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REVIEW ARTICLE

Invadopodia, Regulation, and Assembly in Cancer Cell Invasion

Bechara A. Saykali and Mirvat El-Sibai

Department of Natural Sciences, The Lebanese American University, Beirut, Lebanon

Abstract

The occurrence of invadopodia has been, since its characterization, a hallmark of cancerous cell invasion and metastasis. These structures are now the subject of a controversy concerning their cellular function, molecular regulation, and assembly. The terms invadopodia and podosomes have been used interchangeably since their discovery back in 1980. Since then, these phenotypes are now more established and accepted by the scientific community as vital structures for 3D cancer cell motility. Many characteristics relating to invadopodia and podosomes have been elucidated, which might prove these structures as good targets for metastasis treatment. In this review, we briefly review the actin reorganization process needed in most types of cancer cell motility. We also review the important characteristics of invadopodia, including molecular components, assembly, markers, and the signaling pathways, providing a comprehensive model for invadopodia regulation.

Keywords: invadopodia, podosome, 3D motility, cancer motility, signaling

WHAT ARE INVADOPODIA?

For cells to move, scavenge for nutrients, undergo wound healing etc. many events should occur, some of which are actin cytoskeleton remodeling, and formation of actin-based structures. These structures are necessary for proper adhesion to the subcellular matrix as well as sensing the surrounding matrix for growth hormones and nutrients (Hall, 2009). They can be classified into two groups, where the first group results in two-dimensional locomotion, and the other resulting in motility in the third dimension. The former includes actin assemblies such as lamellipodia and filopodia, while the latter relies on other specified actin structures. Many characteristics distinguish them from each other, namely, location of formation, protrusive dimension, duration, proteins involved, and function (Murphy & Courtneidge, 2011). In the case of 3D locomotion, two main structures are to be noted, podosomes and invadopodia. Many references would consider them as being two forms for one phenotype, but others differentiate between them according to duration of action and the ability to produce matrix-degrading enzymes (Chen et al., 1985).

The series of events that led to the characterization of podosomes began with David-Pfeuty and Singer back in 1980 (Singer & David-Pfeuty, 1980). Their Transformation of v-Src into CEF cells (chicken embryo fibroblast) via Rous sarcoma virus (RSV) caused relocation of vinculin and α-Actinin from focal adhesions to form circular clusters known as rosettes. They were not referred to as podosomes until 1985. Tarone et al. (1985) considered them to be feet-like, and thus were dubbed podosomes. They were also termed invadopodia by Chen (1989), to reflect the adhesive and degrading ability of these structures, in cancer cell lines (Chen et al., 1985). In the subsequent years, podosomes and invadopodia were described in other cell types, namely, macrophages, dendritic cells, endothelial cells and vascular smooth muscles (Gimona et al., 2008). In an attempt to clear the confusion between the two nomenclatures (podosome and invadopodia) because many authors use the two terms interchangeably, Murphy and Courtneidge (2011) suggested the following: The use of the term podosome would be linked strictly to normal cell lines while invadopodia would be linked to cancer cell lines. Other studies published by the Condeelis lab further distinguish between invadopodia, podosomes, and other degradative structures on a molecular level. According to their findings, endogenous Nck1 are only present in invadopodia, Grb2 only localizes to Src-transformed fibroblast and PMA-stimulated HUVEC cells, while neither, Nck1 nor Grb2, are involved in podosome assembly (Oser et al., 2011). Before we delve into what has been presented so far for invadopodia, a brief description of the actin cycle involved is of benefit.

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Address correspondence to Mirvat El-Sibai, Assistant Professor, Department of Natural Sciences, Lebanese American University, P.O. Box: 13-5053, Chouran 1102 2801, Beirut, Lebanon. Phone: + 961-1-786456, Ext. 1253. Fax: + 961-1-867098. E-mail: mirvat.elsibai@lau.edu.lb

THE ACTIN MODEL

As mentioned above, the cell would move in 2D or 3D according to its need and the environment. Different cells

use different ways of locomotion, but the rearrangement of the cytoskeleton is common to all those mechanisms (Hall, 2009), so it is beneficial to review these events.

For the cell to migrate in two dimensions, a shift is first required from a non-polarized into a polarized state. This transition will acquire the cell a motile phenotype, where the cell's front extends and attaches just behind the leading edge, forming focal contacts with the ECM (Parker & Sukumar, 2003). The cell's rear would detach and retract. When the cell's rear detaches, and focal contacts mature into focal adhesions at the leading edge, the cell can now pull itself forward. For these events to occur, major rearrangement of the cytoskeleton should happen, including actin, myosin, tubulin, and their binding proteins (Insall & Machesky, 2009). Two structures are typically described in two 2D migration, namely, lamellipodia and filopodia (Small, 1994). For these structures to form, reorganization of the actin cytoskeleton is needed. First a nucleation step should occur where monomeric ATP-globular actin (G-actin) will polymerize to form a short strand of filamentous ADP-bound actin (F-actin). More specifically, actin monomers will nucleate via the actin-binding proteins Arp 2/3, Ena/VASP proteins or DRFs, forming a faster growing filament, which has a fast growing, barbed or +-end and a slow growing, pointed end. The main activators of the Arp2/3 complex are the WASP family proteins. Once incorporated, ADP-actin molecules remain in the filament and dissociated after reaching the negative end. Profilin will then enhance the nucleotide exchange process leading to ATP-actin monomers, which are either stored with thymosin β, shuttled to the barbed end, or as profilin-actin complexes (Singer & David-Pfeuty, 1980; Lambrechts et al., 2004). In addition to actin reorganization, and for the cell to move, a contractile force is needed. This contractile force is established by the formation of actin–myosin complexes. Once established, these complexes use energy from ATP hydrolysis to drive the contraction (Hall, 2009). For optimal migration to occur, polymerization and contractility must be synchronized, both temporally and spatially. According to Olson, one explanation could be that both procedures are activated by the same GTPase but with different kinetics. For example, the activation of RhoA (GTPase), results in actin polymerization, via RhoA's binding to DRF1, whereas, actomyosin contractility is activated through ROCK or LMK (Olson & Sahai, 2009). This can lead to the activation of polymerization prior to contraction. Another explanation given also by Olson and Sahai (2009) also states that activators of polymerization could also be inhibitors of contraction. For example, activation of Rac1 activates WAVE-dependent actin nucleation, while it also inhibits RhoA through production of ROS, which reduces contractility in this phase. Another theory, proposed by Alan Hall (2009), states that polymerization occurs at the anterior end while contraction occurs at the posterior and sides. Hence even though Rho activation is observed both at the anterior and posterior (El-Sibai et al., 2008; Pertz et al., 2006), it is postulated that the function of this activation is polymerization at the anterior and contraction at the posterior.

Another important concept in cell motility and actin dynamics is chemotaxis. Cells exhibit a remarkable ability to detect a gradient around it, which would almost always trigger a cell motility or cell polarization response. This response involves several complex, interrelated processes including motility, polarity and directional movement (Iijima et al., 2002). Type I PI3K have been viewed for quite a while as being the key link connecting signaling pathways to cell movement and thus orchestrating chemotaxis (Ferguson et al., 2007). Other findings restrict the role of PI3K in chemotaxis in less motile cells such as fibroblasts, and imply the involvement of other regulators such as Ras, TOR complex 2, and SCAR/WAVE (Schneider & Haugh, 2006; Kamimura et al., 2008; Millius et al., 2009). The process of chemotaxis has been also found to affect cell migration and invadopodia formation, in the third dimension. According to John Condeelis (DesMarais et al., 2009), in a gradient of EGF, invadopodia will assemble more toward the source of the stimulant.

Till date four major types of membrane protrusions have been identified, namely, lamellipodia, filopodia, blebs and invadopodia or podosomes. While lamellipodia and filopodia are involved in forward movement, invadopodia couples actin polymerization with the delivery of matrix-degrading metalloproteases. Although these protrusions are most likely to act together to achieve cell movement, still each projection display a specific array of signaling molecules and actin dynamics (Ridley, 2011; Bear & Gertler, 2009). The accepted model for lamellipodium formation depends heavily on Arp 2/3 activity. Actin filaments are first severed by cofilin, which provides new fast growing ends. Arp2/3 will bind these sites and initiate actin polymerization and nucleation, creating new branched filaments (Mullins et al., 1998). Arp2/3 activating is mediated by the WAVE complex, which in turn, is regulated by Rac1 (Campellone & Welch, 2010). Formins can also nucleate actin filaments independent of the Arp2/3 complex, generating unbranched filaments. Actin monomers are delivered to the site of polymerization after cofilin severing and profilin activation (Pollard & Borisy, 2003; Ponti et al., 2004; Yang et al., 2007). As for filopodium formation, the main actors involved are the formin family along with several others. mDia2, Cdc42 and Cdc42 targets are recruited to the cell membrane, which will then stimulate actin polymerization (Breitsprecher et al., 2011). Actin filaments are polymerized either *de novo* or through using lamellipodium as actin source. Filopodia extend through addition of monomeric actin (G-actin) onto filamentous actin (F-actin), through several proteins (Machesky & Li, 2010). Two main pathways are known to drive actin filaments extension in filopodia. One of these is through mDia2, where VASP and mDia2 will drive actin nucleation at the

filopodial tip, and then profilin would directly bind mDia2 providing it with actin monomers for extension. Another pathway is through Arp2/3 where Cdc42 will stimulate WASP/Arp2/3 actin polymerization, in addition to mDia2-mediated actin polymerization (Wang et al., 2010; Takenawa & Suetsugu, 2007).

INVADOPODIA COMPONENTS AND ASSEMBLY

Because there is no single marker for invadopodia, we usually look for multiple key components of these structures. One of the most recognized markers for invadopodia is localization and expression of Tks adaptor proteins (Courtneidge et al., 2005; Lock et al., 1998). Important factors characterizing invadopodia, such as its assembly structure, can be visualized after rhodamine–Phalloidin staining. Another prime factor of invadopodia is its metalloprotease activity, although this is not restricted to invadopodia. Recent studies show that focal adhesions also exhibit this type of activity (Wang & McNiven, 2012). Matrix-degrading proteases are delivered to the site of adhesion through vesicles, in particular the membrane type 1 metalloprotease (MT1-MMP; Wolf & Friedl, 2009). These metalloproteases are observed after stimulation with several stimuli such as, platelet-derived growth factor (PDGF), transforming growth factor-β (TGFβ), and epidermal growth factor (EGF). These signals are relayed to the inner cell mainly through two pathways; the Src, and PKC-related pathways (Schachtner et al., 2013). Stating that Src is involved in invadopodial regulation does not come as a surprise at this stage of research. Tks5 was identified as one of its substrate, along with other proteins such as cortactin, the actin-binding and crosslinking protein 110 kDa actin filament-associated protein (AFAP110; also known as AFAP1) and the integrin effector and adaptor protein p130CAS. All these proteins are implicated previously in invadopodia formation (Destaing et al., 2008; Lock et al., 1998). As for PKC, it acts in concert with Src to regulate the invadopodia assembly. After phorbol ester stimulation, PKC α will induce AFAP110 to colocalize with and activate Src, through a mechanism requiring PI3K activity (Tatin et al., 2006; Gatesman et al., 2004). Thus, PKC–Src-mediated pathways seem to have a key role in the formation of podosomes and invadopodia. It is now possible to sum up the different events that occur in order to assemble the invadopodial structure.

Stage 1: Polarization and Src signaling

For the cell to start the assembly of invadopodia, it should shift from a quiescent to a migratory phase, where the adhesions at the leading edge are dissolved and the formation of invadopodia initiated (Block et al., 2008). An established regulator of adhesion structures is the focal adhesion kinase (FAK). FAK is thought to regulate invadopodia initiation because it has "suitable characteristics": On one side FAK is responsible for the formation and dissolution of focal adhesions (Tomar & Schlaepfer, 2009), and on the other side it is known to regulate Src kinase (Wang & McNiven, 2012), which is directly linked to invadopodia formation. Although theoretically this seems feasible, contradictory data have been presented about the involvement of FAK in invadopodia formation. FAK has been described, as a negative regulator of invadopodia through the spatial control of Src (Chan et al., 2009). Other studies have shown that FAK is actually found in invadopodia and that overexpressing FAK would promote invadopodia formation (Vitale et al., 2008). Taking the presented data, for this stage, into consideration, the following model is proposed: When the cell is quiescent, the cell will signal for upregulation of focal adhesions. This is done through activation of FAK by phosphorylation at the Y397 residue; leading to the binding of FAK to Src. FAK-mediated phosphorylation of Src at Y416 activates its near focal adhesion (FAs) sites (Tomar & Schlaepfer, 2009). This complex has been found to phosphorylate the scaffolding protein p130Cas. Binding of adaptor proteins to p130Cas will facilitate activation of small Rho GTPases, which in turn will lead to upregulation and maturation of adhesion structure (Ricono et al., 2009). The switch the cell undergoes from quiescent to invasive is linked with depletion of FAK. Depletion of FAK from the cell will induce the redistribution of Src from FAs to invadopodia. According to a paper published in 2009, after FAK depletion in MTLn3 cells, tyrosine phosphorylation of p130Cas and Paxillin will drop at focal adhesion. This will be replaced by tyrosine phosphorylation of cortactin and Tks5 at invadopodia (Chan et al., 2009). Although it is established that FAK is important for Src localization to focal adhesion, it is still not clear what determines Src localization to invadopodia, despite strong evidence showing involvement of integrins (Qin et al., 2014).

Stage 2: Invadopodia assembly and phosphoinositide 3-kinase signaling

Invadopodia assembly initiation has been linked with PI3K activity. The use of PI3K inhibitors or PI3K-specific siRNA has been shown to reduce invadopodia formation and ECM degradation (Hoshino et al., 2012; Yamaguchi et al., 2011). Class I PI3K will phosphorylate phosphatidylinositol 4,5-bisphosphate [$PIP(3.4)_2$] to create phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) (Fruman et al., 1998). PIP_3 will recruit kinases involved in invadopodia regulation, such as Akt serine threonine kinases and phosphoinositide-dependent protein kinase 1 (PDK1) (Cantley, 2002; Yamaguchi et al., 2011). In addition to recruiting these kinases, PIP_3 can also play as a precursor lipid for $PIP(3.4)_2$, which localizes to invadopodia in Src-transformed fibroblasts (Oikawa et al., 2008). Two Src substrates, Tks4 and Tks5, were identified as invadopodia organizers. The lab of Sara Courtneidge studies Tks adaptor proteins in depth, and has been able to show that Tks5

and Tks4 are required for both invadopodia formation and the invasive property for multiple human cancer cell lines (Seals et al., 2005; Buschman et al., 2009). Activation of Tks5 alone will lead to aggregation of several invadopodia proteins but not the activation ECM degradation. Src phosphorylation of both Tks5 and Tks4 couples the assembly of the invading structure and degrading ability. $PIP(3.4)_2$ may recruit the scaffold protein Tks5 by binding its PX domain, which will target it for localization with cortactin to the cell membrane (Clark & Weaver, 2008). After localization, it is thought that Tks5 regulates invadopodia formation by binding to key actin regulators such as Nck1, Nck2, NWASP, and Grb2, via its third SH_3 domain (Oikawa et al., 2008; Stylli et al., 2009). It has been also shown that both Tks5 and TKs4 can bind the ADAMs family of metalloproteases in Src-transformed cells (Abram et al., 2003) and regulate the production of reactive oxygen species (ROS). The regulation of ROS by Tks proteins is not directly linked to invadopodial formation, yet the role of ROS in promoting signal transduction, in transiently inhibiting the activity of some phosphatases, and in increasing matrix metalloprotease synthesis should prove promising (Gianni et al., 2009). In certain instances, maturation of invadopodia has been linked with the production of certain metalloproteases, where silencing Tks4 has been shown to result in the formation of immature invadopodia. These were found to have Tks5 and cortactin localized to the site of invasion, and the secretion of MMPs, but no MT1-MMP and the loss of matrix degrading ability (Buschman et al., 2009). Though Tks5 has been well characterized since it was first described in 2005 (Seals et al., 2005), it still does not have a clear role, since new data were recently published suggesting a different role for Tks5. New evidence presented by the Condeelis laboratory describes a different role for Tks5 in invadopodial regulation in breast carcinoma cells. Data presented by this laboratory argue that Tks5 is not actually needed for invadopodia initiation, but for the maturation of these structures *in vitro.* (Sharma et al., 2013). Further studies involving the role of Tks4 and Tks5 will shed more light on the link between Src and the downstream components of invadopodia.

Another regulator of invadopodia, downstream of Src, is cortactin. An early study published in 1991 shows that neutralizing cortactin via antibodies leads to immature invadopodia, lacking ECM degradation potential (Bowden et al., 1999). More recent studies show the involvement of cortactin in regulating the number and the activity of both invadopodia and podosomes (Magalhaes et al., 2011; Oser et al., 2010). More specifically, cortactin helps in invadopodia regulation through either facilitating actin organization at the site of invasion or through trafficking ECM degrading proteinases. Cortactin was found to regulate actin assembly through the Abelson (Abl) or Abl-related genes (Arg) kinases. Adhesion-related phosphorylation of the Arg–cortactin allows cortactin's SH_3 to bind N-WASp leading to cell edge protrusions (Lapetina et al., 2009). A recent study links directly cortactin to invadopodia formation and maturation through recruitment of NHE1 and the subsequent PH in invadopodia. In addition to what have been presented, the Weaver lab have additionally published data suggesting the involvement of cortactin through regulating the secretion, cell-surface expression and localization to invadopodia of the matrix metalloproteases (MMPs) MT1-MMP, MMP-2 and MMP-9 (Clark & Weaver, 2008; Clark et al., 2007). In general, cortactin appears to be an important regulator of invasion, with many potential mechanisms.

Stage 3: Invadopodia turnover and dissolution

Little is known about the turnover of invadopodia. It has been estimated to range from minutes to hours (Murphy & Courtneidge, 2011). It is thought that the ring structure is uncoupled from the rest of the actin cytoskeleton and dissolved by parts. In this case the actin head would be dissolved first and the filamentous tail last, taking up to hours (Baldassarre et al., 2006). Protein phosphorylation is as important for invadopodia turnover as it is for formation. Tyrosine phosphorylation of cortactin and AFAP110 is important for invadopodia turnover. Studies involving the addition or removal of the phosphate group must be extended in order to shed more light on the turnover process of invadopodia (Calle et al., 2006).

CONCLUSIONS AND PERSPECTIVES

Important breakthroughs have been made since the word invadopodia was first published back in 1980. Many of the structure and components of invadopodia have been deciphered, along with much of the mechanism of the matrix degradation capability. Many pathways are now known, regulating the assembly and maturation of invadopodia, but how the pathway interact and synchronize in currently not yet clear as Ridley (2006) explains (Ridley, 2011). Because lamellipodia, filopodia, FAs, invadopodia, and podosomes are similar in composition, it is possible that these structures interconvert, act synchronically or even come together to form a mega structure called "invadosome" or "invadotron", as McNiven would describe it in one of his reviews (McNiven, 2013). Many other questions are still not answered, such as, what role does the ECM have in the formation of these structures? What do invadopodia look like in vivo? How do invadopodia promote tumor growth and progression? And most importantly how can scientists use these findings in a way to improve cancer treatments. Yet, it is also now known that many of the known components of podosomes and invadopodia are scaffolding or adaptor proteins with no catalytic activity, and are therefore unlikely drug targets (Murphy & Courtneidge 2011). More insight on this topic will certainly increase targeted therapy since cell migration is central to many chronic human diseases, including cancer.

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REFERENCES

Abram C, Seals DF, Pass I, Salinsky D, Maurer L, Roth TM, Courtneidge SA (2003). The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. *J Biol Chem*. 278: 16844–16851.

Baldassarre M, Ayala I, Beznoussenko G, Giacchetti G, Machesky LM, Luini A, Buccione R (2006). Actin dynamics at sites of extracellular matrix degradation. *Eur J Cell Biol*. 85: 1217–1231.

Bear J, Gertler F (2009). Ena/VASP: towards resolving a pointed controversy at the barbed end. *J Cell Sci*. 122: 1947–1953.

Block M, Badowski C, Millon-Fremillon A, Bouvard D, Bouin AP, Faurobert E, Gerber-Scokaert D, Planus E, Albiges-Rizo C (2008). Podosome-type adhesions and focal adhesions, so alike yet so different. *Eur J Cell Biol*. 87: 491–506.

Bowden ET, Barth M, Thomas D, Glazer RI, Mueller SC (1999). An invasion-related complex of cortactin, paxillin and PKCmu associates with invadopodia at sites of extracellular matrix degradation. *Oncogene*. 18: 4440–4449.

Breitsprecher D, Kiesewetter AK, Linkner J, Vinzenz M, Stradal TE, Small JV, Curth U, Dickinson RB, Faix J (2011). Molecular mechanism of Ena/VASP-mediated actin-filament elongation. *EMBO J*. 30: 456–467.

Buschman MD, Bromann PA, Cejudo-Martin P, Wen F, Pass I, Courtneidge SA (2009). The novel adaptor protein Tks4 (SH3PXD2B) is required for functional podosome formation. *Mol Biol Cell*. 20: 1302–1311.

Calle Y, Carragher N, Thrasher A, Jones G (2006). Inhibition of calpain stabilises podosomes and impairs dendritic cell motility. *J Cell Sci*. 119: 2375–2385.

Campellone K, Welch M (2010). A nucleator arms race: cellular control of actin assembly. *Nat Rev Mol Cell Biol*. 11: 237–251.

Cantley LC (2002). The phosphoinositide 3-kinase pathway. *Science*. 296: 1655–1657.

Chan K, Cortesio C, Huttenlocher A (2009). FAK alters invadopodia and focal adhesion composition and dynamics to regulate breast cancer invasion. *J Cell Biol*. 185: 357–370.

Chen W (1989). Proteolytic activity of specialized surface protrusions formed at rosette contact sites of transformed cells. *J Exp Zool*. 251: 167–185.

Chen W-T, Chen J-M, Parsons S, Parsons T (1985). Local degradation of fibronectin at sites of expression of the transforming gene product pp60src. *Nature*. 316: 156–158.

Clark ES, Weaver AM (2008). new role for cortactin in invadopodia: regulation of protease secretion. *Cell Biol*. 87: 581–590.

Clark ES, Whigham AS, Yarbrough WG, Weaver AM (2007). Cortactin is an essential regulator of matrix metalloproteinase secretion and extracellular matrix degradation in invadopodia. *Cancer Res*. 67: 4227–4435.

Courtneidge S, Azucena EF, Pass I, Seals DF, Tesfay L (2005). The SRC substrate Tks5, podosomes (invadopodia), and cancer cell invasion. *Cold Spring Harb Symp Quant Biol*. 70: 167–171.

DesMarais V, Yamaguchi H, Oser M, Soon L, Mouneimne G, Sarmiento C, Eddy R, Condeelis J (2009). N-WASP and cortactin are involved in invadopodium-dependent chemotaxis to EGF in breast tumor cells. *Cell Motil Cytoskeleton*. 66: 303–316.

Destaing O, Sanjay A, Itzstein C, Horne WC, Toomre D, De Camilli P, Baron R (2008). The tyrosine kinase activity of cSrc regulates actin dynamics and organization of podosomes in osteoclasts. *Mol Biol Cell*. 19: 394–404.

El-Sibai M, Pertz O, Pang H, Yip SC, Lorenz M, Symons M, Condeelis JS, Hahn KM, Backer JM (2008). RhoA/ROCK-mediated switching between Cdc42- and Rac1-dependent protrusion in MTLn3 carcinoma cells. *Exp Cell Res*. 314:1540–52.

Ferguson G, Milne L, Kulkarni S, Sasaki T, Walker S, Andrews S, Crabbe T, Finan P, Jones G, Jackson S, Camps M, Rommel C, Wymann M, Hirsch E, Hawkins P, Stephens L (2007). PI(3)Kgamma has an important context-dependent role in neutrophil chemokinesis. *Nat Cell Biol*. 9: 86–91.

Fruman A, Meyers RE, Cantley LC (1998). Phosphoinositide kinases. *Annu Rev Biochem*. 67: 481–507.

Gatesman A., Walker VG, Baisden JM, Weed SA, Flynn DC (2004). Protein kinase Cα activates cSrc and induces podosome formation via AFAP110. *Mol Cell Biol*. 24: 7578–7597.

Gianni D, Diaz B, Taulet N, Fowler B, Courtneidge SA, Bokoch GM (2009). Novel p47(phox)-related organizers regulate localized NADPH oxidase 1 (Nox1) activity. *Sci Signal*. 2: ra54.

Gimona M, Buccione R, Courtneidge S, Linder S (2008). Assembly and biological role of podosomes and invadopodia. *Curr Opin Cell Biol*. 20: 235–241.

Hall A (2009). The cytoskeleton and cancer. *Cancer Metastasis Rev*. 28: 5–14.

Hoshino D, Jourquin J, Emmons SW, Miller T, Goldgof M, Costello K, Tyson DR, Brown B, Lu Y, Prasad NK, Zhang B, Mills GB, Yarbrough WG, Quaranta V, Seiki M, Weaver AM (2012). Network analysis of the focal adhesion to invadopodia transition identifies a PI3K-PKCa invasive signaling axis. *Sci Signal*. 5: ra66.

Iijima M, Huang Y, Devreotes P (2002). Temporal and spatial regulation of chemotaxis. *Dev Cell*. 3: 469–478.

Insall R, Machesky L (2009). Actin dynamics at the leading edge: from simple machinery to complex networks. *Dev Cell*. 17: 310–322.

Kamimura Y, Xiong Y, Iglesias PA, Hoeller O, Bolourani P, Devreotes PN (2008). PIP3-independent activation of TorC2 and PKB at the cell's leading edge mediates chemotaxis. *Curr Biol*. 18: 1034–1043.

Lambrechts A, Van Troys M, Ampe C (2004). The actin cytoskelyon in normal and pathological cell motility. *Int J Biochem Cell Biol*. 36: 1890–1909.

Lapetina S, Mader CC, Machida K, Mayer BJ, Koleske AJ (2009). Arg interacts with cortactin to promote adhesion-dependent cell edge protrusion. *J Cell Biol*. 185: 503–519.

Lock P, Abrahm LC, Gibson T, Courtneidge S (1998). A new method for isolating tyrosine kinase substrates used to identify fish, an SH3 and PX domain-containing protein, and Src substrate. *EMBO J*. 17: 4346–4357.

Machesky L, Li A (2010). Fascin: Invasive filopodia promoting metastasis. *Commun Integr Biol*. 3: 263–270.

Magalhaes MA, Larson DR, Mader CC, Bravo-Cordero JJ, Gil-Henn H, Oser M, Chen X, Koleske AJ, Condeelis J (2011). Cortactin phosphorylation regulates cell invasion through a pH-dependent pathway. *J Cell Biol*. 195: 903–920.

McNiven MA (2013). Breaking away: matrix remodeling from the leading edge. *Trends Cell Biol*. 23: 16–21.

Millius A, Dandekar S, Houk A, Weiner O (2009). Neutrophils establish rapid and robust WAVE complex polarity in an actin-dependent fashion. *Curr Biol*. 19: 253–259.

Mullins R, Heuser J, Pollard T (1998). The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments. *Proc Natl Acad Sci U S A*. 95: 6181–6186.

Murphy D, Courtneidge S (2011). The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. *Nature*. 12: 413–426.

Oikawa T, Itoh T, Takenawa T (2008). Sequential signals toward podosome formation in NIH-src cells. *J Cell Biol*. 182: 157–169.

Olson M, Sahai E (2009). The actin cytoskeleton in cancer cell motility. *Clin Exp Metastasis*. 26: 273–287.

Oser M, Dovas A, Cox D, Condeelis J (2011). Nck1 and Grb2 localization patterns can distinguish invadopodia from podosomes. *Eur J Cell Biol*. 90: 181–188.

Oser M, Mader CC, Gil-Henn H, Magalhaes M, Bravo-Cordero JJ, Koleske AJ, Condeelis J (2010). Specific tyrosine phosphorylation sites on cortactin regulate Nck1-dependent actin polymerization in invadopodia. *J Cell Sci*. 123: 3662–3673.

Parker SB, Sukumar S (2003). Distant metastasis in breast cancer. *Cancer Biol Ther*. 2: 14–21.

Pertz O, Hodgson L, Klemke RL, Hahn KM (2006). Spatiotemporal dynamics of RhoA activity in migrating cells. *Nature* 440:1069–72.

Pollard TD, Borisy GG (2003). Cellular motility driven by assembly and dissassembly of actin filaments. *Cell*. 112: 453–465.

Ponti A, Machacek M, Gupton SL, Waterman-Storer CM, Danuser G (2004). Two distinct actin networks drive the protrusion of migrating cells. *Science*. 305: 1782–1786.

Qin Y, Fu M, Takahashi M, Iwanami A, Kuga D, Rao RG (2014). Epithelial Membrane Protein-2 (EMP2) Activates Src and is a Novel Therapeutic Target for GBM. *J Biol Chem*.00: 1–10 (accessed 6 January 2013).

Ricono JM, Huang M, Barnes LA, Lau SK, Weis SM, Schlaepfer DD, Hanks SK, Cheresh DA (2009). Specific cross-talk between epidermal growth factor receptor and integrin alphavbeta5 promotes carcinoma cell invasion and metastasis. *Cancer Res*. 69: 1383–1391.

Ridley AJ (2006). Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. *Trends Cell Biol*. 16:522–29.

Ridley A (2011). Life at the leading edge. *Cell*. 145: 1012–1023.

Schachtner H, Calaminus S, Thomas S, Machesky L (2013). Podosomes in adhesion, migration, mechanosensing and matrix remodeling. *Cell Motil Cytoskeleton*. 70: 572–589.

Schneider I, Haugh J (2006). Mechanisms of gradient sensing and chemotaxis: conserved pathways, diverse regulation. *Cell Cycle*. 5: 1130–1134.

Seals DF, Azucena EF Jr, Pass I, Tesfay L, Gordon R, Woodrow M, Resau JH, Courtneidge SA (2005). The adaptor protein Tks5/Fish is required for podosome formation and function, and for the protease-driven invasion of cancer cells. *Cancer Cell*. 7: 155–165.

Sharma VP, Eddy R, Entenberg D, Kai M, Gertler FB, Condeelis J (2013). Tks5 and SHIP2 Regulate Invadopodium Maturation, but No Initiation, in Breast Carcinoma Cells. *Curr Biol*. 23: 2079–2089.

Singer S, David-Pfeuty T (1980). Altered distribution of the cytoskeleton proteins vinculin and alfa-actinin in cultured fibroblasts transformed by Rous sarcoma virus. *Proc Natl Acad Sci U S A*. 77: 6687–6691.

Small V (1994). Lamellipodia architecture: Actin filament turnover and the lateral flow of actin filamens durings motility. *Semin Cell Biol*. 5: 157–163.

Stylli S, Stacey TT, Verhagen AM, Xu SS, Pass I, Courtneidge SA, Lock P (2009). Nck adaptor proteins link Tks5 to invadopodia actin regulation and ECM degradation. *J Cell Sci*. 122: 2727–2740.

Takenawa T, Suetsugu S (2007). The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. *Nat Rev Mol Cell Biol*. 8: 37–48.

Tarone G, Cirillo D, Giancotti FG, Comoglio PM, Marchisio PC (1985). Rous sarcoma virus-transformed fibroblasts adhere primarily at discrete protrusions of the ventral membrane called podosomes. *Exp Cell Res*. 159: 141–157.

Tatin F., Varon C., Genot E, Moreau V (2006). A signalling cascade involving PKC, Src and Cdc42 regulates podosome assembly in cultured endothelial cells in response to phorbol ester. *J Cell Sci*. 119: 769–781.

Tomar A, Schlaepfer DD (2009). Focal adhesion kinase: switching between GAPs and GEFs in the regulation of cell motility. *Curr Opin Cell Biol*. 21: 676–683.

Vitale S., Avizienyte E., Brunton V, Frame M (2008). Focal adhesion kinase is not required for Src-induced formation of invadopodia in KM12C colon cancer cells and can interfere with their assembly. *Eur J Cell Biol*. 87: 569–579.

Wang X, He L, Wu YI, Hahn KM, Montell DJ (2010). ight-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. *Nat Cell Biol*. 12: 591–597.

Wang Y, McNiven MA (2012). Invasive matrix degradation at focal adhesions occurs via protease recruitment by a FAK–p130Cas complex. *J Cell Biol*. 196: 375–385.

Wolf K, Friedl P (2009). Mapping proteolytic cancer cell-extracellular matrix interfaces. *Clin Exp Metastasis*. 26: 289–298.

Yamaguchi H, Yoshida S, Muroi E, Yoshida N, Kawamura M, Kouchi Z, Nakamura Y, Sakai R, Fukami K (2011). Phosphoinositide 3-kinase signaling pathway mediated by p110a regulates invadopodia formation. *J Cell Biol*. 193: 1275–1288.

Yang C, Czech L, Gerboth S, Kojima S, Scita G, Svitkina T (2007). Novel roles of formin mDia2 in lamellipodia and filopodia formation in motile cells. *PLoS Biol*. 5: 2624–2645.