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Invadopodia and basement membrane invasion in vivo

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Over 20 years ago, protrusive, F-actin-based membrane structures, termed invadopodia, were identified in highly metastatic cancer cell lines. Invadopodia penetrate artificial or explanted extracellular matrices in 2D culture conditions and have been hypothesized to facilitate the migration of cancer cells through basement membrane, a thin, dense, barrier-like matrix surrounding most tissues. Despite intensive study, the identification of invadopodia in vivo has remained elusive and until now their possible roles during invasion or even existence have remained unclear. Studies in remarkably different cellular contexts—mouse tumor models, zebrafish intestinal epithelia, and *C. elegans* organogenesis—have recently identified invadopodia structures associated with basement membrane invasion. These studies are providing the first in vivo insight into the regulation, function, and role of these fascinating subcellular devices with critical importance to both development and human disease.

Introduction

Basement membrane is a thin, dense, sheet-like form of extracellular matrix that underlies all epithelia and encapsulates muscle, fat, and glial cells.[1-3] The basement membrane arose at the emergence of animal multicellularity[4,5] and is a key regulator of many cellular and tissue-level functions, including cell polarity, differentiation, organ shape, and tissue compartmentalization.[6-11] Despite its dense and highly cross-linked structure, leukocytes and numerous migrating cells in development traffic through basement membranes.[12-14] For example, during the epithelial-to-mesenchymal transitions (EMTs) that occur in gastrulation and neural crest migration, cells acquire the ability to invade through the epithelial basement membrane to initiate their migration to distant sites.[15-17] In a similar manner, cancer cells are also thought to acquire the ability to breach basement membrane to enable metastasis, the most lethal step in cancer progression.[18] Largely because of its importance in cancer, there has been significant interest in understanding the mechanisms cells utilize to invade (or transmigrate) basement membrane barriers. A better understanding of the mechanisms cells use to cross basement membrane should facilitate strategies to therapeutically modulate invasive cellular behavior.[19]

A considerable amount of attention has focused on the potential role of invadopodia, F-actin-based, highly protrusive, matrix-degrading membrane structures, in directing cancer cell invasion through basement membrane.[20-23] Invadopodia depend upon integrin for their formation and are often enriched with the actin regulators Arp2/3 and Wasp, the signaling protein Src, the scaffolding proteins cortactin and Tks5, and the matrix metalloproteinase MT1-MMP.[23] Another membrane-associated structure highly similar to invadopodia, called podosomes, has also been identified. Podosomes, however, are generally associated with non-transformed cells involved with matrix remodeling events, such as osteoclasts and vascular smooth muscle cells, and not basement membrane invasion.[23-29] The term invadosomes has recently been used to encompass both structures,[21,30-32] recognizing that invadopodia and podosomes (and possibly other protrusive membrane structures) likely represent more of a continuum than completely distinct entities.[33]

Invadopodia were first identified over 20 y ago in transformed fibroblasts and human cancer cell lines and have since been observed in primary tumor cells from human patients.[34-37] Reflecting the growing interest in these structures, more than half of the approximately 350 scholarly articles on invadopodia have been published within the last 3 y. Although recent advances have been made in analyzing invadopodia-like protrusions in 3D culture conditions,[19,38] most mechanistic studies on invadopodia have been performed with cancer cell lines in 2D cell culture environments. The advantages in imaging, biochemical approaches, and genetic and pharmacological perturbations in 2D conditions have led to remarkable advances in our understanding of invadopodia.[22,39-42] These findings include the identification of different components of invadopodia,[43-45] elucidation of the stages of invadopodia formation,[46-49] identification of genes associated with cancer metastasis that regulate invadopodia formation,[19,21,50-52] understanding of the trafficking of proteases to invadopodia,[53-57] and examination of invadopodia membrane dynamics.[58] Key insights have also been gained into the regulation of their formation by growth factors, integrin activation, and the microenvironment, including hypoxia, matrix stiffness,

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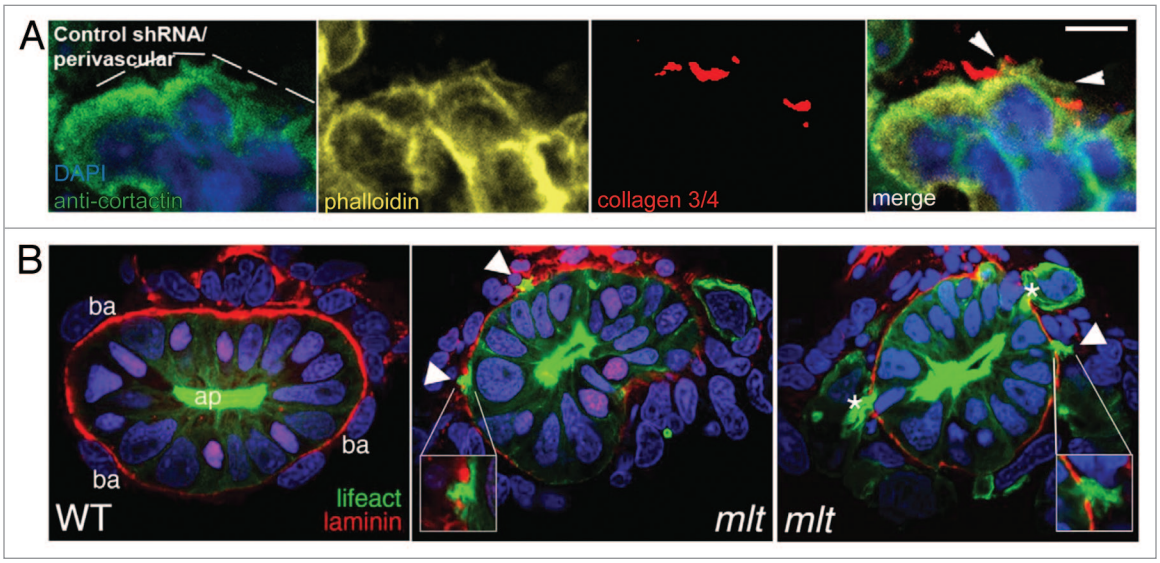

Figure 1. In vivo invadopodia in rat and zebrafish transformed cells. (**A**) Immunohistofluorescence of MTLn3-derived perivascular tumors (DAPI, blue; phallodin, yellow; anti-cortactin, green; dotted line indicates boundary with vasculature, which is encapsulated with a basement membrane) indicate that protrusions (arrowheads) are associated with sites of collagen degradation (an antibody specific for degraded collagen I, Collagen ¾, red). (**B**) Cross sectional images of a zebrafish larvae showing intestinal epithelia in wild-type (left) and *mlt* mutants (center and right). Basement membrane is shown in red (laminin immunostain) and nuclei are stained blue (DAPI). Actin-rich (green, lifeact) protrusions in *mlt* intestine are associated with sites of basement membrane loss (arrowheads and insets). An asterisk labels an epithelial cell invading into the tissue stroma through a cleared region of the basement membrane. Scale bar is 10 μm. Images in (**A**) reprinted with permission, ©Gligorijevic, B., et al. 2012. Originally published in *J Cell Sci.* doi#10.1242/jcs.092726. Images in (**B**) reprinted with permission, ©Sieler, C., et al. 2012. Originally published in *PLOS Biol.* doi#10.1371/journal.pbio.1001386.

and metabolism.[28,59-61] Despite intensive study, one of the greatest gaps in the field has been establishing whether these structures actually exist in vivo, and if so, how they facilitate basement membrane transmigration.[21,62,63] In this review, we present recent work from our lab and others that have indentified invadopodia in vivo. Although the term invadopodia is typically used to describe invasive structures in cancer cells, we propose that invadopodia are components of a normal and likely ancient invasion program utilized by cells to pass through basement membrane during development that is co-opted by transformed cells. Further, we suggest that work in model systems such as zebrafish and *C. elegans* offers new and powerful approaches to understand the role of invadopodia in basement membrane transmigration, as well as insights into the physiological cues and intrinsic programs that govern their formation and function.

The Challenge of Studying Invadopodia In Vivo

The greatest impediment to identifying invadopodia in vivo has been the difficulty of visualizing interactions at the cell–basement membrane interface during the process of invasion. In tumor models, cancer cell invasion events are highly dynamic, rare, and unpredictable; it is thus challenging to catch cells in the act of invading.[64] Further, invasion events often occur deep in complex tissues and require simultaneous visualization of the invasive cell and basement membrane. As live-cell fluorescent-based basement membrane reporters are currently not available in vertebrate models, imaging basement membrane has been limited to ex vivo assays and fixed samples,[65-68] thus hindering the ability to detect and examine the dynamics of invadopodia in native settings.

Cancer Cells Generate Invadopodia Protrusions In Vivo

Despite the challenges of in vivo studies, a significant body of work has shed remarkable insight into cancer cell invasion in physiological settings.[20,62,64,69,70] The Condeelis and Segall groups have pioneered intravital (optical live-animal) imaging techniques, as well as a tumor model where GFP-expressing MTLn3 cells (MTLn3-GFP), a highly invasive rat mammary adenocarcinoma cell line that forms invadopodia in vitro, are injected into the mammary gland of immunocompromised mice and rats and allowed to form tumors.[20,71] Tumors derived from MTLn3 cells metastasize to lung, allowing tumor dissemination and invasion to be studied. Live-cell imaging and intravenous injection of rhodamine dextran have permitted visualization of MTLn3-GFP in the act of invading into vasculature, where these cells must pass through the vascular basement membrane.[72] Recently, utilizing cryosectioning and immunolocalization, protrusions from MTLn3-GFP adjacent to blood vessels have been identified that contain the invadopodia markers cortactin and N-WASP (key F-actin regulators; **Fig. 1A**).[73] The formation of these protrusions also correlates with areas of collagen I degradation, an interstitial matrix component (**Fig. 1A**). Intriguingly, reduction of N-WASP, which regulates invadopodia in vitro,[74] resulted in reduced

protrusions, and correlated with a dramatic reduction in collagen degradation and circulating tumor cells, consistent with an inability of these cells to enter the vasculature. Mouse–tumor model studies with Ras transformed HMLE cells expressing Twist (an immortalized human mammary epithelial cell line), have also revealed punctate subcellular invadopodia-like structures within tumor cells.[75] Inhibition of invadopodia formation through targeting of the PDGF receptor α and the scaffold protein Tks5 inhibited the local invasive ability of the HMLE cells expressing Twist and the tumors remained encapsulated. Stable knockdown of Tks5 also resulted in reduced metastasis in a mouse model for metastatic lung adenocarcinoma.[52] These results strongly support the idea that invadopodia are critical for cancer cell dissemination in vivo and suggest that these structures might be mediating basement membrane transmigration.

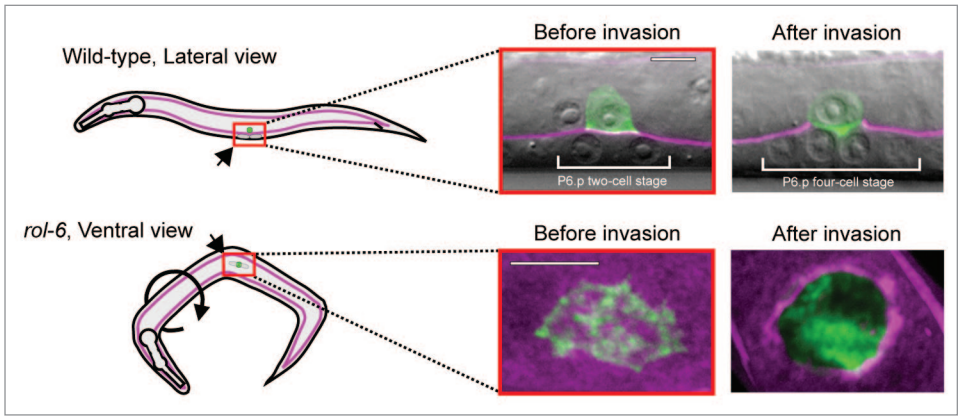

Figure 2. *C. elegans* anchor cell invasion imaged laterally or ventrally. The AC is viewed from two different perspectives for time-lapse imaging, depicted in the schematic (left), and examples of the images from these perspectives are shown (right). The basement membrane is visualized with laminin::GFP (magenta) and F-actin (green) with an AC-specific F-actin probe (*cdh-3* > *mCherry::moeABD*). Fluorescence is overlaid on differential interference contrast images. Wild-type animals (top) lie on their side, resulting in lateral imaging of the AC-basement membrane interaction. Roller mutant animals (bottom) orient randomly, permitting ventral imaging within the plane of the basement membrane. Confocal slices through the AC-basement membrane interface are shown at 2X magnification relative to the lateral view panels. Subsequent panels in this review of the AC are labeled with their perspective used for imaging. Scale Bars, 5 μm. This figure is reprinted with permission, ©Hagedorn, E.J., et al. 2013. Originally published in *J Cell Biol*. doi#10.1083/jcb.201301091.

Invadopodia-Like Protrusions in Transformed Zebrafish Intestinal Epithelium

Recent work by the Pack group has modeled epithelial cell invasion in transformed cells in zebrafish.[76] Intriguingly, zebrafish harboring a mutation that results in constitutive activity of the smooth muscle myosin heavy chain gene (*mlt* mutants) show a striking invasive remodeling of the neighboring intestinal epithelium. Constitutive activation of *mlt* in the smooth muscles causes an increase in smooth muscle contractile tone. Unregulated smooth muscle contractile tone is thought to result in the production of reactive oxygen species, which non-autonomously stimulates invasive remodeling in the neighboring intestinal epithelium.[76] This invasive remodeling includes the appearance of cellular protrusions that contain the invadopodia markers cortactin and Src.[76] These protrusions extend through regions of the intestinal epithelial basement membrane that are devoid of the basement membrane protein laminin, which is consistent with a potential role of invadopodia breaching the basement membrane (**Fig. 1B**). Further, their formation is dependent on Tks5, as well as the non-receptor tyrosine kinase Src, an upstream regulator of invadopodia formation.[23,77]

Recent studies have suggested that loss or disruptions of basement membrane may stimulate protrusions and invasive behavior.[8,78,79] Thus, contractions from the smooth musculature, which is tightly associated with the intestinal epithelia, could tear the basement membrane, inducing epithelial invasive behavior and escape. While this possibility was not formally excluded in this study, a constitutively activate form of Src expressed specifically within the intestinal epithelium in otherwise wild-type animals, led to ectopic F-actin-rich protrusions that were present in fixed samples at sites of apparent breaks in basement membrane.[76] This result adds weight to the notion that the invadopodia-like protrusions from the intestinal epithelial cells may be actively breaching the basement membrane. Interestingly, Src was not sufficient to promote escape of these cells from the epithelium, thus Src might be a specific regulator of invadopodia and does not stimulate a full EMT transition. The work in zebrafish offers further evidence that invadopodia are present in vivo and may mediate breaches in basement membrane.

Invadopodia Breach Basement Membrane During *C. elegans* Organogenesis

During uterine–vulval development in the nematode worm *C. elegans*, a specialized uterine cell called the anchor cell (AC), breaches the juxtaposed uterine and vulval epithelial basement membranes to initiate uterine–vulval attachment.[80,81] AC invasion is highly stereotyped, occurring over a narrow developmental window. The major structural components of basement membrane (laminin and type IV collagen) as well as minor components (SPARC, hemicentin, and fibulin) have been functionally tagged with GFP or GFP-derivatives in C. *elegans*.[82-86] Furthermore, specific proteins of interest can be tagged and expressed within the AC.[87] The ability to easily manipulate the orientation of the worm permits imaging of AC invasion from the lateral viewpoint, as well as within the plane of the cell–basement membrane interface (**Fig. 2**). The combination of these

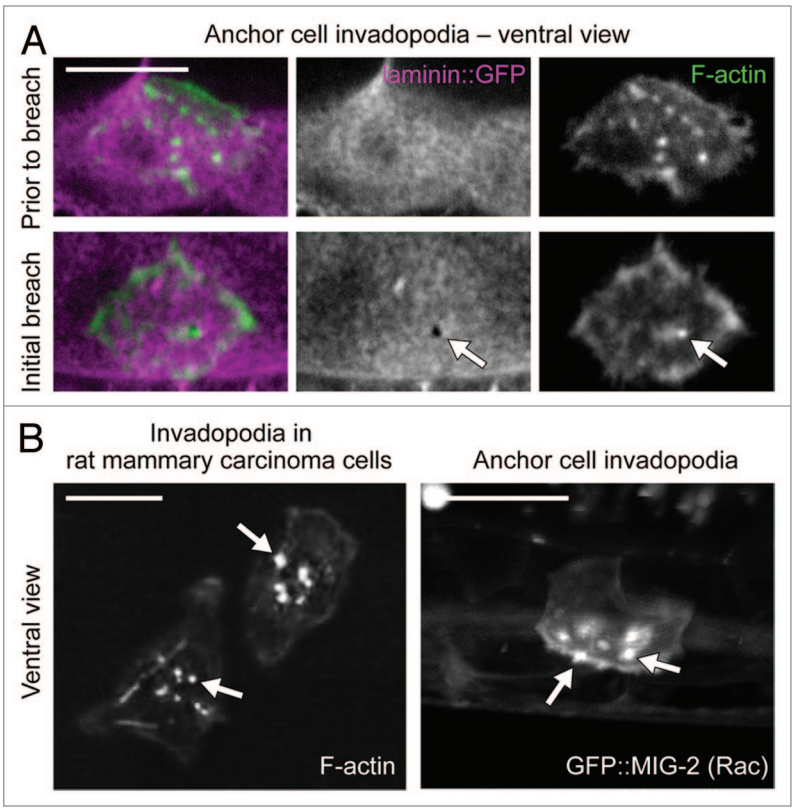

Figure 3. AC invadopodia breach the basement membrane and are similar to cancer cell invadopodia. (**A**) A ventral view time-lapse series of AC invadopodia (actin, green) and the basement membrane (laminin, magenta). Prior to a detectable breach of the basement membrane (top), the AC has many punctate F-actin structures. Upon breach of the basement membrane (bottom), a single F-actin-rich structure occupies the gap in the basement membrane (arrow). (**B**) Phallodin staining of F-actin in MtLn3 plated on fibronectin- and gelatin-coated coverslips labels invadopodia (left). An AC-specific translational reporter for a *C. elegans* Rac ortholog (right), GFP::MIG-2, labels invadopodia in a lateral image tilted 45 degrees back. These two models allow for resolution of individual invadopodia. AC scale bars, 5 um; cancer cell scale bar, 20 um. Left panel of (**B**) is reprinted with permission**, ©Yamaguchi, H., et al. 2005. Originally published in *J Cell Biol.* 168(3):441–52.

perspectives has facilitated the first real-time, high-resolution imaging of cell–basement membrane interactions during invasion in vivo.[12]

Using live-cell imaging of F-actin and actin regulators, we have recently found that the AC breaches the basement membrane with invadopodia (**Fig. 3A**).[88] Prior to invasion, these F-actin-rich foci, which form at the cell–basement membrane interface, look strikingly similar to invadopodia from cancer cells generated upon contact with gelatin and other artificial matrices plated on glass slides (**Fig. 3B**). The *C. elegans* invadopodia also have molecular markers shared by cancer cell invadopodia, including the membrane anchored Rac GTPases, the actin regulatory protein Ena/VASP, and the phospholipid PI(4,5)P_2.[44,89] Similar to cancer cell invadopodia, formation of these structures in the AC is dependent on integrin function.[88,90,91] Validating their ability to breach basement membrane, time-lapse analysis indicated that these structures depress basement membrane during turnover, and one always presages and then occupies the first visible breach in the basement membrane (**Fig. 3A**). These studies confirm that invadopodia breach basement membrane in vivo and strongly suggest that invadopodia are a conserved subcellular device utilized in normal development to penetrate basement membrane barriers.

One of the more interesting aspects of AC invadopodia is how rapidly they turnover and puncture basement membrane. Whereas invadopodia in cancer cells can persist over an hour,[21] the average lifetime of AC invadopodia is approximately 1 min, with the invadopodium that breaches the basement membrane persisting for approximately 5 min.[88] The reasons and significance of these dynamics between in vitro and AC invadopodia are unclear, and may reflect differences in the microenvironment (signaling or matrix properties) or intrinsic factors within the cells. It is notable that the entire process of AC invasion through the juxtaposed uterine and vulval basement membranes is completed in only 90 min.[80,87] In contrast, breast and colon cancer cells plated on native basement membranes in ex vivo assays take days to complete invasion.[65,68] It seems likely that these ex vivo assays may not recapitulate the in vivo signaling environment that mediates rapid invasion. Alternatively, cancer cells might be less efficient in their ability to invade basement membrane.

Over 50 proteins as well as specific phosphoinositide lipids are known to be associated with invadopodia in cancer cells.[43,44] Using genetic, expression, and RNAi based screens, we have identified over 100 genes that regulate AC invasion or are expressed specifically in the AC.[87,92-95] It will be important to determine their possible connection to AC invadopodia, as this will likely increase our understanding of the regulation of invadopodia as well as identify pathways that function in parallel with invadopodia to promote invasion. Most proteins strongly associated with invadopodia in cancer cells are encoded in the *C. elegans* genome and many of these have been identified in screens for genes promoting AC invasion (**Table 1**). Several important regulators, however, are absent. These include the actin regulator and adaptor protein cortactin, the Tks4/5 adaptor proteins, and the transmembrane matrix metalloproteinase MT1-MMP (**Table 1**).[42,44,96] Other proteins may functionally compensate for these absences. For example, similar to cortactin, the highly conserved Abp1 actin-binding protein also activates the Arp2/3 complex.[97,98] In addition, *C. elegans* encodes a predicted GPI membrane anchored matrix metalloproteinase, *zmp-1*, which is specifically expressed in the AC at the time of invasion. Although loss of *zmp-1* does not disrupt invasion in isolation, it is possible that one or more of the five other matrix metalloproteinases in *C. elegans* function redundantly with ZMP-1 during invasion.[99] Finally, it will be important to understand the potential role of PI(4,5)P2 and other phosphosphionositides in AC invadopodia. In cancer cells, these lipids have been proposed to play important roles in recruiting and activating actin regulators within

Table 1. Major cancer cell invadopodia proteins and *C. elegans* orthologs

Human protein	*C. elegans* ortholog	Category	Known role in AC invasion
Src[127]	SRC-1/2	Tyrosine kinase	
Tks4/5[128]	none	Src associated adaptor protein	
Cortactin[129]	none	Cytoskeleton regulation	
Abl family kinases[130,131]	ABL-1	Tyrosine kinase	
MMPs[56,132]	ZMP-1–6	Matrix degradation	+[87]
β1 and β3 integrins[133,134]	PAT-3	Cell adhesion	+[91]
Actin[135]	ACT-1	Cell structure and signaling scaffold	+[91]
Fascin[136]	FASN-1	Cytoskeleton regulation	
Arp2/3[74]	ARX-1–7	Cytoskeleton regulation	+[92]
Cofilin[74]	UNC-60	Cytoskeleton regulation	+[92]
WASp family[137]	WSP-1	Cytoskeleton regulation	
Ena/VASP/Mena[138]	UNC-34	Cytoskeleton regulation	+[93]
PKC[139]	PKC-1/2	Serine/Threonine kinase	
Fak/Pyk2[140,141]	KIN-32	Tyrosine kinase	
Paxilin[139,142]	PXL-1	Cytoskeleton regulation	
Nck[74]	NCK-1	Scaffold protein	
PI3K[143]	AAP-1	Kinase	
Rho family GTPases[144-146]	RHO-1	Cytoskeleton regulation	
	RAC-2, MIG-2, CED-10	Cytoskeleton regulation	+[93]
	CDC-42	Cytoskeleton regulation	+[92]

invadopodia, as well as linking the F-actin-based invadopodium core to the plasma membrane.[47,100]

Invadopodia Are Only One Component of the Invasion Process In Vivo

One of the advantages of examining invadopodia at high resolution in vivo is elucidating the role they play in the context of the complete process of invasion. By following the fate of breached AC invadopodia in *C. elegans*, we have also discovered that soon after penetrating the basement membrane, a large protrusion forms at the site of breach, crosses the basement membrane, and intercalates between cells of the underlying vulval epithelium (**Fig. 4**). Formation of this protrusion correlates with cessation of invadopodia formation, which likely prevents or reduces multiple breaching events. The *C. elegans* ortholog of the vertebrate netrin receptor DCC (UNC-40) plays a critical role in mediating the morphogenetic transition from invadopodia-driven basement membrane breaching to the formation of a protrusion that extends through the basement membrane.[88,93] During BM breaching by invadopodia, UNC-40 traffics to the initial breach and directs the formation of the invasive protrusion by recruiting and concentrating the same F-actin effectors that generate invadopodia (**Fig. 4**).[88] UNC-40 accumulation at the breach site appears to act as a molecular sink that depletes F-actin regulators at invadopodia—in the absence of UNC-40, invadopodia persist, multiple breaching events occur, and a large invasive protrusion fails to form (**Fig. 4**). This elegant mechanism shuts down invadopodia and focuses invasion through a single basement breach. The switch from invadopodia to invasive protrusion-driven changes probably involves mechanisms to dynamically add membrane and actin regulators that facilitate extension of the actin network. Furthermore, in cell culture, microtubules and the intermediate filament protein vimentin are required to extend invasive protrusions.[68] Mechanisms regulating an invadopodia-to-invasive protrusions switch likely occur in other invasive cells as electron microscopy and immunofluorescence studies have observed single protrusions from cancer cells crossing basement membrane in ex vivo invasion assays.[65,68] Whether these are also regulated by the netrin receptor DCC is unknown, but is possible given the strong association of netrin with metastatic cancers and invasive behavior in cell culture.[101-104] Importantly, after breaching basement membrane (a 2D flat, sheet-like surface), cancer cells often enter interstitial tissue, a largely acellular environment dominated by a less dense 3D network of fibrillar collagens.[105] This environment appears to trigger the formation of invadopodia-like structures that promote removal of interstitial matrix at points of cell restriction.[38,106-108] Thus, invadopodia dynamics are likely rapidly tuned to the matrix environment encountered by invasive cells.

The AC also specifically modifies the composition of the basement membrane targeted for invasion. It has been known for sometime that cancer cells secrete normal as well as novel basement membrane proteins and matrix-modifying enzymes.[109-113]

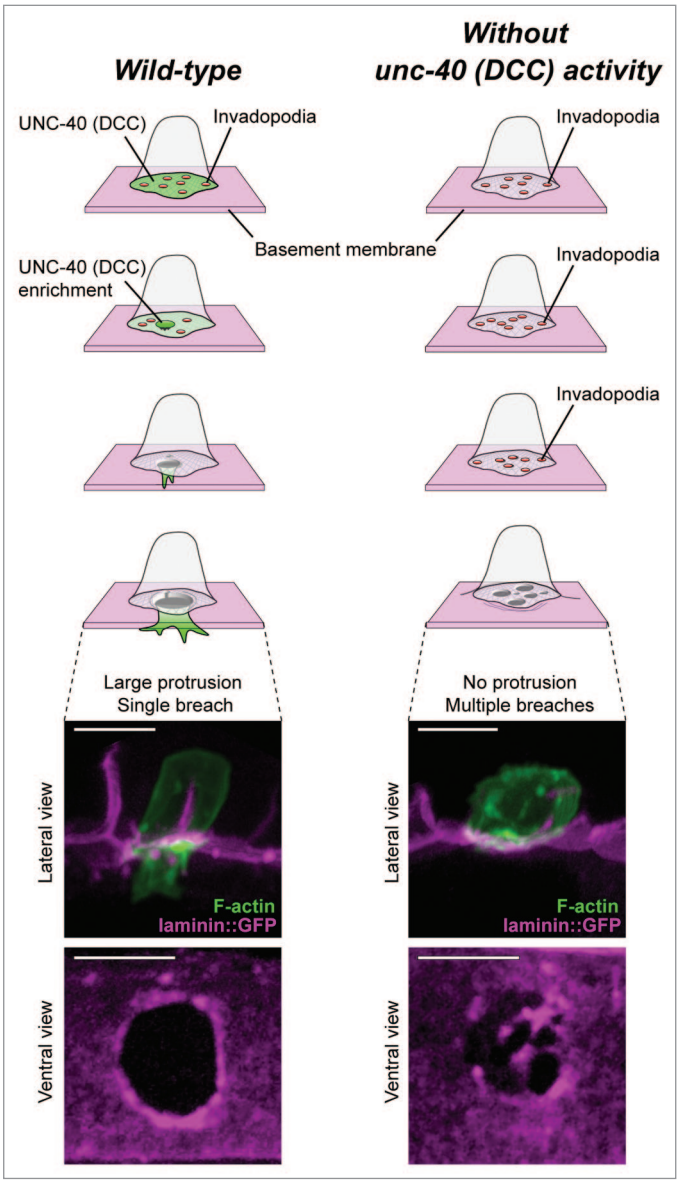

Figure 4. The netrin receptor DCC (UNC-40) mediates invadopodia to invasive protrusion transition. In wild-type animals (left), invadopodia (red circles) are dynamic and rapidly turn over until one breaches the basement membrane. UNC-40 (green) localizes to the initial breach and mediates the transition from many invadopodia to a single invasive protrusion that extends through the single gap in the basement membrane. The invasive protrusion opens a single gap in the basement membrane, allowing the basal membrane of the AC to contact the underlying vulval cells. A lateral view image shows the invasive protrusion extending (actin, green) through a single gap in the basement membrane (laminin, magenta). A ventral view image shows the widened gap through the basement membrane. In the absence of UNC-40 (right), invadopodia persist after the initial breach in the basement membrane, an invasive protrusion fails to form, and multiple breaches through the basement membrane occur. A lateral view image shows the lack of invasive protrusion formation in the AC and a ventral view images shows the multiple breaches through the basement membrane. Scale bars, 5 um. This figure is adapted from and reprinted with permission, ©Morrissey, M.A., et al., 2013. Originally published in *Worm.* doi#20.4262/worm.26169 and ©Hagedorn, E.J., et al. 2013. Originally published in *J Cell Biol.* doi#10.1083/jcb.201301091.

How this may specifically contribute to invasion and tumor metastasis is poorly understood. Just prior to invasion, the AC secretes the conserved extracellular matrix protein hemicentin specifically under the AC in the basement membrane that will be crossed.[84,87] Although the precise function of hemicentin is unclear, deposition of hemicentin assists basement removal during invasion.[87] Active modification of the basement membrane might change its structural properties to facilitate invadopodia penetration and precise removal.

One possible function for hemicentin is to promote physical displacement of the basement membrane. Although it has been generally assumed that basement membrane is dissolved by proteases during invasion,[114] optical highlighting of basement membrane components using a photoconvertible Dendra tag[83,115] has revealed that the basement membrane under the AC is removed, in part, by physical means.[88] The protrusion generated by the AC appears to generate forces that physically shift the basement membrane. Given that the protease *zmp-1* is expressed throughout AC invasion, *zmp-1* and other proteases might assist invasion by weakening the structural make-up of the basement membrane to facilitate physical displacement. Further sensitized screening, as well as profiling gene expression in the AC using single cell isolation techniques,[116] will help reveal mechanisms of how basement membrane is modified to assist in cell invasion events.

Summary and Outlook

The recent studies highlighted here have established that invadopodia and invadopodia-like protrusions exist in vivo. Although examination of invadopodia and cell invasion in natural settings are inherently more challenging due to limitations in visual analysis, experimental manipulations, and examination of large numbers of events, these studies will be essential in establishing how invadopodia are regulated and function during invasion. It is already clear from in vivo studies in zebrafish and *C. elegans* that while invadopodia mediate basement membrane breaching, they are not sufficient to facilitate basement membrane crossing or full removal of this barrier. The use of model systems such as zebrafish and *C. elegans*, with their visual accessibility, simple tissue architecture, transgenics, and amenability to genetic analysis, offers a potent complementary approach to tumor models to answer some of the most pressing questions concerning invadopodia and cell invasion. These include the identities of the signaling pathways that control their formation and regulation, and the cell-intrinsic factors, including transcriptional networks that facilitate invadopodia generation in cells. Identification of other models of invadopodia-basement membrane breaching events in vivo will also be valuable in elucidating the range of invadopodia dynamics and activity in breaching basement membrane. Perhaps most importantly, in vivo studies will be critical in determining how widespread the use of invadopodia are in breaching basement membrane. For example, are invadopodia used in EMT events in development and cancer to facilitate basement membrane crossing? While several studies

have implicated invadopodia with EMT,[75,117] direct visualization of their activity and formation is lacking. Moreover, there is evidence that cells cross basement membrane through a variety of mechanisms, including downregulation of basement membrane receptors,[118] physical forces that shift or breach the basement membrane,[79,83,119] entry through preformed portals,[66,67,120,121] and remodeling events that create absences or holes in the basement membrane.[62,122-124] These studies will be important in evaluating whether targeting invadopodia would be an efficient approach to blocking cancer metastasis. The observation of invadopodia in nematodes, which diverged from the lineage that gave rise to vertebrates approximately 600 million y ago,[125,126] argues strongly that invadopodia are ancient and likely widely utilized structures for basement membrane transmigration. We expect the expansion of in vivo studies will be essential in clarifying the utilization of invadopodia in invasive cell contexts and reveal novel aspects of the regulation and function of these fascinating basement membrane-piercing structures.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer 2003; 3:422-33; PMID:12778132; http://dx.doi.org/10.1038/nrc1094
2. Halfter W, Candiello J, Hu H, Zhang P, Schreiber E, Balasubramani M. Protein composition and biomechanical properties of in vivo-derived basement membranes. Cell Adh Migr 2013; 7:64-71; http://dx.doi.org/10.4161/cam.22479; PMID:23154404
3. Hohenester E, Yurchenco PD. Laminins in basement membrane assembly. Cell Adh Migr 2013; 7:56-63; http://dx.doi.org/10.4161/cam.21831; PMID:23076216
4. Hynes RO. The evolution of metazoan extracellular matrix. J Cell Biol 2012; 196:671-9; http://dx.doi.org/10.1083/jcb.201109041; PMID:22431747
5. Ozbek S, Balasubramanian PG, Chiquet-Ehrismann R, Tucker RP, Adams JC. The evolution of extracellular matrix. Mol Biol Cell 2010; 21:4300-5; http://dx.doi.org/10.1091/mbc.E10-03-0251; PMID:21160071
6. Pastor-Pareja JC, Xu T. Shaping cells and organs in Drosophila by opposing roles of fat body-secreted Collagen IV and perlecan. Dev Cell 2011; 21:245-56; http://dx.doi.org/10.1016/j.devcel.2011.06.026; PMID:21839919
7. Rasmussen JP, Reddy SS, Priess JR. Laminin is required to orient epithelial polarity in the C. elegans pharynx. Development 2012; 139:2050-60; http://dx.doi.org/10.1242/dev.078360; PMID:22535412
8. Egea J, Erlacher C, Montanez E, Burtscher I, Yamagishi S, Hess M, Hampel F, Sanchez R, Rodriguez-Manzaneque MT, Bösl MR, et al. Genetic ablation of FLRT3 reveals a novel morphogenetic function for the anterior visceral endoderm in suppressing mesoderm differentiation. Genes Dev 2008; 22:3349-62; http://dx.doi.org/10.1101/gad.486708; PMID:19056886
9. Gnanaguru G, Bachay G, Biswas S, Pinzón-Duarte G, Hunter DD, Brunken WJ. Laminins containing the β2 and γ3 chains regulate astrocyte migration and angiogenesis in the retina. Development 2013; 140:2050-60; http://dx.doi.org/10.1242/dev.087817; PMID:23571221
10. Hashimoto J, Kariya Y, Miyazaki K. Regulation of proliferation and chondrogenic differentiation of human mesenchymal stem cells by laminin-5 (laminin-332). Stem Cells 2006; 24:2346-54; http://dx.doi.org/10.1634/stemcells.2005-0605; PMID:17071854
11. Spenlé C, Simon-Assmann P, Orend G, Miner JH. Laminin α5 guides tissue patterning and organogenesis. Cell Adh Migr 2013; 7:90-100; http://dx.doi.org/10.4161/cam.22236; PMID:23076210
12. Hagedorn EJ, Sherwood DR. Cell invasion through basement membrane: the anchor cell breaches the barrier. Curr Opin Cell Biol 2011; 23:589-96; http://dx.doi.org/10.1016/j.ceb.2011.05.002; PMID:21632231
13. Rowe RG, Weiss SJ. Breaching the basement membrane: who, when and how? Trends Cell Biol 2008; 18:560-74; http://dx.doi.org/10.1016/j.tcb.2008.08.007; PMID:18848450
14. Khoshnoodi J, Pedchenko V, Hudson BG. Mammalian collagen IV. Microsc Res Tech 2008; 71:357-70; http://dx.doi.org/10.1002/jemt.20564; PMID:18219669
15. Nakaya Y, Sheng G. EMT in developmental morphogenesis. Cancer Lett 2013; 341:9-15; http://dx.doi.org/10.1016/j.canlet.2013.02.037; PMID:23462225
16. Cheung M, Chaboissier MC, Mynett A, Hirst E, Schedl A, Briscoe J. The transcriptional control of trunk neural crest induction, survival, and delamination. Dev Cell 2005; 8:179-92; http://dx.doi.org/10.1016/j.devcel.2004.12.010; PMID:15691760
17. Nakaya Y, Sukowati EW, Wu Y, Sheng G. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. Nat Cell Biol 2008; 10:765-75; http://dx.doi.org/10.1038/ncb1739; PMID:18552836
18. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011; 147:275-92; http://dx.doi.org/10.1016/j.cell.2011.09.024; PMID:22000009
19. Yamaguchi H. Pathological roles of invadopodia in cancer invasion and metastasis. Eur J Cell Biol 2012; 91:902-7; http://dx.doi.org/10.1016/j.ejcb.2012.04.005; PMID:22658792
20. Condeelis J, Segall JE. Intravital imaging of cell movement in tumours. Nat Rev Cancer 2003; 3:921-30; http://dx.doi.org/10.1038/nrc1231; PMID:14737122
21. Linder S, Wiesner C, Himmel M. Degrading devices: invadosomes in proteolytic cell invasion. Annu Rev Cell Dev Biol 2011; 27:185-211; http://dx.doi.org/10.1146/annurev-cellbio-092910-154216; PMID:21801014
22. Weaver AM. Invadopodia: specialized cell structures for cancer invasion. Clin Exp Metastasis 2006; 23:97-105; http://dx.doi.org/10.1007/s10585-006-9014-1; PMID:16830222
23. Murphy DA, Courtneidge SA. The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. Nat Rev Mol Cell Biol 2011; 12:413-26; http://dx.doi.org/10.1038/nrm3141; PMID:21697900
24. Zambonin-Zallone A, Teti A, Carano A, Marchisio PC. The distribution of podosomes in osteoclasts cultured on bone laminae: effect of retinol. J Bone Miner Res 1988; 3:517-23; http://dx.doi.org/10.1002/jbmr.5650030507; PMID:3195364
25. Davies WA, Stossel TP. Peripheral hyaline blebs (podosomes) of macrophages. J Cell Biol 1977; 75:941-55; PMID:925088; http://dx.doi.org/10.1083/jcb.75.3.941
26. Tarone G, Cirillo D, Giancotti FG, Comoglio PM, Marchisio PC. Rous sarcoma virus-transformed fibroblasts adhere primarily at discrete protrusions of the ventral membrane called podosomes. Exp Cell Res 1985; 159:141-57; PMID:2411576; http://dx.doi.org/10.1016/S0014-4827(85)80044-6
27. Wolosewick JJ. Distribution of actin in migrating leukocytes in vivo. Cell Tissue Res 1984; 236:517-25; PMID:6467334; http://dx.doi.org/10.1007/BF00217218
28. Hoshino D, Branch KM, Weaver AM. Signaling inputs to invadopodia and podosomes. J Cell Sci 2013; 126:2979-89; http://dx.doi.org/10.1242/jcs.079475; PMID:23843616
29. Buccione R, Orth JD, McNiven MA. Foot and mouth: podosomes, invadopodia and circular dorsal ruffles. Nat Rev Mol Cell Biol 2004; 5:647-57; http://dx.doi.org/10.1038/nrm1436; PMID:15366708
30. Linder S. Invadosomes at a glance. J Cell Sci 2009; 122:3009-13; http://dx.doi.org/10.1242/jcs.032631; PMID:19692587
31. Destaing O, Block MR, Planus E, Albiges-Rizo C. Invadosome regulation by adhesion signaling. Curr Opin Cell Biol 2011; 23:597-606; http://dx.doi.org/10.1016/j.ceb.2011.04.002; PMID:21550788
32. Saltel F, Daubon T, Juin A, Ganuza IE, Veillat V, Génot E. Invadosomes: intriguing structures with promise. Eur J Cell Biol 2011; 90:100-7; http://dx.doi.org/10.1016/j.ejcb.2010.05.011; PMID:20605056
33. McNiven MA. Breaking away: matrix remodeling from the leading edge. Trends Cell Biol 2013; 23:16-21; http://dx.doi.org/10.1016/j.tcb.2012.08.009; PMID:22999190
34. Mueller SC, Chen WT. Cellular invasion into matrix beads: localization of beta 1 integrins and fibronectin to the invadopodia. J Cell Sci 1991; 99:213-25; PMID:1885668
35. Chen WT. Proteolytic activity of specialized surface protrusions formed at rosette contact sites of transformed cells. J Exp Zool 1989; 251:167-85; http://dx.doi.org/10.1002/jez.1402510206; PMID:2549171
36. Clark ES, Whigham AS, Yarbrough WG, Weaver AM. Cortactin is an essential regulator of matrix metalloproteinase secretion and extracellular matrix degradation in invadopodia. Cancer Res 2007; 67:4227-35; http://dx.doi.org/10.1158/0008-5472.CAN-06-3928; PMID:17483334
37. Kramer RH, Bensch KG, Wong J. Invasion of reconstituted basement membrane matrix by metastatic human tumor cells. Cancer Res 1986; 46:1980-9; PMID:3948175

38. Yu X, Machesky LM. Cells assemble invadopodia-like structures and invade into matrigel in a matrix metalloprotease dependent manner in the circular invasion assay. PLoS One 2012; 7:e30605; http://dx.doi.org/10.1371/journal.pone.0030605; PMID:22347388
39. Sharma VP, Entenberg D, Condeelis J. High-resolution live-cell imaging and time-lapse microscopy of invadopodium dynamics and tracking analysis. Methods Mol Biol 2013; 1046:343-57; http://dx.doi.org/10.1007/978-1-62703-538-5_21; PMID:23868599
40. Weaver AM, Page JM, Guelcher SA, Parekh A. Synthetic and tissue-derived models for studying rigidity effects on invadopodia activity. Methods Mol Biol 2013; 1046:171-89; http://dx.doi.org/10.1007/978-1-62703-538-5_10; PMID:23868588
41. Linder S. The matrix corroded: podosomes and invadopodia in extracellular matrix degradation. Trends Cell Biol 2007; 17:107-17; http://dx.doi.org/10.1016/j.tcb.2007.01.002; PMID:17275303
42. Buccione R, Caldieri G, Ayala I. Invadopodia: specialized tumor cell structures for the focal degradation of the extracellular matrix. Cancer Metastasis Rev 2009; 28:137-49; http://dx.doi.org/10.1007/s10555-008-9176-1; PMID:19153671
43. Hoshino D, Jourquin J, Emmons SW, Miller T, Goldgof M, Costello K, Tyson DR, Brown B, Lu Y, Prasad NK, et al. Network analysis of the focal adhesion to invadopodia transition identifies a PI3K-PKCα invasive signaling axis. Sci Signal 2012; 5:ra66; http://dx.doi.org/10.1126/scisignal.2002964; PMID:22969158
44. Klemke RL. Trespassing cancer cells: 'fingerprinting' invasive protrusions reveals metastatic culprits. Curr Opin Cell Biol 2012; 24:662-9; http://dx.doi.org/10.1016/j.ceb.2012.08.005; PMID:22980730
45. Attanasio F, Caldieri G, Giacchetti G, van Horssen R, Wieringa B, Buccione R. Novel invadopodia components revealed by differential proteomic analysis. Eur J Cell Biol 2011; 90:115-27; http://dx.doi.org/10.1016/j.ejcb.2010.05.004; PMID:20609496
46. Kelley LC, Ammer AG, Hayes KE, Martin KH, Machida K, Jia L, Mayer BJ, Weed SA. Oncogenic Src requires a wild-type counterpart to regulate invadopodia maturation. J Cell Sci 2010; 123:3923-32; http://dx.doi.org/10.1242/jcs.075200; PMID:20980387
47. Sharma VP, Eddy R, Entenberg D, Kai M, Gertler FB, Condeelis J. Tks5 and SHIP2 regulate invadopodium maturation, but not initiation, in breast carcinoma cells. Curr Biol 2013; 23:2079-89; http://dx.doi.org/10.1016/j.cub.2013.08.044; PMID:24206842
48. Artym VV, Zhang Y, Seillier-Moiseiwitsch F, Yamada KM, Mueller SC. Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: defining the stages of invadopodia formation and function. Cancer Res 2006; 66:3034-43; http://dx.doi.org/10.1158/0008-5472.CAN-05-2177; PMID:16540652
49. Oser M, Yamaguchi H, Mader CC, Bravo-Cordero JJ, Arias M, Chen X, Desmarais V, van Rheenen J, Koleske AJ, Condeelis J. Cortactin regulates cofilin and N-WASp activities to control the stages of invadopodium assembly and maturation. J Cell Biol 2009; 186:571-87; http://dx.doi.org/10.1083/jcb.200812176; PMID:19704022
50. Razidlo GL, Schroeder B, Chen J, Billadeau DD, McNiven MA. Vav1 as a Central Regulator of Invadopodia Assembly. Curr Biol 2013; http://dx.doi.org/10.1016/j.cub.2013.11.013; PMID:24332539
51. Brisson L, Driffort V, Benoist L, Poet M, Counillon L, Antelmi E, Rubino R, Besson P, Labbal F, Chevalier S, et al. NaV1.5 Na+ channels allosterically regulate the NHE-1 exchanger and promote the activity of breast cancer cell invadopodia. J Cell Sci 2013; 126:4835-42; http://dx.doi.org/10.1242/jcs.123901; PMID:23902689
52. Li CM, Chen G, Dayton TL, Kim-Kiselak C, Hoersch S, Whittaker CA, Bronson RT, Beer DG, Winslow MM, Jacks T. Differential Tks5 isoform expression contributes to metastatic invasion of lung adenocarcinoma. Genes Dev 2013; 27:1557-67; http://dx.doi.org/10.1101/gad.222745.113; PMID:23873940
53. Poincloux R, Lizárraga F, Chavrier P. Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia. J Cell Sci 2009; 122:3015-24; http://dx.doi.org/10.1242/jcs.034561; PMID:19692588
54. Hoshino D, Kirkbride KC, Costello K, Clark ES, Sinha S, Grega-Larson N, Tyska MJ, Weaver AM. Exosome secretion is enhanced by invadopodia and drives invasive behavior. Cell Rep 2013; 5:1159-68; http://dx.doi.org/10.1016/j.celrep.2013.10.050; PMID:24290760
55. Monteiro P, Rossé C, Castro-Castro A, Irondelle M, Lagoutte E, Paul-Gilloteaux P, Desnos C, Formstecher E, Darchen F, Perrais D, et al. Endosomal WASH and exocyst complexes control exocytosis of MT1-MMP at invadopodia. J Cell Biol 2013; 203:1063-79; http://dx.doi.org/10.1083/jcb.201306162; PMID:24344185
56. Chen WT, Wang JY. Specialized surface protrusions of invasive cells, invadopodia and lamellipodia, have differential MT1-MMP, MMP-2, and TIMP-2 localization. Ann N Y Acad Sci 1999; 878:361-71; PMID:10415741; http://dx.doi.org/10.1111/j.1749-6632.1999.tb07695.x
57. Paz H, Pathak N, Yang J. Invading one step at a time: the role of invadopodia in tumor metastasis. Oncogene 2013; http://dx.doi.org/10.1038/onc.2013.393; PMID:24077283
58. Artym VV, Matsumoto K, Mueller SC, Yamada KM. Dynamic membrane remodeling at invadopodia differentiates invadopodia from podosomes. Eur J Cell Biol 2011; 90:172-80; http://dx.doi.org/10.1016/j.ejcb.2010.06.006; PMID:20656375
59. Lucien F, Brochu-Gaudreau K, Arsenault D, Harper K, Dubois CM. Hypoxia-induced invadopodia formation involves activation of NHE-1 by the p90 ribosomal S6 kinase (p90RSK). PLoS One 2011; 6:e28851; http://dx.doi.org/10.1371/journal.pone.0028851; PMID:22216126
60. van Horssen R, Buccione R, Willemse M, Cingir S, Wieringa B, Attanasio F. Cancer cell metabolism regulates extracellular matrix degradation by invadopodia. Eur J Cell Biol 2013; 92:113-21; http://dx.doi.org/10.1016/j.ejcb.2012.11.003; PMID:23306026
61. Alexander NR, Branch KM, Parekh A, Clark ES, Iwueke IC, Guelcher SA, Weaver AM. Extracellular matrix rigidity promotes invadopodia activity. Curr Biol 2008; 18:1295-9; http://dx.doi.org/10.1016/j.cub.2008.07.090; PMID:18718759
62. Beerling E, Ritsma L, Vrisekoop N, Derksen PW, van Rheenen J. Intravital microscopy: new insights into metastasis of tumors. J Cell Sci 2011; 124:299-310; http://dx.doi.org/10.1242/jcs.072728; PMID:21242309
63. Sibony-Benyamini H, Gil-Henn H. Invadopodia: the leading force. Eur J Cell Biol 2012; 91:896-901; http://dx.doi.org/10.1016/j.ejcb.2012.04.001; PMID:22633185
64. Sahai E. Illuminating the metastatic process. Nat Rev Cancer 2007; 7:737-49; http://dx.doi.org/10.1038/nrc2229; PMID:17891189
65. Hotary K, Li XY, Allen E, Stevens SL, Weiss SJ. A cancer cell metalloprotease triad regulates the basement membrane transmigration program. Genes Dev 2006; 20:2673-86; PMID:16983145; http://dx.doi.org/10.1101/gad.1451806
66. Pflicke H, Sixt M. Preformed portals facilitate dendritic cell entry into afferent lymphatic vessels. J Exp Med 2009; 206:2925-35; http://dx.doi.org/10.1084/jem.20091739; PMID:19995949
67. Wang S, Voisin MB, Larbi KY, Dangerfield J, Scheiermann C, Tran M, Maxwell PH, Sorokin L, Nourshargh S. Venular basement membranes contain specific matrix protein low expression regions that act as exit points for emigrating neutrophils. J Exp Med 2006; 203:1519-32; PMID:16754715; http://dx.doi.org/10.1084/jem.20051210
68. Schoumacher M, Goldman RD, Louvard D, Vignjevic DM. Actin, microtubules, and vimentin intermediate filaments cooperate for elongation of invadopodia. J Cell Biol 2010; 189:541-56; http://dx.doi.org/10.1083/jcb.200909113; PMID:20421424
69. Yamaguchi H, Wyckoff J, Condeelis J. Cell migration in tumors. Curr Opin Cell Biol 2005; 17:559-64; http://dx.doi.org/10.1016/j.ceb.2005.08.002; PMID:16098726
70. Alexander S, Weigelin B, Winkler F, Friedl P. Preclinical intravital microscopy of the tumour-stroma interface: invasion, metastasis, and therapy response. Curr Opin Cell Biol 2013; 25:659-71; http://dx.doi.org/10.1016/j.ceb.2013.07.001; PMID:23896198
71. Wyckoff JB, Jones JG, Condeelis JS, Segall JE. A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. Cancer Res 2000; 60:2504-11; PMID:10811132
72. Wang W, Wyckoff JB, Frohlich VC, Oleynikov Y, Hüttelmaier S, Zavadil J, Cermak L, Bottinger EP, Singer RH, White JG, et al. Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. Cancer Res 2002; 62:6278-88; PMID:12414658
73. Gligorijevic B, Wyckoff J, Yamaguchi H, Wang Y, Roussos ET, Condeelis J. N-WASP-mediated invadopodium formation is involved in intravasation and lung metastasis of mammary tumors. J Cell Sci 2012; 125:724-34; http://dx.doi.org/10.1242/jcs.092726; PMID:22389406
74. Yamaguchi H, Lorenz M, Kempiak S, Sarmiento C, Coniglio S, Symons M, Segall J, Eddy R, Miki H, Takenawa T, et al. Molecular mechanisms of invadopodium formation: the role of the N-WASP-Arp2/3 complex pathway and cofilin. J Cell Biol 2005; 168:441-52; http://dx.doi.org/10.1083/jcb.200407076; PMID:15684033
75. Eckert MA, Lwin TM, Chang AT, Kim J, Danis E, Ohno-Machado L, Yang J. Twist1-induced invadopodia formation promotes tumor metastasis. Cancer Cell 2011; 19:372-86; http://dx.doi.org/10.1016/j.ccr.2011.01.036; PMID:21397860
76. Seiler C, Davuluri G, Abrams J, Byfield FJ, Janmey PA, Pack M. Smooth muscle tension induces invasive remodeling of the zebrafish intestine. PLoS Biol 2012; 10:e1001386; http://dx.doi.org/10.1371/journal.pbio.1001386; PMID:22973180
77. Frame MC. Newest findings on the oldest oncogene; how activated src does it. J Cell Sci 2004; 117:989-98; http://dx.doi.org/10.1242/jcs.01111; PMID:14996930
78. Nguyen-Ngoc KV, Cheung KJ, Brenot A, Shamir ER, Gray RS, Hines WC, Yaswen P, Werb Z, Ewald AJ. ECM microenvironment regulates collective migration and local dissemination in normal and malignant mammary epithelium. Proc Natl Acad Sci U S A 2012; 109:E2595-604; http://dx.doi.org/10.1073/pnas.1212834109; PMID:22923691
79. Hiramatsu R, Matsuoka T, Kimura-Yoshida C, Han SW, Mochida K, Adachi T, Takayama S, Matsuo I. External mechanical cues trigger the establishment of the anterior-posterior axis in early mouse embryos. Dev Cell 2013; 27:131-44; http://dx.doi.org/10.1016/j.devcel.2013.09.026; PMID:24176640
80. Sherwood DR, Sternberg PW. Anchor cell invasion into the vulval epithelium in C. elegans. Dev Cell 2003; 5:21-31; PMID:12852849; http://dx.doi.org/10.1016/S1534-5807(03)00168-0

81. Schindler AJ, Sherwood DR. Morphogenesis of the caenorhabditis elegans vulva. Wiley Interdiscip Rev Dev Biol 2013; 2:75-95; http://dx.doi.org/10.1002/wdev.87; PMID:23418408
82. Kao G, Huang CC, Hedgecock EM, Hall DH, Wadsworth WG. The role of the laminin beta subunit in laminin heterotrimer assembly and basement membrane function and development in C. elegans. Dev Biol 2006; 290:211-9; PMID:16376872; http://dx.doi.org/10.1016/j.ydbio.2005.11.026
83. Ihara S, Hagedorn EJ, Morrissey MA, Chi Q, Motegi F, Kramer JM, Sherwood DR. Basement membrane sliding and targeted adhesion remodels tissue boundaries during uterine-vulval attachment in Caenorhabditis elegans. Nat Cell Biol 2011; 13:641-51; http://dx.doi.org/10.1038/ncb2233; PMID:21572423
84. Vogel BE, Hedgecock EM. Hemicentin, a conserved extracellular member of the immunoglobulin superfamily, organizes epithelial and other cell attachments into oriented line-shaped junctions. Development 2001; 128:883-94; PMID:11222143
85. Fitzgerald MC, Schwarzbauer JE. Importance of the basement membrane protein SPARC for viability and fertility in Caenorhabditis elegans. Curr Biol 1998; 8:1285-8; PMID:9822581; http://dx.doi.org/10.1016/S0960-9822(07)00540-4
86. Hesselson D, Newman C, Kim KW, Kimble J. GON-1 and fibulin have antagonistic roles in control of organ shape. Curr Biol 2004; 14:2005-10; http://dx.doi.org/10.1016/j.cub.2004.11.006; PMID:15556862
87. Sherwood DR, Butler JA, Kramer JM, Sternberg PW. FOS-1 promotes basement-membrane removal during anchor-cell invasion in C. elegans. Cell 2005; 121:951-62; PMID:15960981; http://dx.doi.org/10.1016/j.cell.2005.03.031
88. Hagedorn EJ, Ziel JW, Morrissey MA, Linden LM, Wang Z, Chi Q, Johnson SA, Sherwood DR. The netrin receptor DCC focuses invadopodia-driven basement membrane transmigration in vivo. J Cell Biol 2013; 201:903-13; http://dx.doi.org/10.1083/jcb.201301091; PMID:23751497
89. Yamaguchi H, Yoshida S, Muroi E, Kawamura M, Kouchi Z, Nakamura Y, Sakai R, Fukami K. Phosphatidylinositol 4,5-bisphosphate and PIP5-kinase Ialpha are required for invadopodia formation in human breast cancer cells. Cancer Sci 2010; 101:1632-8; http://dx.doi.org/10.1111/j.1349-7006.2010.01574.x; PMID:20426790
90. Destaing O, Planus E, Bouvard D, Oddou C, Badowski C, Bossy V, Raducanu A, Fourcade B, Albiges-Rizo C, Block MR. β1A integrin is a master regulator of invadosome organization and function. Mol Biol Cell 2010; 21:4108-19; http://dx.doi.org/10.1091/mbc.E10-07-0580; PMID:20926684
91. Hagedorn EJ, Yashiro H, Ziel JW, Ihara S, Wang Z, Sherwood DR. Integrin acts upstream of netrin signaling to regulate formation of the anchor cell's invasive membrane in C. elegans. Dev Cell 2009; 17:187-98; http://dx.doi.org/10.1016/j.devcel.2009.06.006; PMID:19686680
92. Matus DQ, Li XY, Durbin S, Agarwal D, Chi Q, Weiss SJ, Sherwood DR. In vivo identification of regulators of cell invasion across basement membranes. Sci Signal 2010; 3:ra35; http://dx.doi.org/10.1126/scisignal.2000654; PMID:20442418
93. Ziel JW, Hagedorn EJ, Audhya A, Sherwood DR. UNC-6 (netrin) orients the invasive membrane of the anchor cell in C. elegans. Nat Cell Biol 2009; 11:183-9; http://dx.doi.org/10.1038/ncb1825; PMID:19098902
94. Schindler AJ, Sherwood DR. The transcription factor HLH-2/E/Daughterless regulates anchor cell invasion across basement membrane in C. elegans. Dev Biol 2011; 357:380-91; http://dx.doi.org/10.1016/j.ydbio.2011.07.012; PMID:21784067
95. Ziel JW, Matus DQ, Sherwood DR. An expression screen for RhoGEF genes involved in C. elegans gonadogenesis. Gene Expr Patterns 2009; 9:397-403; http://dx.doi.org/10.1016/j.gep.2009.06.005; PMID:19540360
96. Stylli SS, Kaye AH, Lock P. Invadopodia: at the cutting edge of tumour invasion. J Clin Neurosci 2008; 15:725-37; http://dx.doi.org/10.1016/j.jocn.2008.03.003; PMID:18468901
97. Olazabal IM, Machesky LM. Abp1p and cortactin, new "hand-holds" for actin. J Cell Biol 2001; 154:679-82; http://dx.doi.org/10.1083/jcb.200105061; PMID:11514584
98. Kessels MM, Engqvist-Goldstein AE, Drubin DG. Association of mouse actin-binding protein 1 (mAbp1/SH3P7), an Src kinase target, with dynamic regions of the cortical actin cytoskeleton in response to Rac1 activation. Mol Biol Cell 2000; 11:393-412; PMID:10637315; http://dx.doi.org/10.1091/mbc.11.1.393
99. Altincicek B, Fischer M, Fischer M, Lüersen K, Boll M, Wenzel U, Vilcinskas A. Role of matrix metalloproteinase ZMP-2 in pathogen resistance and development in Caenorhabditis elegans. Dev Comp Immunol 2010; 34:1160-9; http://dx.doi.org/10.1016/j.dci.2010.06.010; PMID:20600277
100. Yamaguchi H, Oikawa T. Membrane lipids in invadopodia and podosomes: key structures for cancer invasion and metastasis. Oncotarget 2010; 1:320-8; PMID:21307399
101. Dumartin L, Quemener C, Laklai H, Herbert J, Bicknell R, Bousquet C, Pyronnet S, Castronovo V, Schilling MK, Bikfalvi A, et al. Netrin-1 mediates early events in pancreatic adenocarcinoma progression, acting on tumor and endothelial cells. Gastroenterology 2010; 138:1595-606, e1-8; http://dx.doi.org/10.1053/j.gastro.2009.12.061; PMID:20080097
102. Kaufmann S, Kuphal S, Schubert T, Bosserhoff AK. Functional implication of Netrin expression in malignant melanoma. Cell Oncol 2009; 31:415-22; http://dx.doi.org/10.3233/CLO-2009-0491; PMID:19940358
103. Ramesh G, Berg A, Jayakumar C. Plasma netrin-1 is a diagnostic biomarker of human cancers. Biomarkers 2011; 16:172-80; http://dx.doi.org/10.3109/1354750X.2010.541564; PMID:21303223
104. Shimizu A, et al. Netrin-1 Promotes Glioblastoma Cell Invasiveness and Angiogenesis by Multiple Pathways Involving Activation of RhoA, Cathepsin B and CREB. J Biol Chem 2012; 288:2210-22 http://dx.doi.org/ 10.1074/jbc.M112.397398; PMID:23195957
105. Even-Ram S, Yamada KM. Cell migration in 3D matrix. Curr Opin Cell Biol 2005; 17:524-32; PMID:16112853; http://dx.doi.org/10.1016/j.ceb.2005.08.015
106. Li A, Dawson JC, Forero-Vargas M, Spence HJ, Yu X, König I, Anderson K, Machesky LM. The actin-bundling protein fascin stabilizes actin in invadopodia and potentiates protrusive invasion. Curr Biol 2010; 20:339-45; http://dx.doi.org/10.1016/j.cub.2009.12.035; PMID:20137952
107. Wolf K, Wu YI, Liu Y, Geiger J, Tam E, Overall C, Stack MS, Friedl P. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat Cell Biol 2007; 9:893-904; http://dx.doi.org/10.1038/ncb1616; PMID:17618273
108. Tolde O, Rösel D, Veselý P, Folk P, Brábek J. The structure of invadopodia in a complex 3D environment. Eur J Cell Biol 2010; 89:674-80; http://dx.doi.org/10.1016/j.ejcb.2010.04.003; PMID:20537759
109. Mercurio AM, Rabinovitz I. Towards a mechanistic understanding of tumor invasion--lessons from the alpha6beta 4 integrin. Semin Cancer Biol 2001; 11:129-41; http://dx.doi.org/10.1006/scbi.2000.0364; PMID:11322832
110. Fujita M, Khazenzon NM, Bose S, Sekiguchi K, Sasaki T, Carter WG, Ljubimov AV, Black KL, Ljubimova JY. Overexpression of beta1-chain-containing laminins in capillary basement membranes of human breast cancer and its metastases. Breast Cancer Res 2005; 7:R411-21; http://dx.doi.org/10.1186/bcr1011; PMID:15987446
111. Marinkovich MP. Tumour microenvironment: laminin 332 in squamous-cell carcinoma. Nat Rev Cancer 2007; 7:370-80; http://dx.doi.org/10.1038/nrc2089; PMID:17457303
112. Oikawa Y, Hansson J, Sasaki T, Rousselle P, Domogatskaya A, Rodin S, Tryggvason K, Patarroyo M. Melanoma cells produce multiple laminin isoforms and strongly migrate on α5 laminin(s) via several integrin receptors. Exp Cell Res 2011; 317:1119-33; http://dx.doi.org/10.1016/j.yexcr.2010.12.019; PMID:21195710
113. Hamasaki H, Koga K, Aoki M, Hamasaki M, Koshikawa N, Seiki M, Iwasaki H, Nakayama J, Nabeshima K. Expression of laminin 5-γ2 chain in cutaneous squamous cell carcinoma and its role in tumour invasion. Br J Cancer 2011; 105:824-32; http://dx.doi.org/10.1038/bjc.2011.283; PMID:21829200
114. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144:646-74; PMID:21376230; http://dx.doi.org/10.1016/j.cell.2011.02.013
115. Gurskaya NG, Verkhusha VV, Shcheglov AS, Staroverov DB, Chepurnykh TV, Fradkov AF, Lukyanov S, Lukyanov KA. Engineering of a monomeric green-to-red photoactivatable fluorescent protein induced by blue light. Nat Biotechnol 2006; 24:461-5; PMID:16550175; http://dx.doi.org/10.1038/nbt1191
116. Zhang S, Kuhn JR. Cell isolation and culture. WormBook 2013; 1-39; http://dx.doi.org/10.1895/wormbook.1.157.1; PMID:23430760
117. Pignatelli J, Tumbarello DA, Schmidt RP, Turner CE. Hic-5 promotes invadopodia formation and invasion during TGF-β-induced epithelial-mesenchymal transition. J Cell Biol 2012; 197:421-37; http://dx.doi.org/10.1083/jcb.201108143; PMID:22529104
118. Nakaya Y, Sukowati EW, Sheng G. Epiblast integrity requires CLASP and Dystroglycan-mediated microtubule anchoring to the basal cortex. J Cell Biol 2013; 202:637-51; http://dx.doi.org/10.1083/jcb.201302075; PMID:23940118
119. Wong CW, Song C, Grimes MM, Fu W, Dewhirst MW, Muschel RJ, Al-Mehdi AB. Intravascular location of breast cancer cells after spontaneous metastasis to the lung. Am J Pathol 2002; 161:749-53; http://dx.doi.org/10.1016/S0002-9440(10)64233-2; PMID:12213701
120. Madsen CD, Sahai E. Cancer dissemination--lessons from leukocytes. Dev Cell 2010; 19:13-26; PMID:20643347; http://dx.doi.org/10.1016/j.devcel.2010.06.013
121. Nourshargh S, Hordijk PL, Sixt M. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. Nat Rev Mol Cell Biol 2010; 11:366-78; PMID:20414258; http://dx.doi.org/10.1038/nrm2889
122. Stoletov K, Kato H, Zardouzian E, Kelber J, Yang J, Shattil S, Klemke R. Visualizing extravasation dynamics of metastatic tumor cells. J Cell Sci 2010; 123:2332-41; http://dx.doi.org/10.1242/jcs.069443; PMID:20530574
123. Stoletov K, Montel V, Lester RD, Gonias SL, Klemke R. High-resolution imaging of the dynamic tumor cell vascular interface in transparent zebrafish. Proc Natl Acad Sci U S A 2007; 104:17406-11; http://dx.doi.org/10.1073/pnas.0703446104; PMID:17954920
124. Kelley LC, Lohmer LL, Hagedorn EJ, Sherwood DR. Traversing the basement membrane in vivo: A diversity of strategies. J Cell Biol 2014; 204:291-302; http://dx.doi.org/10.1083/jcb.201311112; PMID:24493586

125. Peterson KJ, Lyons JB, Nowak KS, Takacs CM, Wargo MJ, McPeek MA. Estimating metazoan divergence times with a molecular clock. Proc Natl Acad Sci U S A 2004; 101:6536-41; http://dx.doi.org/10.1073/pnas.0401670101; PMID:15084738
126. Aris-Brosou S, Yang Z. Bayesian models of episodic evolution support a late precambrian explosive diversification of the Metazoa. Mol Biol Evol 2003; 20:1947-54; http://dx.doi.org/10.1093/molbev/msg226; PMID:12949122
127. Rohrschneider LR, Eisenman RN, Leitch CR. Identification of a Rous sarcoma virus transformation-related protein in normal avian and mammalian cells. Proc Natl Acad Sci U S A 1979; 76:4479-83; PMID:92031; http://dx.doi.org/10.1073/pnas.76.9.4479
128. Abram CL, Seals DF, Pass I, Salinsky D, Maurer L, Roth TM, Courtneidge SA. The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. J Biol Chem 2003; 278:16844-51; http://dx.doi.org/10.1074/jbc.M300267200; PMID:12615925
129. Wu H, Parsons JT. Cortactin, an 80/85-kilodalton pp60src substrate, is a filamentous actin-binding protein enriched in the cell cortex. J Cell Biol 1993; 120:1417-26; PMID:7680654; http://dx.doi.org/10.1083/jcb.120.6.1417
130. Mader CC, Oser M, Magalhaes MA, Bravo-Cordero JJ, Condeelis J, Koleske AJ, Gil-Henn H. An EGFR-Src-Arg-cortactin pathway mediates functional maturation of invadopodia and breast cancer cell invasion. Cancer Res 2011; 71:1730-41; http://dx.doi.org/10.1158/0008-5472.CAN-10-1432; PMID:21257711
131. Smith-Pearson PS, Greuber EK, Yogalingam G, Pendergast AM. Abl kinases are required for invadopodia formation and chemokine-induced invasion. J Biol Chem 2010; 285:40201-11; http://dx.doi.org/10.1074/jbc.M110.147330; PMID:20937825
132. Monsky WL, Kelly T, Lin CY, Yeh Y, Stetler-Stevenson WG, Mueller SC, Chen WT. Binding and localization of M(r) 72,000 matrix metalloproteinase at cell surface invadopodia. Cancer Res 1993; 53:3159-64; PMID:8391388
133. Mueller SC, Chen WT. Cellular invasion into matrix beads: localization of beta 1 integrins and fibronectin to the invadopodia. J Cell Sci 1991; 99:213-25; PMID:1885668
134. Zambonin-Zallone A, Teti A, Grano M, Rubinacci A, Abbadini M, Gaboli M, Marchisio PC. Immunocytochemical distribution of extracellular matrix receptors in human osteoclasts: a beta 3 integrin is colocalized with vinculin and talin in the podosomes of osteoclastoma giant cells. Exp Cell Res 1989; 182:645-52; PMID:2542071; http://dx.doi.org/10.1016/0014-4827(89)90266-8
135. Marchisio PC, Cirillo D, Teti A, Zambonin-Zallone A, Tarone G. Rous sarcoma virus-transformed fibroblasts and cells of monocytic origin display a peculiar dot-like organization of cytoskeletal proteins involved in microfilament-membrane interactions. Exp Cell Res 1987; 169:202-14; PMID:3028844; http://dx.doi.org/10.1016/0014-4827(87)90238-2
136. Li A, Dawson JC, Forero-Vargas M, Spence HJ, Yu X, König I, Anderson K, Machesky LM. The actin-bundling protein fascin stabilizes actin in invadopodia and potentiates protrusive invasion. Curr Biol 2010; 20:339-45; http://dx.doi.org/10.1016/j.cub.2009.12.035; PMID:20137952
137. Mizutani K, Miki H, He H, Maruta H, Takenawa T. Essential role of neural Wiskott-Aldrich syndrome protein in podosome formation and degradation of extracellular matrix in src-transformed fibroblasts. Cancer Res 2002; 62:669-74; PMID:11830518
138. Philippar U, Roussos ET, Oser M, Yamaguchi H, Kim HD, Giampieri S, Wang Y, Goswami S, Wyckoff JB, Lauffenburger DA, et al. A Mena invasion isoform potentiates EGF-induced carcinoma cell invasion and metastasis. Dev Cell 2008; 15:813-28; PMID:19081071; http://dx.doi.org/10.1016/j.devcel.2008.09.003
139. Bowden ET, Barth M, Thomas D, Glazer RI, Mueller SC. An invasion-related complex of cortactin, paxillin and PKCmu associates with invadopodia at sites of extracellular matrix degradation. Oncogene 1999; 18:4440-9; http://dx.doi.org/10.1038/sj.onc.1202827; PMID:10442635
140. Chan KT, Cortesio CL, Huttenlocher A. FAK alters invadopodia and focal adhesion composition and dynamics to regulate breast cancer invasion. J Cell Biol 2009; 185:357-70; http://dx.doi.org/10.1083/jcb.200809110; PMID:19364917
141. Duong LT, Rodan GA. PYK2 is an adhesion kinase in macrophages, localized in podosomes and activated by beta(2)-integrin ligation. Cell Motil Cytoskeleton 2000; 47:174-88; http://dx.doi.org/10.1002/1097-0169(200011)47:3<174::AID-CM2>3.0.CO;2-N; PMID:11056520
142. Mueller SC, Yeh Y, Chen WT. Tyrosine phosphorylation of membrane proteins mediates cellular invasion by transformed cells. J Cell Biol 1992; 119:1309-25; PMID:1447304; http://dx.doi.org/10.1083/jcb.119.5.1309
143. Nakahara H, Otani T, Sasaki T, Miura Y, Takai Y, Kogo M. Involvement of Cdc42 and Rac small G proteins in invadopodia formation of RPMI7951 cells. Genes Cells 2003; 8:1019-27; PMID:14750956; http://dx.doi.org/10.1111/j.1365-2443.2003.00695.x
144. Berdeaux RL, Díaz B, Kim L, Martin GS. Active Rho is localized to podosomes induced by oncogenic Src and is required for their assembly and function. J Cell Biol 2004; 166:317-23; http://dx.doi.org/10.1083/jcb.200312168; PMID:15289494
145. Head JA, Jiang D, Li M, Zorn LJ, Schaefer EM, Parsons JT, Weed SA. Cortactin tyrosine phosphorylation requires Rac1 activity and association with the cortical actin cytoskeleton. Mol Biol Cell 2003; 14:3216-29; http://dx.doi.org/10.1091/mbc.E02-11-0753; PMID:12925758
146. Linder S, Nelson D, Weiss M, Aepfelbacher M. Wiskott-Aldrich syndrome protein regulates podosomes in primary human macrophages. Proc Natl Acad Sci U S A 1999; 96:9648-53; PMID:10449748; http://dx.doi.org/10.1073/pnas.96.17.9648