Published in final edited form as:
Eur J Cell Biol. 2014 October ; 93(0): 438–444. doi:10.1016/j.ejcb.2014.07.003.

Digging a little deeper: the stages of invadopodium formation and maturation

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Abstract

Invadopodia are actin-rich protrusions that degrade the extracellular matrix and are required for penetration through the basement membrane, stromal invasion and intravasation. Invadopodia are enriched in actin regulators, such as cortactin, cofilin, N-WASp, Arp2/3 and fascin. Much of the work to date has centered around identifying the proteins involved in regulating actin polymerization and matrix degradation. Recently, there have been significant advances in characterization of the very early stages of invadopodium precursor assembly and the role of adhesion proteins, such as β1 integrin, talin, FAK and Hic-5, in promoting invadopodium maturation. This review summarizes these findings in the context of our current model of invadopodial function and highlights some of the important unanswered questions in the field.

Keywords

invadopodia; invasion; metastasis; β1 integrin; Arg; talin; moesin; NHE-1; cofilin; cdc42

Introduction

Tumor cell metastasis is a multistep process that involves escape from the primary tumor, migration through the stroma, entry into the vasculature and dissemination to distant sites. Invadopodia are finger-like, actin-rich protrusions that are formed by metastatic tumor cells to degrade the extracellular matrix (ECM). Analogous to podosomes formed by hematopoietic cells and rosettes found in Src-transformed fibroblasts, smooth muscle and endothelial cells, invadopodia are formed by tumor cells to facilitate breach of the basement membrane surrounding carcinoma *in situ*, invasive cancer cell migration through the dense

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stromal ECM and degradation of the endothelial basement membrane for entry into the blood (Bravo-Cordero et al., 2012; Destaing et al., 2010; Eckert et al., 2011; Linder et al., 1999; Moreau et al., 2003; Yamaguchi et al., 2005). Pioneering work by Chen and colleagues demonstrated that invadopodia are capable of degrading many different types of ECM, including collagen types I and IV, laminin and fibronectin (Kelly et al., 1994). Subsequent work demonstrated that invadopodia also degrade native, tissue-derived basement membranes (Parekh et al., 2011; Schoumacher et al., 2010). As a number of reviews have discussed the similarities and differences between invadopodia and podosomes (Block et al., 2008; Destaing et al., 2011; Gimona et al., 2008; Linder et al., 2011; Murphy and Courtneidge, 2011), this review will focus on recent advances in characterizing the early stages of invadopodium precursor assembly as well as the invadopodium maturation phase (actin polymerization and matrix degradation).

Invadopodium precursor formation

Stimuli of invadopodium assembly

Invadopodium precursors are defined as complexes of invadopodial proteins that do not degrade the ECM. Many stimuli have been reported to induce invadopodium precursor formation (Figure 1 - Stage 1). These stimuli can be grouped into the following categories: growth factors, oncogenic transformation, induction of the epithelial-mesenchymal transition (EMT), hypoxia and matrix metalloprotease (MMP) activity (presumably through the generation of degraded ECM fragments; Clark et al., 2007; Eckert et al., 2011; Pignatelli et al., 2012b; Yamaguchi et al., 2005). Although the epidermal growth factor (EGF) is the best characterized growth factor stimulus, transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF) and heparin binding EGF (HB-EGF) have been shown to induce invadopodium precursor formation in a number of different tumor cell types (Diaz et al., 2013; Eckert et al., 2011; Pignatelli et al., 2012b; Rajadurai et al., 2012; Yamaguchi et al., 2005). EGF receptor (EGFR) ligands, EGF and HB-EGF, induce the formation of invadopodium precursors in starved breast cancer cells and are sufficient to activate the pathways leading to actin polymerization and ultimately matrix degradation (Busco et al., 2010; Zhou et al., 2013). TGF-β and PDGFR ligands, on the other hand, are able to induce *de novo* invadopodium formation in normal breast and mammary epithelial cells, respectively, which do not normally form these structures (Eckert et al., 2011; Pignatelli et al., 2012b). Finally, it should be noted that tumor cells can also form invadopodia in the absence of external stimuli via autocrine signaling, likely involving the abovementioned growth factors.

Invadopodium-like invasive protrusions were initially identified in chicken embryonic fibroblasts that were transformed with Rous sarcoma virus (Chen, 1989). In breast and pancreatic epithelial cells, Src and Ras transformation are sufficient to induce *de novo* invadopodium precursor formation (Neel et al., 2012; Pignatelli et al., 2012b). TGF-β-induced transformation or induction of EMT by Twist1 can also stimulate invadopodium formation in epithelial cells (Eckert et al., 2011; Pignatelli et al., 2012b). Finally, other microenvironmental factors, such as hypoxia and perhaps degraded ECM products, can promote invadopodium formation (Arsenault et al., 2013; Clark et al., 2007; Diaz et al.,

2013). Hypoxia, for example, stimulates invadopodium function via ADAM12-dependent release of HB-EGF in multiple cancer cell lines (Diaz et al., 2013).

Although invadopodia form in response to multiple extracellular cues, these pathways appear to converge at the level of the Rho family GTPase, Cdc42. In contrast to the other major Rho GTPases (RhoA, RhoC and Rac; Box 1), Cdc42 depletion in mammary adenocarcinoma cells completely abrogates EGF-induced invadopodium precursor formation as well as invadopodium formation at steady state (Desmarais et al., 2009; Yamaguchi et al., 2005). Similarly, Cdc42 is required for actin punctum formation in pancreatic tumor cells (Razidlo et al., 2014).

Rho GTPases are activated by guanine exchange factors (GEFs), which stabilize the GTP-bound (active) form of the GTPase, leading to activation of downstream GTPase targets (Rossman et al., 2005). A number of Cdc42 GEFs have been implicated in invadopodium formation, including Vav1, β-PIX and Fgd1 (Ayala et al., 2009; Md Hashim et al., 2013; Razidlo et al., 2014). Recently, Src has been shown to activate Vav1, which, in turn, activates Cdc42 to induce invadopodium formation (Razidlo et al., 2014). β-PIX is essential for hypoxia-induced invadopodium formation, while the Cdc42-specific GEF Fgd1 also promotes invadopodium formation (Ayala et al., 2009; Md Hashim et al., 2013). Interestingly, as all three GEFs are activated by EGF-induced Src phosphorylation (Feng et al., 2010; Miyamoto et al., 2003; Razidlo et al., 2014), it is tempting to speculate that the EGFR-Src-GEF-Cdc42 axis may represent a major pathway for initiation of invadopodium precursor assembly (Figure 2). However, since Src is not required for precursor formation in all cases (Mader et al., 2011), an important future direction will be to better characterize the multiple input pathways that initiate assembly of the precursor core structure.

Invadopodium precursor assembly and anchoring

Recently, high temporal resolution microscopy has demonstrated that invadopodium precursors are assembled in a highly orchestrated manner (Sharma et al., 2013). Invadopodial core proteins, cortactin, cofilin and N-WASp form an initial nucleus, or core structure, that is associated with an actin filament (Figure 1 - Stage 1; Artym et al., 2006; Sharma et al., 2013). The adaptor protein Tks5 joins the complex approximately 20 seconds later in order to anchor it to the phosphoinositide $PI(3,4)P_2$ via its PX domain (Sharma et al., 2013). Formation of $PI(3,4)P_2$ at the plasma membrane is thought to occur in a step-wise manner, in which EGFR activates phosphoinositide-3 kinase (PI-3K) to convert $PI(4,5)P_2$ into $PI(3,4,5)P_3$. PIP_3 is then dephosphorylated by the 5'-phosphatases Ship2 or synaptojanin2 to form $PI(3,4)P_2$ (Figure 2; Chuang et al., 2004; Sharma et al., 2013; Yamaguchi et al., 2011). Around 2-3 minutes after invadopodium precursor assembly begins, Tks5 binds to $PI(3,4)P_2$ to anchor the structure to the membrane (Sharma et al., 2013). The adhesion receptor β1 integrin is then recruited to the structure, activated and binds to ECM ligands to further stabilize the structure and trigger the maturation process (i.e. actin polymerization and matrix degradation; Figure 1 - Stage 2; Figure 2; Beaty et al., 2013; Sharma et al., 2013).

Invadopodium maturation

An emerging role for adhesion proteins at invadopodia

A long-standing question in the invadopodium field has been: do invadopodia adhere to the ECM (Gimona et al., 2008; Linder et al., 2011)? Many adhesion proteins have been reported to localize to invadopodia, including vinculin, paxillin, filamin, NEDD9, as well as integrins (α3, α5 and β1 integrins, but not β3 integrin; Beaty et al., 2013; Branch et al., 2012; Coopman et al., 1996; Mueller et al., 1999; Sharma et al., 2013; Takkunen et al., 2010). Integrins are adhesion receptors that have three conformational states: inactive (bent/closed), activated (extended) and adherent (extended and bound to the ECM; Askari et al., 2010; Frelinger et al., 1988; Mould and Humphries, 2004; Nishida et al., 2006; Xiong et al., 2001). We have recently shown that β1 integrin is activated in the invadopodium core and that stimulation of β1 integrin-mediated adhesion accelerates precursor maturation into matrix-degrading invadopodia (Figure 1 - Stages 2-4; Beaty et al., 2013). Although β1 integrin does not affect invadopodium precursor assembly, it is required for invadopodium stability through adhesion to the ECM and activating actin polymerization (see below).

β1 integrin promotes invadopodium maturation, specifically by interacting with the tyrosine kinase Arg (Beaty et al., 2013). Interestingly, β1 integrin-EGFR crosstalk is required for Arg activation, as neither β1 integrin activation nor EGF stimulation alone is sufficient to induce Arg activation for invadopodium maturation (Beaty et al., 2013). Arg binding to the β1 integrin cytoplasmic tail is thought to disrupt its autoinhibitory conformation, unmasking Y272 for Arg autophosphorylation (Tanis et al., 2003). EGFR-Src-mediated phosphorylation of Arg Y439 on its activation loop then results in full Arg activation (Bradley and Koleske, 2009; Tanis et al., 2003). Arg phosphorylates cortactin on Y421 and Y466, which recruit Nck1, an adapter protein that binds N-WASp to facilitate Arp2/3 activation (Mader et al., 2011; Oser et al., 2010). This ultimately leads to synergistic cofilin-Arp2/3-dependent actin polymerization (Figure 2).

Like β1 integrin, the focal adhesion protein talin does not regulate precursor assembly (Beaty et al., 2014); rather, talin is essential for invadopodium maturation and tumor cell metastasis, as depletion of talin blocks invadopodial matrix degradation, invasion through 3D ECM and intravasation and spontaneous lung metastasis *in vivo* (Beaty et al., 2014). Talin is recruited to precursors by binding to actin via the I/LWEQ domain in its C-terminus (talin rod; Beaty et al., 2014). Talin binds directly to the ezrin-radixin-moesin (ERM) family protein, moesin, *in vitro* and is required for moesin recruitment to invadopodia in MDA-MB-231 cells (Beaty et al., 2014). Moesin, in turn, recruits the sodium-hydrogen exchanger-1 (NHE-1) to invadopodia, where it extrudes H+ from the cell in order to acidify the ECM as well as increase the intracellular pH to disrupt the inhibitory interaction between cortactin and the F-actin severing protein, cofilin (Beaty et al., 2014; Busco et al., 2010; Denker et al., 2000; Magalhaes et al., 2011). This allows active cofilin to sever F-actin to generate free actin barbed ends, which elongate new filaments that support nucleation by the Arp2/3 complex (and potentially direct nucleation by cofilin) for dendritic actin polymerization (Andrianantoandro and Pollard, 2006; Bravo-Cordero et al., 2013; DesMarais et al., 2004). Thus, by regulating moesin and NHE-1 recruitment to invadopodia,

talin promotes cofilin-dependent actin polymerization and matrix degradation (Figure 1 - Stages 3-4; Figure 2).

Cofilin activity is tightly regulated in a spatiotemporal manner. In addition to being regulated by binding to cortactin, cofilin activity is controlled by phosphorylation on serine 3 (Yang et al., 1998). This residue is phosphorylated by LIM domain kinases (LIMK) and TES kinases (TESK) and is dephosphorylated by slingshot and chronophin (Gohla et al., 2005; Niwa et al., 2002; Toshima et al., 2001; Yang et al., 1998). At invadopodia, cofilin activity is stimulated by the talin-moesin-NHE-1 complex and suppressed by the RhoC-ROCK-LIMK pathway (Beaty et al., 2014; Bravo-Cordero et al., 2011; Magalhaes et al., 2011). p190RhoGEF, a RhoC GEF, localizes in a ring around the invadopodium core to locally activate RhoC, whereas p190RhoGAP, an inactivator of RhoC, is enriched at the invadopodium core (Bravo-Cordero et al., 2011). The result is RhoC-ROCK-mediated LIMK activation and cofilin phosphorylation (inactivation) outside of invadopodia and active cofilin concentrated at the invadopodium core, where RhoC is inactive (Bravo-Cordero et al., 2011). Interestingly, β1 integrin induces Arg-dependent p190RhoGAP phosphorylation and activation in fibroblasts and neurons (Bradley et al., 2006; Kerrisk et al., 2013; Warren et al., 2012). Thus, it will be interesting to explore the possibility that β1 integrin-Arg signaling may act as a master upstream regulator of invadopodial cofilin activity through Arg-mediated phosphorylation of both cortactin on Y421 and p190RhoGAP at the invadopodia core to relieve cortactin- and RhoC-dependent suppression of cofilin activity, respectively.

The focal adhesion proteins, focal adhesion kinase (FAK), Hic-5, integrin-linked kinase (ILK) and NEDD9, also regulate invadopodia. FAK regulates invadopodium formation indirectly by sequestering active Src at focal adhesions (Chan et al., 2009). FAK knockdown leads to redistribution of Src from focal adhesions to invadopodia, resulting in increased invadopodium formation, but impaired invasion through fibronectin due to reduced focal adhesion turnover and degradation capacity per invadopodium (Chan et al., 2009; Oser et al., 2009). Ectopic Hic-5 expression or knockdown of the endocytic adaptor protein β2-adaptin induce invadopodium matrix degradation via Src activation in normal MCF10A epithelial cells (Pignatelli et al., 2012a; Pignatelli et al., 2012b). Thus, FAK, Hic-5 and β2-adaptin regulate Src-dependent invadopodium function.

ILK and the docking protein NEDD9 regulate MMP surface expression at invadopodia. ILK recruits the scaffold protein IQGAP to invadopodia to induce membrane type-I MMP (MT1-MMP) exocytosis, while NEDD9 limits the accumulation of tissue inhibitor of metalloproteinase-2 (TIMP2), an endogenous MT1-MMP inhibitor, at invadopodia to promote MT1-MMP-mediated matrix degradation (Branch et al., 2012; McLaughlin et al., 2014). Taken together, adhesion proteins regulate invadopodium maturation by enhancing actin polymerization and MMP-mediated matrix degradation, while having limited effects on invadopodium precursor assembly.

Unbranched actin polymerization

While much of the work done on invadopodia has focused on the dendritic actin network generated by Arp2/3, there is strong evidence that invadopodia also contain linear, bundled

actin filaments (Li et al., 2010; Schoumacher et al., 2010). Diaphanous-related formins (DRF) are a family of actin nucleators that induce the formation of linear actin networks, such as those found in stress fibers and filopodia (Lizarraga et al., 2009). mDia2 (mouse orthologue of DRF3) localizes to invadopodia, and DRF1-3 have been shown to promote invadopodium maturation in MDA-MB-231 cells (Lizarraga et al., 2009). More specifically, mDia2 has been shown to promote invadopodial elongation and stability in 3D ECM (Figure 1 – Stage 4; Lizarraga et al., 2009; Schoumacher et al., 2010). Similarly, fascin, an actin bundling protein, localizes to invadopodia to promote stability, elongation and matrix degradation (Li et al., 2010; Schoumacher et al., 2010). Thus, regulators of linear actin filaments play an important role in invadopodium maturation.

The role of microtubules and intermediate filaments in invadopodium maturation

Mature invadopodia also contain microtubules and intermediate filaments, namely vimentin (Schoumacher et al., 2010). Although these cytoskeletal elements have not been investigated as extensively as actin-associated proteins, a number of recent studies point to their importance in invadopodium function (Kikuchi and Takahashi, 2008; Schoumacher et al., 2010). Interestingly, disruption of microtubules by treating cells with the microtubule-stabilizing agent paclitaxel, microtubule-destabilizing agent nocodazole, or knockdown of vimentin does not affect initial invadopodium formation (Kikuchi and Takahashi, 2008; Schoumacher et al., 2010); however, microtubules and intermediate filaments are required for invadopodial elongation (Figure 1 - Stages 4; Schoumacher et al., 2010). Taken together, data indicate that actin is critical for all stages of invadopodium formation and maturation, whereas microtubules and intermediate filaments regulate the later stages of invadopodium maturation.

Protease recruitment: the culmination of invadopodium maturation

Invadopodium maturation is a complex process that requires coordination of many different proteins and culminates in the accumulation of a number of proteases, including seprase, cathepsins and MMPs (Brisson et al., 2013; Mueller et al., 1999; Sakurai-Yageta et al., 2008). RhoA and Cdc42 stimulate the association of the polarity protein IQGAP1 with the exocyst complex, which cooperates with endosomal Wiskott-Aldrich syndrome protein and Scar homolog (WASH) to promote MT1-MMP delivery to invadopodia (Figure 2; Monteiro et al., 2013; Sakurai-Yageta et al., 2008). Cortactin also regulates invadopodial MT1-MMP trafficking and MMP-2 and MMP-9 secretion (Clark et al., 2007). The v-SNARE VAMP7 then localizes to invadopodia and facilitates MT1-MMP vesicle anchoring at invadopodia for MT1-MMP insertion into the plasma membrane (Steffen et al., 2008). Last, MMP-2 and MMP-9 are delivered to invadopodia, through a Rab40b- and VAMP4-dependent mechanism, and synergize with MT1-MMP to degrade the surrounding ECM at invadopodia (Jacob et al., 2013; Marrero-Diaz et al., 2009; Sakurai-Yageta et al., 2008).

MT1-MMP trafficking is also regulated by Cdc42-interacting protein-4 (CIP4). CIP4 is an F-BAR protein that promotes MT1-MMP endocytosis to limit ECM degradation; however, when Src is activated at invadopodia, it phosphorylates and inactivates CIP4, resulting in MT1-MMP accumulation at the plasma membrane (Hu et al., 2011). Integrins have also been shown to regulate MT1-MMP trafficking. β1 integrin abrogates MT1-MMP

endocytosis in human endothelial cells, and integrin-mediated adhesion induces MT1-MMP exocytosis at 3D invadopodia in a Rab8-dependent manner (Bravo-Cordero et al., 2007; Galvez et al., 2002). As β1 integrin localizes to invadopodia (Coopman et al., 1996), this raises the intriguing possibility that β1 integrin may cooperate with Src-CIP4 to stabilize MT1-MMP at the cell surface of invadopodia and facilitate matrix degradation.

Conclusion

Significant advances have been made in understanding the initiation and function of invadopodia in tumor cells. Recent advances in characterizing the early stages of invadopodium precursor formation and the molecular mechanisms of invadopodium maturation have increased our understanding of the regulation of these structures and their *in vivo* functions in cancer progression. An important role for adhesion proteins has recently emerged: these proteins localize to late invadopodium precursors at the onset of the maturation process (Figure 1 – Stage 2) to promote actin polymerization and matrix degradation (Figure 1 - Stages 3-4). However, there are a number of open questions in the field that remain unresolved.

First, how is the early invadopodium precursor assembled? While a role for EGFR-Src-Vav1-Cdc42 has been recently identified (Desmarais et al., 2009; Razidlo et al., 2014), the molecular mechanisms by which Cdc42 induces precursor assembly are poorly understood. The upstream GEFs and GAPs that regulate Cdc42 activity and its downstream effectors during the very early stages of invadopodium precursor assembly are not known. For example, it is assumed that N-WASp is the primary Cdc42 effector activated during invadopodium precursor formation, yet Cdc42 knockdown completely abrogates precursor formation, whereas N-WASp depletion only partially blocks their formation in mammary adenocarcinoma cells (Desmarais et al., 2009). This suggests that Cdc42 activates multiple downstream targets at invadopodia. In addition, it is not clear what other Cdc42-independent pathways stimulate invadopodium formation in other tumor types. Thus, an important area of future study is to identify the proteins that are necessary for invadopodium precursor formation using synchronized starvation-growth factor stimulation assays and to determine the relative contributions of the different Cdc42 effectors in precursor assembly.

Another important issue is the development of consensus markers that uniquely identify invadopodia. In the past, matrix degradation was thought to be a distinguishing feature of invadopodia; however, recent work has demonstrated that structures traditionally considered to be “non-degradative” (e.g. focal adhesions and filopodia) actually degrade matrix in some contexts (Starnes et al., 2014; Wang and McNiven, 2012). Thus, a minimum of two invadopodial markers is needed to differentiate these structures. Using actin or cortactin as solitary markers is not sufficient since these proteins are present in other structures that degrade the matrix. We and others have used actin-associated proteins (e.g. cortactin) together with an actin-independent marker (e.g. Tks5 or MT1-MMP) to definitively identify *bona fide* invadopodium precursors (not associated with ECM degradation) or mature invadopodia (associated with ECM degradation; Artym et al., 2006; Beaty et al., 2013; Sakurai-Yageta et al., 2008).

Second, what determines the subcellular location of invadopodium formation in 2D and 3D ECM contexts? Although there is evidence that invadopodia and podosomes form at the proximal tips of focal adhesions in smooth muscle cells and MTLn3 cells (Burgstaller and Gimona, 2004; Sharma et al., 2013), this is not the case for many other cell types, including MDA-MB-231 cells (unpublished observation). Moreover, it is not clear that focal adhesions *per se* are required for invadopodium precursor formation. When MTLn3 and MDA-MB-231 cells are plated on poly-L-lysine to prevent integrin-mediated adhesion and focal adhesion formation (Lo et al., 1998), invadopodium precursors still form in response to growth factor stimulation (unpublished observation). Consistent with this finding, disruption of focal adhesion signaling by knockdown of key adhesion proteins either has no effect on invadopodium precursors (i.e. talin and β1 integrin) or increases precursor formation (i.e. FAK; Beaty et al., 2013; Beaty et al., 2014; Chan et al., 2009). This suggests that the formation of invadopodium precursors at the proximal tips of focal adhesions is due to the specific characteristics of this site (e.g. decreased contractility), rather than their specific association with adhesion proteins (Burgstaller and Gimona, 2004). In support of this notion, myosin II is dispersed from and p190RhoGAP is enriched at sites of prospective podosome formation to reduce local cytoskeletal contractility and allow for podosome assembly to occur (Burgstaller and Gimona, 2004). The generality of this finding has yet to be confirmed, but testing this hypothesis in tumor cells will provide important insights into potential mechanisms of invadopodium assembly.

Third, what proteins recruit RhoGTPase GEFs and GAPs to invadopodia? In other systems, p190RhoGEF and p190RhoGAP are recruited to focal adhesions through binding to FAK, but since FAK does not localize to invadopodia, it is not clear how these proteins are recruited (Chan et al., 2009; Lim et al., 2008; Tomar et al., 2009; Yu et al., 2011). Fourth, how are matrix degradation, adhesion, protrusion and translocation through degraded ECM coordinated in a 3D matrix setting? Finally, do invadopodia play a role in extravasation and colonization of secondary organs? While a role for invadopodia has been demonstrated in the case of stromal invasion and intravasation (Eckert et al., 2011; Roh-Johnson et al., 2013), it is yet to be determined if invadopodia are required for later stages of the metastatic cascade. Answers to these questions will add to our growing understanding of invadopodium function and allow us to better evaluate how specific invadopodial proteins may be targeted for clinical applications.

ACKNOWLEDGEMENTS

We thank Jose-Javier Bravo-Cordero, Jeanine Pignatelli and Ved Sharma for their thoughtful discussion. Research in the Condeelis lab has been funded by NIH CA150344 (J.C.) and the MSTP training grant T32-GM007288 (B.B.).

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Box 1. Rho GTPases in invadopodia

Rho GTPases are important regulators of actin dynamics at invadopodia. Cdc42 is one of the few proteins that is essential for initial invadopodium precursor assembly (see main text for further details; Desmarais et al., 2009; Sakurai-Yageta et al., 2008). RhoA, on the other hand, is dispensable for invadopodium precursor formation, but drives invadopodium maturation. Using a RhoA biosensor, we demonstrated that RhoA activity is low in the core of invadopodium precursors (Bravo-Cordero et al., 2011). Rather, RhoA appears to be specifically important for invadopodium maturation, as RhoA knockdown impairs ECM degradation, but only has a modest effect on the total number of invadopodia/cell (e.g. precursors and mature invadopodia; Bravo-Cordero et al., 2011). Consistent with this finding, RhoA cooperates with Cdc42 to stimulate exocyst-mediated MT1-MMP delivery to invadopodia (Sakurai-Yageta et al., 2008), and heterotypic cell contact between tumor cells and macrophages induces RhoA activation, leading to increased numbers of mature invadopodia (Roh-Johnson et al., 2013).

RhoC is another Rho GTPase isoform that regulates invadopodium function. A role for RhoC in invasion was first described by the Hynes group (Clark et al., 2000). Their work demonstrated that RhoC is necessary for extravasation in melanoma cells. In mammary adenocarcinoma cells, RhoC confines cofilin-dependent actin polymerization to the invadopodium core for efficient protrusion formation and focused matrix degradation (see main text for further details; Bravo-Cordero et al., 2011). RhoC depletion leads to the formation of shorter invadopodium protrusions associated with larger areas of shallow matrix degradation, leading to inefficient tumor cell invasion (Bravo-Cordero et al., 2011). Thus, similar to FAK knockdown, RhoC depletion results in increased matrix degradation, but impaired invasion, highlighting the importance of coordination between the actin machinery and matrix degradation during tumor cell invasion.

Although Rac1 has been shown to promote matrix degradation in melanoma and glioma cells (Chuang et al., 2004; Nakahara et al., 2003), Rac1 knockdown dramatically increases invadopodium matrix degradation in breast cancer cells, without affecting invadopodium precursor formation (Moshfegh et al., 2014). Using a Rac1 biosensor, we were able to show that Rac1 is inactive in invadopodium precursors, but is transiently activated immediately prior to invadopodium disassembly (Moshfegh et al., 2014). Localized stimulation of Rac1 by photoactivatable Rac induces rapid invadopodium disassembly, suggesting that the primary role of Rac1 in breast cancer cells is to regulate invadopodium disassembly, not formation or maturation (Moshfegh et al., 2014).

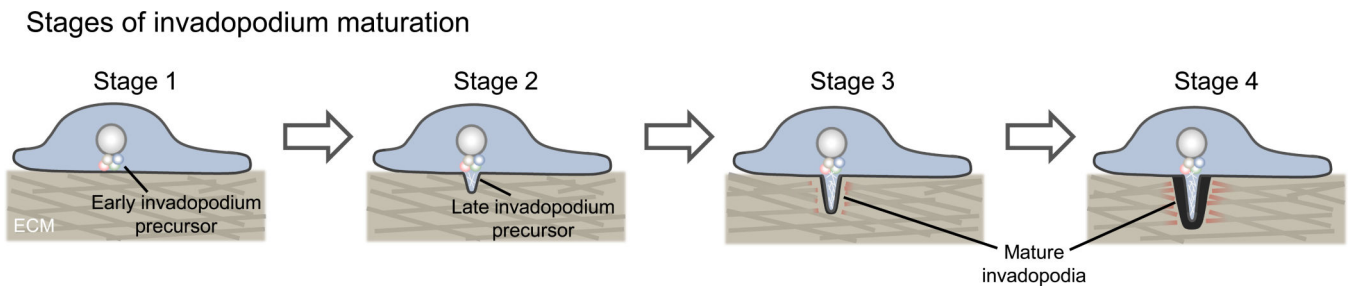

Figure 1. Stages of invadopodium maturation

Stage 1 (early precursor stage): invadopodia initially form as non-degradative precursors that consist of a core structure containing actin, cortactin, cofilin, N-WASp, Tks5 and other proteins. Stage 2 (late precursor stage): kinases are activated, β1 integrin and talin are recruited and Tks5 anchors the precursor to $PI(3,4)P_2$. Stages 3-4 (mature invadopodium stage): in stage 3, actin polymerization is activated by stimulation of the NHE-1-cofilin pathway, and continued actin polymerization drives invadopodial elongation and stabilization. In stage 4, microtubule and intermediate filament recruitment facilitates further elongation of the protrusion, and matrix proteases are recruited to degrade the ECM (modified from Artym et al., 2006; Oser et al., 2009; Schoumacher et al., 2010; Sharma et al., 2013).

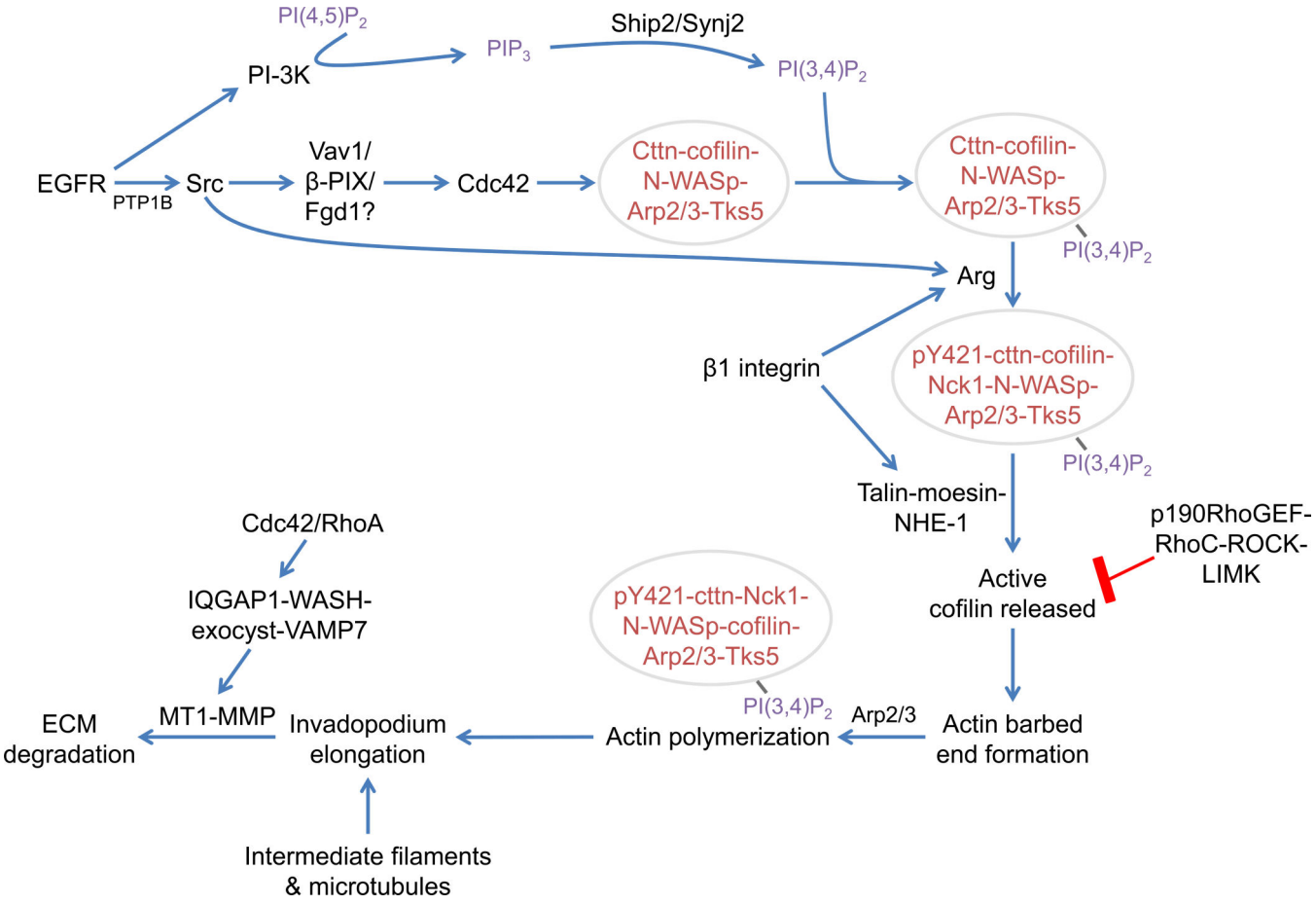

Figure 2. Integrative signaling diagram of invadopodial assembly and maturation

Invadopodia initially form as precursors in response to EGF or other stimuli (e.g. TGF-β or PDGF). Src is activated either directly by EGFR or by PTP1B (Cortesio et al., 2008). These stimuli induce Cdc42 activation, leading to assembly of the precursor core structure (red text within circle; Razidlo et al., 2014; Yamaguchi et al., 2005). The invadopodium precursor is then anchored by binding to $PI(3,4)P_2$ and further stabilized by β1 integrin-mediated adhesion to the ECM (Beaty et al., 2013; Sharma et al., 2013). Invadopodium maturation begins as β1 integrin activates Arg, which phosphorylates cortactin on Y421 to recruit Nck1 (Beaty et al., 2013; Oser et al., 2010). Talin localizes to the structure and recruits a complex of moesin and NHE-1 through a direct binding interaction with moesin (Beaty et al., 2014). The intracellular pH increases in response to NHE-1 activity, which disrupts the inhibitory interaction between cortactin and cofilin (Busco et al., 2010; Magalhaes et al., 2011). Cofilin severs F-actin to form barbed ends that are used to elongate filaments, on which Nck1 induces N-WASp-Arp2/3-dependent dendritic nucleation (DesMarais et al., 2004). Actin polymerization is required for MMP-dependent matrix degradation at invadopodia, possibly through MMP recruitment (Oser et al., 2009; Sakurai-Yageta et al., 2008; Yamaguchi et al., 2005). MT1-MMP is delivered by the IQGAP1-WASH-exocyst complex and fuses to the membrane via the v-SNARE VAMP7, resulting in matrix degradation (Monteiro et al., 2013; Sakurai-Yageta et al., 2008; Steffen et al., 2008). Cortactin, cttn; synaptojanin2, synj2.