replacing α-catenin in the E-cadherin/β-catenin complex to SAJ could be eplin, a newly identified actin-binding protein [26]. The juxtamembrane domain of E-cadherin binds to p120-catenin which is important in surface tracking, lysosomal degradation and correct membrane localization of E-cadherin [27–30]. Furthermore, p120-catenin plays an elementary role in the stability of epithelial cell-cell adhesion by repressing the activity of RhoA and activating Rac and Cdc42 [31–33]. All three GTPases are key regulators of actin assembly and play an essential role in the stability of cell-cell adhesion by enforcing actin stress fibers (RhoA) and the formation of migratory membrane protrusions, such as lamellipodia and filopodia (Rac and Cdc42, respectively), as discussed below.

Besides its adhesive function, E-cadherin also encompasses signaling capabilities, transduced predominantly by proteins interacting with its intracellular domain, such as β-

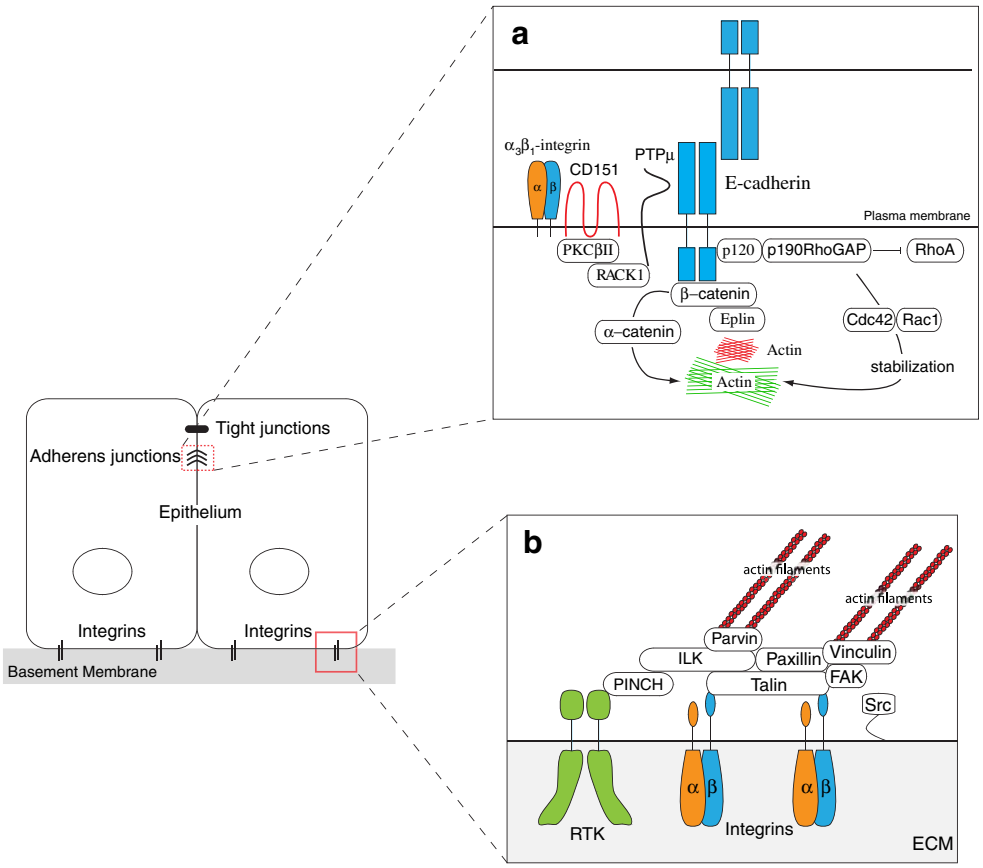

Fig. 1 Differentiated, polarized epithelial cells are tightly attached to their neighboring cells by E-cadherin-mediated cell-cell adhesion complexes and to the extracellular matrix via integrins. **a** Epithelial cell-cell adhesion. E-cadherin is connected via β-catenin and Eplin to stable, electron-dense actin microdomains called spot adherens junctions (SAJ) in an α-catenin-independent manner. These E-Cadherin-SAJs complexes are attached and correctly positioned to an underlying dynamic actin framework via α-catenin. E-cadherin-mediated cell adhesion is stabilized by (i) p120-catenin recruited p190RhoGAP, which stabilizes the actin cytoskeleton underlying the adhesion complex by balancing the activities of RhoA and Rac1/Cdc42, and by (ii) protein tyrosine phosphatase PTPμ, which keeps β-catenin in an dephosphorylated state thereby preventing its degradation. PTPμ is correctly positioned to β-catenin by a multimeric protein complex consisting of receptor of activated protein kinase C-1 (RACK1), protein kinase C-βII (PKCβII), the tetraspanin CD151 and $\alpha_3\beta_1$-integrin. **b** Epithelial cell-matrix adhesion. Integrin-mediated cell-matrix adhesion and linkage to the actin cytoskeleton is accomplished by a multiprotein complex consisting of the adaptor proteins talin, paxillin, vinculin and the ternary complex of pinch, parvin and integrin-linked kinase (ILK), called tIPP complex. The interaction and phosphorylation status of focal adhesion kinase (FAK) and the non-receptor tyrosine kinase Src is critical for integrin complex assembly and turnover

catenin, or receptors that form multimeric complexes with E-cadherin, such as c-Met, the cognate receptor for HGF, IGF1R or integrins [34]. Notably, E-cadherin has been shown to interact with a multimeric complex that consists of $\alpha_3\beta_1$-integrin, the tetraspanin CD151, which recruits protein kinase C-βII (PKCβII), receptor of activated protein kinase C-1 (RACK1), and the transmembrane protein tyrosine phosphatase PTPμ [35] (Fig. 1 a). This multimeric complex promotes association of the cadherin-catenin complex with the actin cytoskeleton and supports cadherin mediated cell-cell adhesion. CD151 appears to be important for the filopodia-based "adhesion zipper formation", a process by which initial filopodia-mediated contacts of epithelial cells develop into mature cell-cell junctions [36, 37]. Moreover, CD151 expression accelerates E-cadherin-mediated intercellular adhesion by inducing Cdc42-induced filopodia extensions which form initial cell-cell contacts. Consistent with these observations, E-cadherin colocalizes and interacts with cortactin, a key regulator of actin-cytoskeleton assembly and remodeling [38].

E-cadherin also associates with c-Met, IGF1R and α_v-integrin at the plasma membrane [39, 40]. Interestingly, in the absence of α-catenin the E-cadherin/IGF1R complex does not form, suggesting that α-catenin besides its function as actin-anchoring protein also exerts a function as an important scaffolding protein. Upon stimulation with IGF-I, α_v-integrin dissociates from the cell-cell adhesion complex and translocates to focal contact sites of invasive structures, such as invadopodia (see below). These findings expand E-cadherin's functional repertoire beyond its adhe-

sive functions and emphasize the critical role of E-cadherin as a regulator of signaling complexes.

3 Loss of E-cadherin function and its consequences

Loss of E-cadherin gene expression or of E-cadherin protein is frequently found during tumor progression in most epithelial cancers. Hence, loss of E-cadherin function is a clinical indicator for poor prognosis and metastasis [41–43]. Since E-cadherin plays a key role in epithelial structure and homeostasis its expression underlies a strict control. As many other proteins, E-cadherin can be regulated at the transcriptional as well as at the post-translational level, yet both mechanisms usually cooperate for an efficient repression of E-cadherin function.

3.1 Transcriptional control of E-cadherin

Transcriptional repression of E-cadherin is mediated by a list of transcription factors, among them intensely studied factors like Snail1 (Snail), Snail2 (Slug), ZEB1 (δEF1), ZEB2 (Sip1), E47, and Twist [44] (Fig. 2 a). The expression of these repressors can be induced by a variety of stimuli, including activation of the TGFβ, HGF, EGF, Wnt, and Notch signaling pathways. Moreover, they seem to regulate each other's expression in positive and negative feedback loops. Engagement of these transcriptional repressors at the E-cadherin gene promoter eventually leads to epigenetic silencing of the gene by histone modifications (acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation) and subsequently by DNA hypermethylation [45–47]. Such silencing of the E-cadherin promotor is a complex process [48]. As a first step, Snail1 recruits the histone deacetylase HDAC to the E-cadherin promotor complex, thereby inducing histone deacetylation. Subsequently, the polycomb repressor complex 2 (PRC2) is recruited to the site and methylates histones, thus supporting E-cadherin repression. Upon initial down-regulation of E-cadherin gene expression, Snail1 induces ZEB1 expression, 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 the protein arginine methyltransferase 5 (PRMT5) to support Snail1-mediated transcriptional repression [49]. In a large variety of human cancers, the E-cadherin gene is found to be highly hypermethylated, yet how the initial silencing of the gene promoter converts into a more long-term repression by DNA hypermethylation remains to be resolved [50]. In the context of EMT and the metastatic dissemination of tumor cells, the molecular basis of the reversibility or irreversibility of E-cadherin's epigenetic silencing is thus a future challenge. This complexity and/or variability in E-cadherin regulation indicates that the cell has a dynamic range of E-cadherin expression and depending on the actual need can either totally suppress or temporally attenuate its expression.

3.2 Post-translational control of E-cadherin

On the post-translational level, the transport of newly synthesized E-cadherin to the cell membrane can be inhibited via O-glycolysation [51] or mature, membrane-bound E-cadherin can be degraded by proteolytic cleavage or endocytosed from the plasma membrane [52–55]. The proteolytic cleavage of E-cadherin can also produce E-cadherin fragments which exert signaling functions. For example, γ-secretase-mediated cleavage of E-cadherin produces a C-terminal, cytoplasmic fragment (CTF2) that is transported into the nucleus in a p120-catenin-dependent manner (Fig. 2 b). In the nucleus, CTF2 modulates the interaction between p120-catenin and Kaiso, a transcriptional repressor, thereby affecting for example cell survival [56]. Destabilization of the E-cadherin adhesion complex is also accomplished by receptor tyrosine kinase or Src-mediated phosphorylation of E-cadherin, followed by its ubiquitylation by the E3 ligase Hakai and subsequent degradation [57, 58] (Fig. 2 b). Moreover, integrin-activated focal adhesion kinase (FAK) can phosphorylate β-catenin and thus induce its ubiquitylation and degradation and the disassembly of the E-cadherin cell adhesion complex [59]. Endocytosis of E-cadherin can occur via clathrin or caveolin-dependent mechanisms [60–62]. A key player in clathrin-mediated E-cadherin endocytosis is Arf6, a Ras-related small GTPase. It promotes endocytosis via recruitment of Nm23-H1, a nucleoside diphosphate kinase (and the first metastasis suppressor gene ever identified [63]), which in turn activates dynamin-dependent fission of vesicles and destabilization of cortical actin by recruiting the guanine nucleotide exchange factor (GEF) Tiam1, a Rac1 inhibitor [64]. Recently, a new GTPase activating protein (GAP) for Arf6, Smap1, has been identified, which plays an essential role in E-cadherin endocytosis, revealing a new player in post-translational E-cadherin control [65, 66].

The downregulation of E-cadherin not only leads to a mechanical disruption of AJ, it also liberates proteins from the cytoplasmic cell adhesion complex which exert ambivalent functions depending on their subcellular localization. The probably most prominent cytoplasmic interaction partner of E-cadherin is β-catenin, well known for its dual role in cell adhesion and Wnt signaling [67]. Stabilized by active Wnt signaling or by mutations in the β-catenin phosphorylation/degradation pathway, β-catenin accumulates in the cytoplasm and enters the nucleus where it

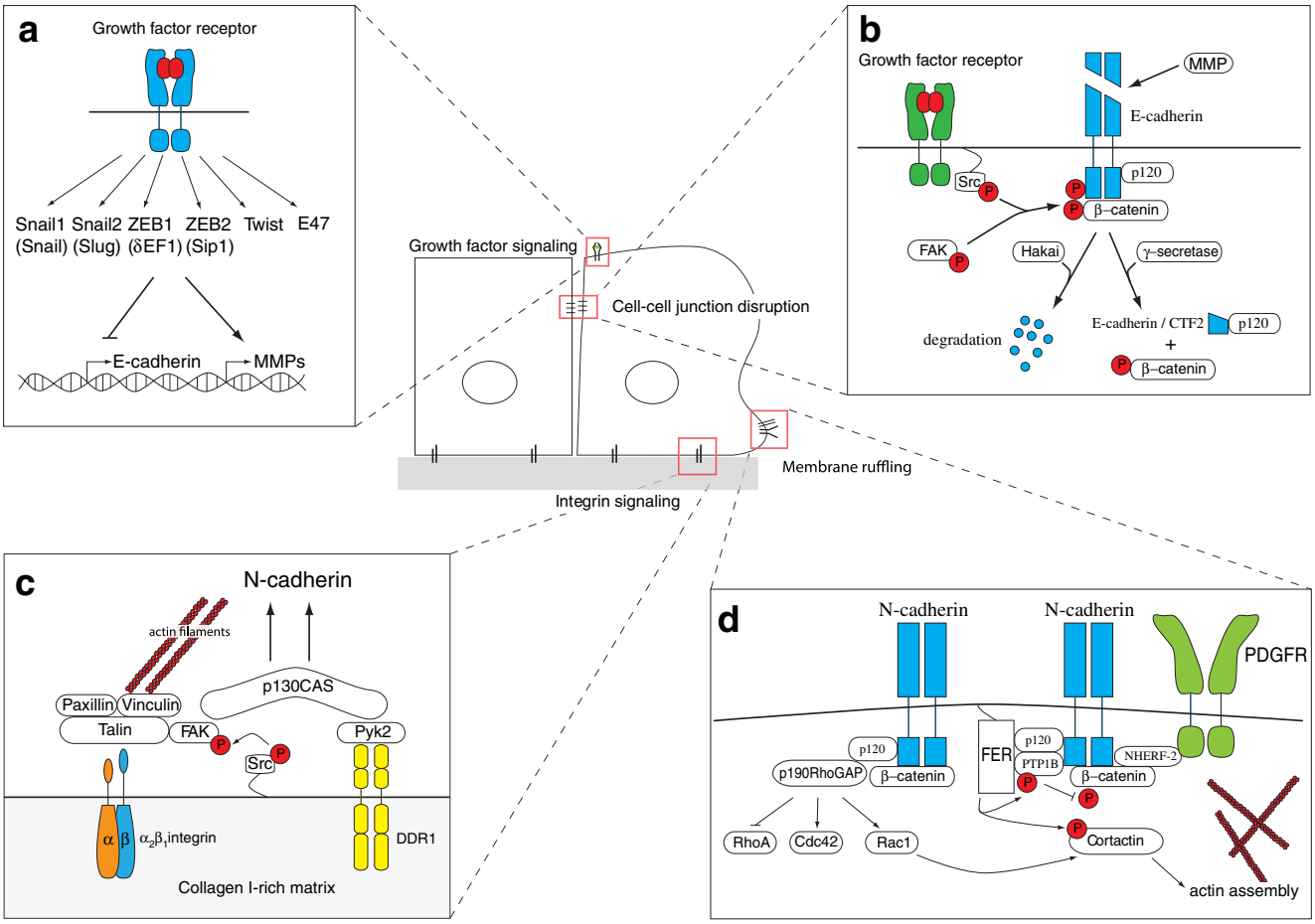

Fig. 2 Induction of epithelial-mesenchymal transition (EMT). **a** Repression of E-cadherin gene expression. Growth factor stimulation induces various signaling cascades leading to the induction of EMT. Among the regulated genes are transcription factors that repress E-cadherin gene expression and induce expression of genes such as matrix metalloproteinases (MMP). **b** Loss of E-cadherin function. The E-cadherin cell-cell adhesion complex is destabilized by Src and/or FAK-mediated phosphorylation of either E-cadherin or β-catenin. The phosphorylation of the cytoplasmic E-cadherin domain induces its proteasomal degradation mediated by the E3-ubiquitin-ligase Hakai. Phosphorylation of β-catenin induces its detachment from E-cadherin and relocalization into the nucleus, where together with Tcf transcriptions factors modulates the expression of pro-invasive genes. Intracellular cleavage of E-cadherin by γ-secretase also results in a loss of E-cadherin function, yet its newly generated C-terminal fragment (CTF2) together with p120-catenin translocates into the nucleus, where it modifies the activity of the transcriptional repressor Kaiso. **c** Integrin-mediated support of EMT. Enrichment of collagen I in the extracellular matrix supports EMT via the coordinated signaling of β_1-integrins and the collagen receptor DDR1. These signals converge via FAK and Pyk2 kinases on the $p130^{Crk}$-associated scaffold (CAS) protein, resulting in the induction of N-cadherin expression. **d** The formation of protrusive membrane structures. Upon PDGF stimulation, N-cadherin co-localizes with PDGFR in membrane ruffles. The interaction of both N-cadherin and PDGFR relies on Na^+/H^+ exchanger regulatory factor-2 (NHERF-2) which is recruited to the complex by p120-catenin. Localized in membrane ruffles, N-cadherin contributes to actin cytoskeleton remodeling (i) by recruiting p190RhoGAP and thereby favoring Rac1 and or Cdc42-mediated actin remodeling and (ii) by employing p120-catenin to recruit the non-receptor tyrosine kinase Fer. Fer in turn phosphorylates and activates cortactin which induces actin assembly. Morover, Fer stabilizes the N-cadherin complex by phosphorylating and activating protein tyrosine phosphatase PTP1B, thus preventing β-catenin phosphorylation and degradation

interacts with members of the Tcf/Lef family of transcription factors and modulates expression of a large number of genes involved in cell proliferation, migration, invasion, and morphogenesis, including cyclin D1, the cell adhesion molecule L1-CAM, matrix metalloproteases (MMP) and the metastasis gene S100A4 [68, 69]. Another recently discovered target of β-catenin/Tcf signaling is Fascin, a actin-bundling protein which is essential for filopodia formation and cancer cell invasion [70, 71].

Similar to β-catenin, upon loss of E-cadherin function, p120-catenin is also freed from the cytoplasmic cell adhesion complex and accumulates in the cytoplasm. In addition to its functions on Rho family GTPases and the actin cytoskeleton (see above), p120-catenin can traffic to the nucleus where it binds to 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 de-repression. However, the nature of p120/Kaiso target genes is still poorly defined [72].

4 The cadherin switch and its consequences

The loss of epithelial E-cadherin and the gain of mesenchymal N-cadherin expression is a major hallmark of EMT. This cadherin switch leads to a drastic change in the adhesive properties of a cell, as it loses its affinity for epithelial neighbors and gains affinity for mesenchymal cells, such as fibroblasts or vascular endothelial cells. Besides the change of the adhesive repertoire, the gain of N-cadherin expression also provokes increased cell migration and invasion [73, 74]. Like E-cadherin, N-cadherin belongs to the family of classical cadherins and forms homophilic cell-cell adhesion junctions. N-cadherin is normally expressed in nervous tissue, in vascular endothelial cells, and in skeletal and cardiac muscle cells. Its expression is upregulated in the cells of the primitive streak during mesoderm formation and during progression of a variety of cancers, where its expression correlates with poor prognosis [74–76]. Investigations into the molecular mechanisms underlying the induction of N-cadherin expression during EMT have only recently begun. It has been shown that N-cadherin can be upregulated by collagen I via the coordinated engagement of the collagen receptor discoidin domain receptor 1 (DDR1) and $\alpha_2\beta_1$-integrin based on a $p130^{Crk}$-associated substrate (CAS) scaffold [10, 77] (Fig. 2 c). On the transcriptional level, the transcriptional repressor Twist appears to be involved in the induction of N-cadherin gene expression in an β_1-integrin-dependent manner [78–80].

Like its epithelial counterpart, N-cadherin is connected via α-catenin and β-catenin to the cytoskeleton and functions as both a mechanical cell adhesion component and a signaling molecule. Recently, it has been reported that during neurite extension the traction forces generated by retrograde actin flow are directly transmitted to N-cadherin adhesions, thereby mechanically linking N-cadherin with the formation of motile structures [81]. Interestingly, the authors show that the mechanical engagement of N-cadherin induces local actin polymerization and thereby ensures the integrity of the adhesion complex. Engagement of N-cadherin activates the RhoGTPase Rac1, which in turn recruits the actin remodeling protein cortactin to the N-cadherin adhesion complex [82] (Fig. 2 d). Within this complex, the non-receptor tyrosine kinase Fer associates with N-cadherin via p120-catenin [83]. Fer then phosphorylates and activates cortactin, thereby inducing actin remodeling, and increasing the mobility of N-cadherin molecules to extend the adhesion zone and, finally, to promote the formation of stable cell-cell adhesion. In line with these observations, N-cadherin is found to be localized in the lamellipodia of adjacent, contacting myoblasts [84]. Fer also phosphorylates the phosphatase protein tyrosine phosphatase 1B (PTP1B) and promotes its binding to N-cadherin. The PTP1B/N-cadherin interaction protects β-catenin from degradation by keeping it in a dephosphorylated state and thereby ensures the stability of the N-cadherin-mediated cell-cell junctions [85, 86] (Fig. 2 d).

Also similar to E-cadherin, N-cadherin interacts with a number of signal transduction molecules and contributes to various signaling pathways. For example, Na+/H+ exchanger regulatory factor (NHERF)-2 has been shown to physically link N-cadherin to PDGF receptor (PDGFR) by binding both β-catenin in the N-cadherin/β-catenin complex and PDGFR [87] (Fig. 2 d). PDGFR activation is known to induce actin reorganization and cell proliferation and differentiation and to play an important role in EMT [88, 89]. Interestingly, PDGF stimulation of NIH3T3 cells leads to colocalization of N-cadherin, p120-catenin and p190RhoGAP in dorsal circular ruffles (DCRs), structures known to depend on growth factor-induced Rac activity and subsequent RhoA inhibition [90–92]. This is interesting because p120-catenin is not only important for the recruitment of Fer to the N-cadherin complex, but together with p190RhoGAP also coordinates the antagonistic functions between Rac and RhoA [31, 93]. This antagonism plays a critical role in defining the structure of the actin cytoskeleton. The active form of RhoA stimulates focal-adhesion (FA) formation and contractility via assembly of predominantly radially-oriented actin stress fibers (ASF), whereas Rac activation induces cell spreading, migration and membrane ruffling via actin polymerization at the cell periphery. Moreover, Rac activation inhibits Rho activity, which can also be achieved by p120-catenin over-expression [94] (Fig. 2 d).

Besides PDGFR, N-cadherin also interacts with FGF receptors (FGFR) in a complex with neural cell adhesion molecule (NCAM), a immunoglobulin domain cell adhesion molecule [42, 95]. The interaction between N-cadherin and FGFR leads to stabilization of FGFR at the membrane surface by preventing its internalization upon ligand binding. As a result, sustained MAPK pathway activation and increased cell motility and MMP secretion promote invasiveness of N-cadherin-expressing cells [96–98]. NCAM accomplishes a similar induction of cell migration and invasion by directly binding and stimulating FGFR via its fibronectin type III domains [95]. Thereby, NCAM-mediated stimulation of FGFR signaling differs substantially from FGF-induced FGFR signal transduction resulting into different cellular outcomes, such as increased cell-substrate adhesion, migration and invasion by NCAM and cell proliferation by FGF [99]. The interaction between N-

cadherin and NCAM with FGFR relies on a CAM-domain proximally located to the acid box region of the FGFR, which is not required for FGF ligand binding [100]. Such complex interactions between different cell adhesion molecules and tyrosine kinases raise another level of complexity in the regulation of cell migration, invasion and metastasis formation. For a more detailed insight into specific aspects of cell adhesion and signaling complexes at the invasive cancer front we refer the reader to recently published reviews [42, 101].

Like E-cadherin, N-cadherin is also proteolytically processed to generate shedded extracellular domain and intracellular domain fragments with potential signaling functions. In neurons, N-cadherin is cleaved by ADAM10 and by PS1/γ-secretase to produce a cytoplasmic fragment of N-cadherin, N-Cad/CTF2, for example stimulated by bone morphogenic protein-4 (BMP4) [102, 103]. N-Cad/CTF2 is able to interfere with the CPB/CREB transcription complex, by binding the transcription factor CBP and inducing its proteasomal degradation. N-Cad/CTF2-induced repression of CPB/CREB-mediated transcriptional control suppresses expression of genes important for proliferation and differentiation, such as c-Fos. N-Cad/CTF2 also promotes migration of neural crest cells by increasing the expression of β-catenin and therewith inducing the expression of β-catenin target genes like cyclin D1. Interestingly, N-Cad/CTF2 facilitates β-catenin-dependent signaling (i) by inhibiting β-catenin phosphorylation, (ii) by increasing β-catenin transcription and (iii) by reducing full-length N-cadherin protein levels to prevent sequestration of β-catenin to cell-cell junctions [104].

These data describe N-cadherin as a critical protein in the regulation of EMT and cell invasiveness. Besides simply exercising a cell adhesion function that changes a cell's affinity from epithelial cells to mesenchymal cells, it is actively involved in delineating a cell's migratory state by modulating growth factor signaling and remodeling the actin cytoskeleton. Such pleiotropic functions certainly warrant detailed future experimental investigations.

5 Integrin-mediated cell-matrix adhesion and signaling

The extracellular matrix (ECM) is a constantly remodeled 3D structure consisting of a variety of specialized proteins and proteoglycans which are able to regulate many cellular processes, including cell proliferation, survival, differentiation, and migration. This control is mainly based on a constant communication between the adhesive adaptors of a cell, integrins, and the ECM. Changes of a cell's integrin repertoire or the composition of the ECM can have drastic consequences for the cell and, in extremis, can lead to cell death as well as transformation. Besides its scaffolding function, the ECM is also able to bind and sequester diverse growth factors and chemokines which can be retrieved by local proteolysis and, dependent on activation or inactivation, affect cell behavior. Integrins are heterodimeric type-I transmembrane proteins consisting of an α-and a β-chain. Mammals have 18 α and 8 β chains which are combined to generate 24 different combinations which bind in a specific, yet partially overlapping, manner various components of the ECM.

Similar to cadherins, also integrins function as both mechanical adhesion and signaling molecules. Importantly, integrins switch between an inactive, low ligand-affinity conformation and an active, high ligand-affinity conformation. Activation can be achieved by binding of intracellular proteins to integrins, such as talin, or by MMP-mediated proteolytic cleavage [105, 106]. Integrins are linked to the actin cytoskeleton and signal via the ternary complex of integrin-linked kinase (ILK), pinch and parvin, also named the tIPP complex [107] (Fig. 1 b). In the tIPP, integrins are linked through ILK to the actin cytoskeleton, either via parvin itself or via a paxillin/vinculin/parvin complex. ILK plays a major role as a scaffold protein in assembling the multimeric protein complex which is necessary for the integrin-actin cytoskeleton linkage (via parvin, paxillin and vinculin). Moreover, ILK is required for the formation of signaling complexes with receptor tyrosine kinases via pinch. The signaling cascades triggered by the activation of integrins and its cytoplasmic partners are complex. Among the direct targets downstream of integrins are FAK, Src-family kinases, glycogen synthase-kinase-3 β (GSK3 β) and protein kinase B (AKT/PKB). Effectors of these signaling cascades include MAPK, NFκB, Jun, β-catenin and others and together they modulate cell proliferation, cell survival, cell migration and invasion. Among the mitogenic integrins are $\alpha_6\beta_4$ or $\alpha_v\beta_3$-integrins which cooperate with diverse growth factor receptors, including EGFR, ErbB2, and c-Met [108–111]. On the other hand, growth inhibitory signals can be transmitted, for example via $\alpha_2\beta_1$-integrin, which in turn activates p38-MAPK-mediated cell-cycle inhibition [112, 113]. Integrin $\alpha_v\beta_6$ or $\alpha_v\beta_8$ engagement leads to the activation of latent TGFβ thereby executing its cytostatic effect [114, 115]. Faced by this dichotomy of integrin signaling, cancer cells switch their integrin expression to a pro-oncogenic repertoire in order to invade and survive in the surrounding tissue.

6 Integrins in EMT and cell invasion

The function of integrins during EMT is diverse and dynamic as they are able to initiate and enforce EMT and invasion. For example, engagement of integrins $\alpha_1\beta_1$ or $\alpha_2\beta_1$ by collagen type I results in a loss of E-cadherin

mediated cell-cell contacts, along with the activation of the β-catenin/Tcf pathway in pancreatic cancer cells [59, 116]. Furthermore, as described above, collagen type I is also able to induce N-cadherin expression upon activation of the integrin $\alpha_2\beta_1$ together with the collagen receptor discoidin domain receptor 1 (DDR1), a receptor tyrosine kinase [77]. Both, the downregulation of E-cadherin and upregulation of N-cadherin play important roles in the initiation and execution of EMT. Interestingly, Snail1, the transcriptional repressor of E-cadherin expression and a potent inducer of EMT, is able to induce the expression of $\alpha_v\beta_3$-integrin which is well known for its pro-invasive functions and its localization in the invading front of cancers [117, 118].

Along with their function as mechanical anchor proteins for cell migration and invasion, integrins play an important role in the correct localization of proteases. Several reports demonstrate that the colocalization and cooperation of β1-integrin and MT1-MMP1 is necessary for cancer cell invasion into a collagen matrix and that both MT1-MMP and β_1-integrins have important roles in EMT [119–123]. The localization of MT1-MMP to β_1-integrins is an exocytic event dependent on the activity of the GTPase Rab8 [124]. Integrin-mediated recruitment of ECM remodeling proteases is also responsible for the liberation and/or activation of matrix bound growth factors and chemokines. As described above, the cooperation of MT1-MMP and $\alpha_v\beta_8$-integrin leads to the activation of latent TGFβ [115, 125]. TGFβ usually acts as a cytostatic, tumor suppressing factor, but it promotes tumor progression and invasion, if the tumor cells overcome its cytostatic and apoptotic effects [126]. In fact TGFβ is one of the most potent inducers of EMT in cultured cells *in vitro* and in animal models *in vivo* [1]. Other integrins which are upregulated during EMT, such as $\alpha_v\beta_6$-integrin, are also able to increase protease expression and to liberate and activate TGFβ [127–129]. Also, the activities of the cytoplasmic interaction partners of integrins, ILK and pinch, have been implicated in the process of EMT [130–132].

7 EMT and the actin cytoskeleton

The actin cytoskeleton is a highly dynamic structure, which is constantly remodeled in a living cell. This dynamics are based on a well-balanced and highly controlled equilibrium of local assembly and disassembly of actin filaments. Obviously, such regulation is a prerequisite for processes like endocytosis, cell motility, and cancer cell invasion.

7.1 RhoGTPases and EMT

The members of the Rho GTPase family are mainly responsible for integrating and transmitting signals from chemokine and growth factor receptors and from adhesion receptors to effector proteins of actin remodeling. RhoGTPases are activated upon GTP binding and inactive in their GDP-bound form. RhoGTPase activation is tightly controlled by three groups of regulatory proteins, guanine nucleotide exchange factors (GEF), GTPase-activating proteins (GAP), and guanine nucelotide dissociation inhibitors (GDI). GEF are responsible for the activation of RhoGTPases by promoting the exchange of Rho-bound GDP by GTP. This is counteracted by GAP which raise the intrinsic GTPase activity of RhoGTPases and the hydrolysis of bound GTP to GDP. Finally, GDI bind inactive Rho-GDP and prevent the interaction with RhoGEFs and thus its activation. RhoA, Rac1 and Cdc42 are best studied among the 23 family members of RhoGTPases. The complexity of RhoGTPase signaling arises not only from the size of its family members and number of effector proteins (~ 70 proteins), but also from the numbers of GEF (~ 70 members), GDI (~3 members) and GAP (~ 60 members) which modulate their activity. Depending on which GEF, GDI or GAP is interacting with the RhoGTPase the biological response can be different. In the GTP-bound form, RhoGTPases activate effector proteins, which are often serine/threonine kinases, such as the p21-activated kinases (PAK) for Rac1 and Cdc42 and the ROCK kinases for RhoA. In general, RhoGTPases affect almost all cell biological processes in a cell's life [133–135]. With regard to migration and invasion and in a simplified view, RhoA induces actin stress fiber formation and regulates cytoskeletal changes affecting cell-cell or cell-matrix adhesion, Rac1 is involved in lamellipodia and membrane ruffle formation, and Cdc42 is involved in filopodia formation [136, 137]. Based on their central function in actin remodeling and their ability to induce MMPs, Rho GTPases play an important role in EMT as well [138].

During growth factor-induced EMT, tight control of the activities of RhoGTPases is critical. 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 [139]. 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, respectively. The recruitment of p190-RhoGAP results in the activation of Rac1 which leads to a stabilization of E-cadherin junctions by inhibiting the activities of IQ-domain GTPase-activating protein 1 (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 [140].

During EMT, p120-catenin binds to mesenchymal cadherins at the cell membrane but is also found localized in the cytoplasm. Cytoplasmic p120-catenin functions as a RhoA-GDI that binds and represses RhoA activity [141]. Simultaneously, p120-catenin bound to mesenchymal cadherins at the cell membrane promotes Rac1 activity and induces the formation of motile, protrusive membrane structures, such as 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 (ROS), which in turn activate p190RhoGAP by inhibiting the low-molecular weight protein tyrosine phosphatase (LMW-PTP) [90, 142]. Moreover, the expression of Snail1, the transcriptional repressor of E-cadherin gene expression and potent inducer of EMT, is is increased upon Rac1-mediated ROS production [143]. The importance of RhoGTPases in EMT is also underscored by the notions that Rac1b, a splice variant of Rac1, is highly expressed in malignant breast tissue, that RhoA down-regulation is required for EMT in colon carcinoma progression, and that Rock activity is critical for cell migration, invasion and metastasis after EMT [141, 144, 145].

The activity of RhoA during EMT not only effects cell-cell adhesion but also microtubule-mediated cell-matrix adhesion and basement membrane integrity [146]. RhoA, when localized at the cell-basement membrane (BM) gets in contact with Net1, a RhoA-specific GEF, thereby exerting an important function for the integrity of the BM in epiblasts. During gastrulation, a process resembling EMT, RhoA at the cell basis loses its activity, which leads to microtubule destabilization, cell-BM contact disruption and BM breakdown. Notably, destabilization of the cell-BM contacts precedes breakdown of cell-cell adhesions.

Tiam1, a GEF for Rac1, 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 [147, 148]. Interestingly, ablation of Tiam-1 in a mouse model of chemical-induced skin carcinogenesis reduces tumor incidence yet increases tumor malignancy, demonstrating the ambivalent role of Rac1 in tumor formation and tumor progression [149].

Despite major progress, the detailed role of Rho family GTPases in EMT and tumor progression still remains unresolved. The sophistication of tumor cell motility and invasion on one side and the immense complexity of the regulation of RhoGTPases on the other side obscure a simple solution. The formation of membrane protrusions and other RhoGTPase-dependent activities during EMT are not linear processes, and rather depend on the integrated activities of many members of the RhoGTPase family and their interaction with various regulatory proteins which will eventually determine the time and localization of their specific activities.

7.2 Membrane ruffles

The onset of motility requires a relaxation of static actin structures in order to form pliable membrane protrusions. Rigid actin stress fibers are disassembled upon dorsal circular ruffle (DCR) formation leaving a fine cortical actin meshwork behind, from which cell membrane protrusions like lamellipodia can emerge [150, 151]. DCRs are short-lived actin structures formed at the dorsal surface of growth factor (PDGF, HGF, EGF)-stimulated cells. DCRs, formed at the leading edge of growth factor-stimulated cells, are also able to secrete MMP (e.g. MMP-2), revealing their potential role for the onset of cell invasion [152]. Besides relaxing static membrane structures, DCRs are also important for macropinocytosis in growth factor-stimulated epithelial cells [153, 154]. Macropinocytosis plays an important role in the modulation of cell signaling; it may either inhibit signal transduction via degradation of growth factor receptor complexes or it may potentiate signaling, as shown in the case of EGF [154]. DCRs are enriched with actin assembly proteins, such as Arp2/3, WASP and cortactin, suggesting that DCR formation requires actin assembly. As mediators of growth factor signaling, RhoGTPases, like Rac1 or Cdc42, are required for DCRs formation as well [155]. Other reports indicate the involvement of protein kinase A (PKA) and the cytoplasmic tyrosine kinase Abl in DCR formation [156]. Both, PKA and Abl, are known to be involved in EMT [157–159].

β-catenin in a complex with APC is also found to be localized at the leading edge of migratory cells, implying an important role for cell polarization and migration by linking microtubules to the actin cytoskeleton [160–162]. Notably, β-catenin seems to exert its function at the leading edge of cells by co-localizing with N-cadherin and IQGAP1 in membrane ruffles [118]. IQGAP1, an effector of Cdc42 and Rac1, here acts as a key regulator of internalization of N-cadherin and APC. The fact that PDGFR also co-localizes with N-cadherin in membrane ruffles suggests an interesting overlap of proteins known to be important in both EMT and ruffle or podosome formation [87].

Other newly identified players in DCR formation and important for motility are palladin and its interaction partner Eps8 [163, 164]. Palladin binds F-actin and cross-links actin filaments into bundles. It also interacts with Src, thereby affecting Src-mediated actin remodeling [165].

Both proteins are also involved in podosome formation which highlights the kinship between DCRs and podosomes [163]. The cancer relevance of Eps8 and palladin is underlined by their increased expression in various human cancers, including breast and thyroid cancer [166–170]. Interestingly, palladin expression appears to be induced by treatment of myofibroblasts with TGFβ1, suggesting a link between TGFβ signaling, a major inducer of EMT, and the formation of ruffles and podosomes [171].

7.3 Lamellipodia and filopodia

To be able to leave the primary tumor and to disseminate to secondary sites, tumor cells have to break down cell-cell and cell-matrix junctions, as described above, and they have to invade into the surrounding tissue by gaining motility and forming invasive, matrix degrading structures. On planar two-dimensional culture substrates, cells utilize two well-known organelles to explore and move into the surrounding environment, filopodia and lamellipodia. Both are actin-rich membrane protrusions that are formed upon remodeling of the actin cytoskeleton beneath the plasma membrane.

Lamellipodia are flat, sheet-like protrusion and they are the main organelle for cell locomotion. The unbranched, long actin filaments at the base of the lamellipodium are progressing into a highly, lateral branched actin network at the leading edge, giving lamellipodia their typical structure (Fig. 3 b). Here, actin assembly and lateral branching (dendritic nucleation) are mainly controlled by the Arp2/3 complex, a seven subunit protein and major initiator of actin assembly. The activity of the Arp2/3 complex itself is controlled by actin nucleation-promoting factors, such as the N-WASP or the Scar/WAVE complexes which are themselves recruited to and activated at the membrane by Rac1 [172–174]. Increased expression of Arp2/3 and WAVE2 has been shown to correlate with poor prognosis in breast and liver carcinomas underlining the relevance of lamellipodia-like structures in cancer progression [175, 176]. Furthermore, the formation of lamellipodia is also observed upon ErbB2-driven EMT in epithelial cells, indicating that the formation of lamellipodia-like structures underlies the increased invasiveness observed during EMT [177].

Lamellipodia interact and attach to their environment via different adhesion molecules, including integrins and cadherins [84, 178]. Also, CD44, the hyaluronan receptor was identified to be in a complex with the protease MT1-MMP in lamellipodial protrusions [179]. Here, MT1-MMP binds directly to CD44 and mediates its proteolytic cleavage, thereby stimulating migration (Fig. 3 b).

In contrast, filopodia are rod-like extension consisting of tight bundled actin fibers which penetrate into the surrounding environment originating from the basis of lamellipodia. Filopodia can be considered as a sensory organ of the cell that are used to detect and assimilate signals like chemoattractants or nutrients released from e.g. blood vessels. Interestingly, metastatic cells are rich in filopodia-like structures, which correlates with their invasiveness [180, 181]. Filopodia formation is based on non-branched, processive actin assembly, controlled by fascin, diaphanous and Ena/VASP [173, 182] (Fig. 3 b). Actin bundling via fascin, diaphanous and Ena/VASP, on the other hand, is controlled by Rac and Cdc42. Fascin upregulation correlates with poor prognosis in different cancer types, including gastric cancer, lung cancer and breast cancer [183–185]. Notably, fascin is a direct target the β-catenin/Tcf signaling pathway, which is active in the process of EMT as well [71]. Recently, it has been reported that filopodia can transform into lamellipodia by initiating dendritic actin nucleation, characteristic for lamellipodia formation, demonstrating that both filopodia and lamellipodia are highly interactive, inter-convertible structures [186].

7.4 Podosomes and invadopodia

To move into or through a three-dimensional matrix, the real life situation, cells have to gain the capability to remodel the ECM via the expression of proteinases. In the last few years, actin-rich protrusion with ECM proteolytic activity, called invadopodia, have drawn particular attention in the context of cancer cell invasion. Invadopodia are considered the transformed counterparts of podosomes, which are formed by non-transformed yet highly migratory cells, such as macrophages, dendritic cells, osteoclasts, and activated smooth muscle and endothelial cells. The exact functions of podosomes are still rather elusive, yet it seems that they are formed when cell adhesion junctions and matrix needs to be concomitantly degraded. This may be the case, when immune cells like monocytes or dendritic cells have to cross tissue boundaries or during bone resorption by osteoclasts for the control of bone homeostasis [187]. The similarities or non-similarities of podosomes and invadopodia are still under debate, yet the most striking differences between both lies in the degree of ECM degradation and their half-lives. Invadopodia are strong degraders of ECM and persist for up to one h, whereas podosomes exhibit less ECM degradation activity and are stable only for minutes. Common for invadopodia and podosomes is their architecture. They share an actin filament-rich core containing the actin assembly machinery (WASP, cortactin and Arp2/3) and a multimeric protein complex surrounding the actin core, consisting of integrins and integrin-associated proteins like vinculin, talin and paxillin (Fig. 3 a). The multimeric adhesive ring complex is

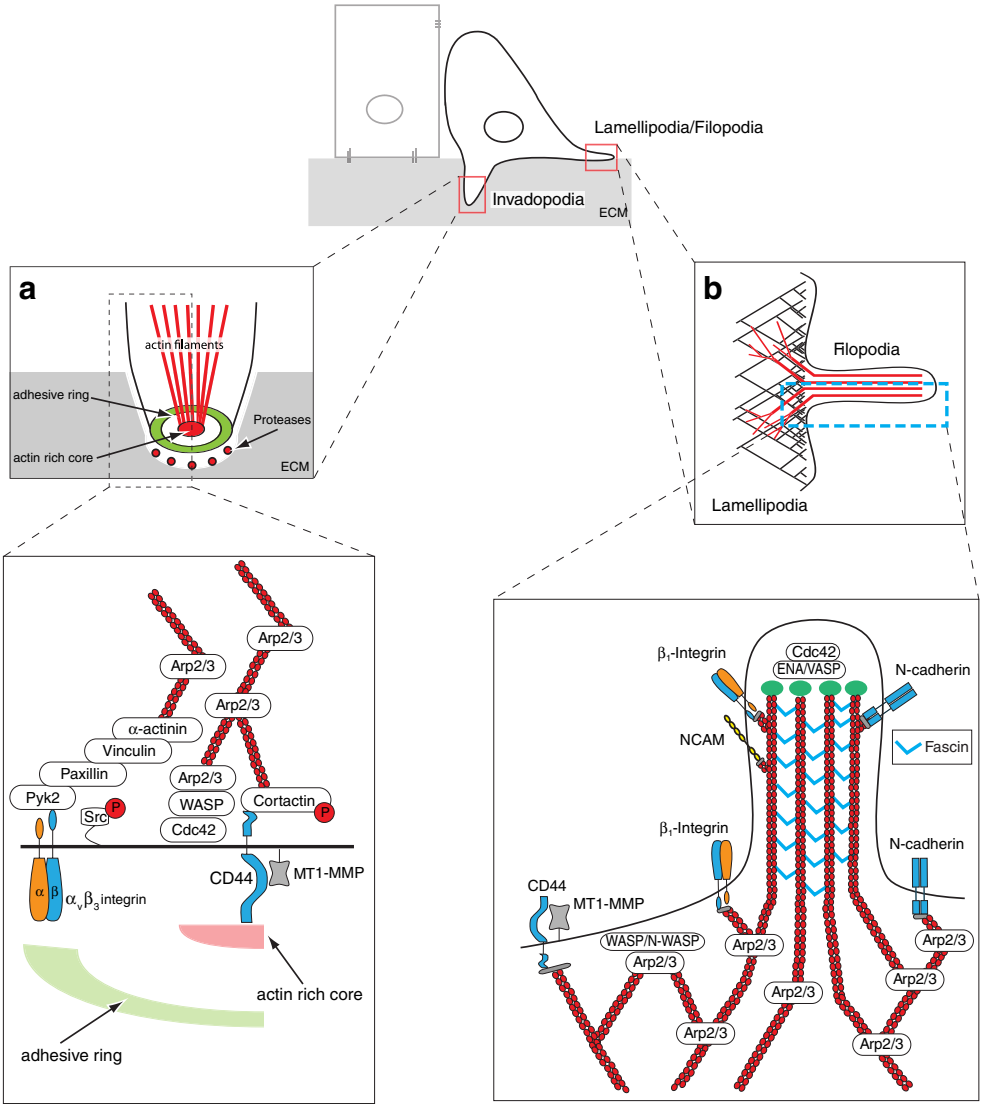

Fig. 3 Upon completion of EMT, spindle-shaped, invasive cells migrate and invade into the surrounding environment by utilizing (i) membrane protrusion like lamellipodia and filopodia for horizontal cell movement and (ii) podosome-derived invasive structures, invadopodia, for ventral invasion. **a** Invadopodia-mediated invasion. Invadopodia are actin and phosphotyrosine-rich membrane protrusions with a high extracellular matrix degrading capacity. Invadopodia consist of an outer adhesive ring and a central actin-rich core. The non-receptor tyrosine kinase Src, the actin binding protein cortactin and the membrane bound matrix metalloproteinase MT1-MMP play an essential role in the formation and function of invadopodia. Integrins (like $\alpha_v\beta_3$-integrin), along with their cytoplasmic interaction partners, are positioned in the adhesive ring where they mediate adhesion. The actin organization and nucleation is controlled by the Arp2/3 protein complex (adapted from Block et al. [191]). **b** Lamellipodia/filopodia-mediated migration. Lamellipodia are the major organelles for cell movement and build upon highly branched dendritic actin networks, which are initiated by the nucleation promoting factors WASP/N-WASP and the Arp2/3 complex. Lamellipodia interact with their environment via N-cadherin, β_1-integrin or the hyaluronan receptor CD44, the latter also important for the correct positioning of the membrane-bound MT1-MMP. Filopodia are stiff, rod-like extension formed by tightly bundled actin filaments. They function as sensory organs of cells that penetrate into the surrounding microenvironment. They originate from the root of lamellipodia and are initiated and controlled by the concerted activity of the RhoGTPase Cdc42 and nucleation-promoting factor ENA/VASP. The major determinant for actin bundling and filopodia morphology is fascin. Filopodia interact with their environment via the cell adhesion molecules N-cadherin, NCAM, and β_1-integrins

connected to the actin core via radial actin filaments [188–191].

Invadopodia of invading tumors cells mediate proteolysis of the ECM via the expression of different MMP, the most prominent being MT1-MMP collagenase and one of its direct targets, MMP2 gelatinase [192]. Invadopodia have been identified in numerous cancer cell lines, including malignant melanoma, breast cancer, glioma, and head and neck cancer [193–196]. The formation of invadopodia can be initiated by various signals, for example by EGF, HGF or TGFβ-induced signal transduction [193, 197, 198] or by $\alpha_6\beta_1$-integrin engagement [199]. Invadopodia-inducing

growth factors are not only produced by cancer cells themselves but are frequently supplied by tumor-associated macrophages (TAM), which themselves are attracted to the tumor microenvironment by tumor-released chemokines, such as colony stimulating factor 1 (CSF-1) or placental growth factor (PlGF). TAM are well-known to increase cancer cell invasion for example by inducing EGF driven invadopodia formation in the cancer cells [200, 201]. Besides their important role in primary tumor invasion and tumor cell intravasation into the blood or the lymphatic circulation, invadopodia are also critical for extravasation at secondary sites. Thereby, the chemokines S100A8 and S100A9, produced by endothelial or myeloid cells at the premetastatic niche, attract circulating tumor cells and facilitate their extravasation and invasion at the secondary site by inducing the formation of tumor cell invadopodia [202].

The formation of invadopodia follows distinct steps [194]. First, a local enrichment of actin and cortactin at sites of cell-ECM contact initiates the recruitment of MT1-MMP. This stage is named the pre-invadopodia stage and is then followed by further recruitment of actin, cortactin and MT1-MMP, which leads to matrix degradation and the formation of mature, invading invadopodia. Late invadopodia are defined by dispersal of actin and cortactin leaving an MT1-MMP enriched structure that maintains matrix degradation. A recent report proposes important roles for the calcium-dependent protease calpain-2, protein tyrosine phosphatase PTP1B, and Src for the onset of invadopodia formation and the regulation of invadopodia turnover [203]. Apparently, activation of integrins and/or EGF receptor leads to increased calpain-2 activity, which subsequently proteolytically processes and activates PTP1B. Activated PTP1B wields dephosphorylation of an inhibitory phosphotyrosine of Src resulting in increased Src tyrosine kinase activity, Src-mediated phosphorylation of cortactin and, subsequently, to actin assembly. Conversely, activated Src phosphorylates calpain-2, resulting in proteolytic cleavage and inactivation of cortactin and, thus, invadopodia disassembly. Both Src and cortactin are essential for invadopodia formation and their increased activity can be used as a potential marker for the onset of invadopodia formation [204, 205]. Downstream targets of activated Src which are known to be essential for invadopodia formation and maintenance include cortactin, N-WASP, ArfGAPs like AMAP1/AMAP2, paxillin and Tks5/Fish [197, 206–209].

Invadopodia are known to be enriched with integrins of the β_1-integrin family (α_3, α_5, α_6) or with $\alpha_v\beta_3$-integrin [207, 210, 211]. β_1-integrins are receptors for laminin, collagen and fibronectin, $\alpha_v\beta_3$-integrins preferentially bind to vitronectin. Furthermore, the interaction of $\alpha_v\beta_3$-integrin with MT1-MMP leads to the activation of immature MMP2, thereby promoting collagen proteolysis in invasive breast carcinomas [211]. Each of both proteins, $\alpha_v\beta_3$-integrin as well as MT1-MMP, have been shown to be important for EMT [122, 212]. The co-expression and most likely co-operation of MT1-MMP and β_1-integrin in EMT-driven invasiveness, under the control of the transcription factor Twist, suggests that proteins relevant for invadopodia formation are important for EMT as well [213].

Besides their function as mechanical anchors in the ECM, integrins actively participate in invadopodia formation. For example, engaged $\alpha_6\beta_1$-integrin induces phosphorylation of p190RhoGAP and promotes its localization in invadopodia, where it represses RhoA activity and supports the formation of membrane protrusions [214]. As key regulators of actin assembly, the major RhoGTPases RhoA, Rac1, and Cdc42 play essential roles in invadopodia formation. Elimination of the activities of Rac1 or Cdc42 results in a reduction of invadopodia formation in glioma cells and mammary carcinoma cells, respectively [197, 215]. On the other hand, RhoA is known to be important for actin assembly and exocytosis of MMP in invadopodia by controlling IQGAP1 activity [216]. For more detailed insights into invadopodia formation and function, we refer the reader to several excellent recent reviews [188, 217–219].

7.5 Lamellipodia, filopodia and their overlap with invadopodia

The kinship between the two-dimensional filopodia and lamellipodia with three-dimensional invadopodia is still under debate. It seems that invadopodia represent a hybrid of both structures. Like lamellipodia, invadopodia are built upon a branched actin network, yet they employ N-WASP for actin assembly instead of WAVE-2 used by lamellipodia. On the other hand, both invadopodia and filopodia engage Cdc42 for the induction of membrane protrusions which are long and thin in their appearance, suggesting that actin bundling could play an important role in invadopodia formation. However, whether filopodial actin-bundling proteins like fascin are located in invadopodia remains to be determined [220].

7.6 Invadopodia and their role in EMT

As illustrated above, EMT leads to increased motility and invasiveness of epithelial cells by dissolving epithelial cell-cell adhesion, modulating cell-matrix adhesion and by inducing the secretion of ECM degrading proteinases. Thus, it can be assumed that EMT provokes the formation of podosome or invadopodia-like structures. Surprisingly, up to date there is no experimental evidence that directly links EMT with the formation of invadopodia or podosomes. Nonetheless, a number of proteins that are important

for invadopodia formation and for the execution of EMT have been identified, strongly suggesting that invadopodia formation could be involved in EMT-driven cell invasion. Both EMT and invadopodia formation can be stimulated by various growth factors, including EGF, HGF, PDGF, and TGFβ [5, 197, 221]. The engagement of these growth factors leads to the activation of the non-receptor tyrosine kinase Src. The potency of Src to induce EMT and/or invadopodia formation is well established [203, 205, 222]. Moreover, downstream targets of Src-signaling, such as calpain, are upregulated during TGFβ-induced EMT and are required for invadopodia formation [203, 223]. Another protein, able to induce invadopodia and also involved in EMT, is endoglin, a cell surface adhesion molecule and co-receptor for TGFβ signaling [224]. Endoglin is needed for EMT during heart development, and its expression correlates with increased invasiveness of breast cancer cells via the increased formation of invadopodia [198, 225].

Most likely, invadopodia and podosomes are not only matrix degrading structures but also function as guidance organelles that provide a cell with the ability to probe its microenvironment by exploring potential cell-cell and cell-matrix adhesions and by sensing chemoattractive cues. Interestingly, PDGF induces the translocation of cortactin to podosomes via the GAP BPGAP1 and Rac1, indicating that PDGF receptor is involved in the formation of podosomes [226, 227]. As described above, PDGFR colocalizes with N-cadherin in membrane ruffles, and both N-cadherin and cortactin are found in the growth cones of emerging neuronal axons and exert a crucial role in neuron guidance [81, 228]. The co-localization of cortactin with the N-cadherin/NCAM/FGFR complex and NCAM-mediated FGFR signaling have been shown to contribute to neurite outgrowth [95]. Thus, consistent with its upregulated expression during EMT (the cadherin switch, see above), N-cadherin is likely to be involved in the formation of invadopodia.

8 Outlook

In this review, we have attempted to summarize recent novel insights into the functional contribution of cellular processes, such as the formation of membrane protrusions and the reorganization of the actin cytoskeleton to EMT and tumor metastasis. While there is an increasing functional correlation between the formation of migratory and invasive membrane protrusions and the process of EMT, a direct link between the cellular and molecular processes underlying the formation of lamellipodia, filopodia and invadopodia on one hand, and EMT and tumor metastasis on the other hand, still remains to be established. Apparently, if such link can be ascertained, the molecular mechanisms and genes and factors underlying the cellular organization of the cytoskeleton and the formation of membrane protrusions move into the spotlight of medical relevance, i.e. the design and development of innovative therapeutic approaches to interfere with cancer cell invasion and metastatic dissemination. Hence, future research to resolve these issues is urgently warranted.

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