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The interplay between the proteolytic, invasive, and adhesive domains of invadopodia and their roles in cancer invasion

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Invadopodia are actin-based protrusions of the plasma membrane that penetrate into the extracellular matrix (ECM), and enzymatically degrade it. Invadopodia and podosomes, often referred to, collectively, as "invadosomes," are actin-based membrane protrusions that facilitate matrix remodeling and cell invasion across tissues, processes that occur under specific physiological conditions such as bone remodeling, as well as under pathological states such as bone, immune disorders, and cancer metastasis. In this review, we specifically focus on the functional architecture of invadopodia in cancer cells; we discuss here three functional domains of invadopodia responsible for the metalloproteinase-based degradation of the ECM, the cytoskeleton-based mechanical penetration into the matrix, and the integrin adhesome-based adhesion to the ECM. We will describe the structural and molecular organization of each domain and the cross-talk between them during the invasion process.

Preface

Invadopodia are actin-based protrusions of the plasma membrane that penetrate into the extracellular matrix (ECM) and enzymatically degrade it.[1-3] They belong to a family of structures, called invadosomes,[4] which facilitate cell invasion through tissues, a process that occurs under specific physiological conditions such as wound repair, pathogen infection, embryogenesis, and cell differentiation, as well as under pathological conditions such as cancer metastasis.[5]

The first indication of invadosome family structures came in 1980, when David-Pfeuty and Singer observed, in chicken embryo fibroblasts, the relocalization of vinculin and α-actinin from focal adhesions into circular "rosettes," following transformation with Rous sarcoma virus (RSV).[6] Five years later, these v-src induced structures were defined by Tarone and Marchisio as "podosomes"[7], due to their foot-like morphology. Later, more of their structural and signaling components, such as actin,[8] fimbrin,[9] and the oncogenic v-src itself,[10,11] were identified, and RSV-induced podosomes were shown by Parsons et al. to be ECM contacts that also serve as sites of local matrix degradation.[11] Their capacity to invade and degrade the ECM led Chen, in 1989, to name these structures "invadopodia."[12] Chen and colleagues continued to characterize these structures, and demonstrated their dependence on metalloproteinase degradation activity,[12] tyrosine phosphorylation,[12] and matrix adhesion.[13-16] Similar adhesive and degradative structures were found in normal cells such as macrophages,[17] osteoclasts,[14,18] dendritic cells,[19] vascular smooth muscle cells,[20] and endothelial cells,[21] as well as in a wide variety of metastatic cancer cell lines such as breast carcinoma,[22] melanoma,[16] bladder carcinoma,[23] head and neck carcinoma,[24] prostate cancer,[25] and a variety of primary tumors.[26]

Despite the overall similarity between invadopodia, podosomes, and src-induced invadosomes, they do vary in their size, shape, density, and stability.[1,4] Under a fluorescent microscope, invadopodia of cultured cancer cells usually appear as dot-like structures, with an F-actin core, containing several actin-binding proteins and signaling molecules.[2,5] The cores can be scattered or clustered at the cell center, usually in close proximity to the nucleus.[1] Podosomes, on the other hand, can be distributed in various fashions. They can be located throughout the cell in marcrophages, located at the cell periphery in smooth muscle cells, forming rosette-shaped clusters in endothelial cells, and in unique super-structures, such as sealing zones, in osteoclasts.[4] Src-induced invadosomes in fibroblasts display rosette-shaped peripheral invadosomes.[4]

Invadopodia are long-lived, stable structures that last up to a few hours,[27,28] as opposed to podosomes, whose lifespans is in the order of few minutes.[5] The podosome actin core is surrounded by an adhesion ring containing plaque proteins such as vinculin, paxilin, and talin,[4,29] whereas the adhesion component of invadopodia is still poorly characterized. Invadopodia and podosomes also differ in their degree of penetration into the ECM: invadopodia were shown to penetrate deep into the matrix[30] and form filament-like processes, while podosomes display shallow appearance[4] and, fail to form long membrane protrusions.[31] Both podosomes and invadopodia degrade the ECM by local secretion of proteolytic enzymes; as indicated, podosomes tend to degrade the exposed surface of the matrix,

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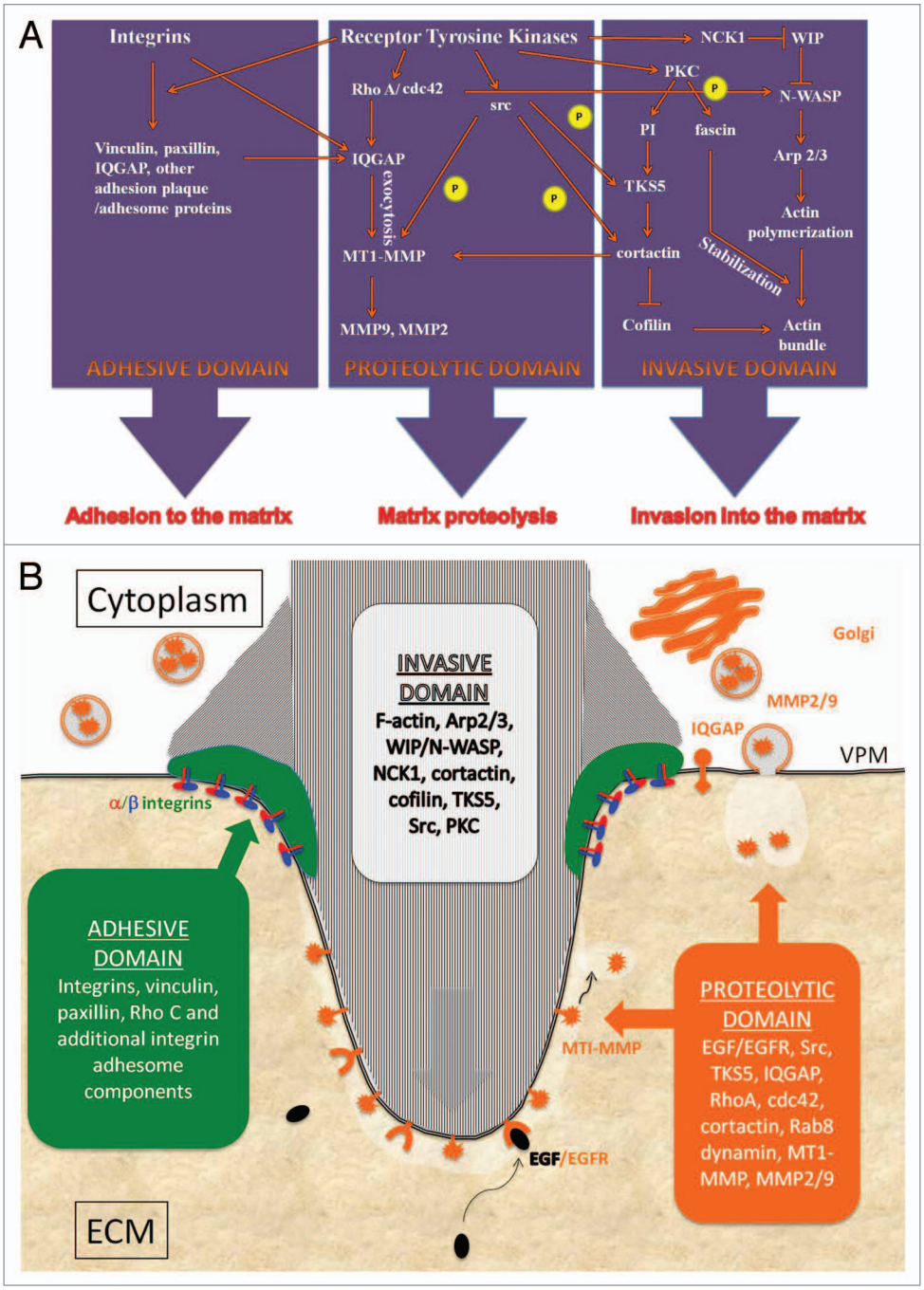

Figure 1. See opposite page for figure legend.

while invadopodia, usually, penetrate deeper, into the ECM.[1,4] Invadopodia, podosomes, and src-induced invadosome also display different activation pathways for core components such as the arp 2/3 complex, whose activation in invadopodia require NCK1, while in src-induced invadosomes the activation is mediated by Grb2. In podosomes of macrophages, the activation is not mediated by either one of the proteins.[32] This emphasizes the fact that while these structures are molecularly similar, and perform similar functions there might act via different pathways.

In this review, we will focus on the functional architecture of invadopodia in cancer cells, addressing the mechanisms underlying the proteolytic and mechanical remodeling of the ECM by them. We will describe the structural and molecular organization of invadopodia and the roles of the proteolytic domain, the invasive domain and the adhesive domain (**Fig. 1A and B**;

Figure 1 (Opposite page). Mapping of the signaling cascades associated with the proteolytic, invasive, and adhesive domains of invadopodia. The proposed signaling and protein interaction cascades associated with the formation of invadopodia, their adhesion to the matrix, and their penetration into it. Key components involved in these processes, their upstream regulators and downstream targets are shown. Proteolytic domain: ECM degradation by invadopodia is attributed to the activation and function of matrix metalloproteinases (MMPs). The membrane-bound form MT1-MMP is the master regulator of the degradation process, together with the secreted proteases MMP9 and MMP2. The synthesis and secretion of proteases is mediated by the ER and Golgi systems that deliver MMP-containing vesicles along microtubules. One of the central regulators of this process is src tyrosine kinase, which is activated upon receptor tyrosine kinase (RTK) activation. Src phosphorylates MT1-MMP and promotes its clathrin-mediated endocytosis and recycling, a process that promotes the transport of active MT1-MMP to the plasma membrane. Other downstream effectors of the RTK signaling cascades are the small GTPases, Rho A and CDC42, which can activate IQGAP interaction with sec8, a component of the exocytosis machinery that further promotes the delivery of MT1-MMP to the plasma membrane. Another mechanism for MT1-MMP recruitment to invadopodia involves phosphorylated cortactin, in a mechanism that is src- and Arg kinase-dependent. As a master regulator, MT1-MMP can also cleave and activate the secreted MMP9 and MMP2. Invasive domain: Assembly of an invasive actin network is induced by activation of RTKs (e.g., the EGF receptor), which leads to src and PKC kinase activation, as well as recruitment of adaptor molecules such as NCK1. NCK1 binds to WIP (WASP interacting protein) and block its inhibitory interaction with N-WASP, thus enabling N-WASP binding to Arp 2/3 and activating actin nucleation. The Arp 2/3 complex can nucleate actin polymerization on existing filaments, forming a branched actin network. The Arp 2/3 actin nucleation activity is promoted by the serving activity of cofillin, which forms free barbed ends on existing actin filaments. In response to PKC kinase activation, phosphoinositides (PI) are generated at the inner aspect of the plasma membrane of newly forming invadopodia. The adaptor protein TKS5 is recruited to the plasma membrane by binding to PIs, and is phosphorylated by src. The phosphorylated and membrane-associated TKS5 recruits cortactin that is also phosphorylated by src. In its unphosphorylated form, cortactin binds to cofilin, and inhibits it. Upon phosphorylation, cortactin detaches from cofilin, enabling it to sever actin filaments. Active cortactin facilitates both actin filament bundling and recruitment of MT1-MMP (see "proteolytic domain"). PKC can also activate the actin bundling protein fascin, which stabilizes the actin bundle. Adhesive domain: The adhesive domain of invadopodia is a ring-shaped structure associated with the periphery of the interface between the actin core bundle and the ventral plasma membrane (see "invasive domain"). This domain assembles within seconds to minutes following core formation. The assumption is that the adhesion ring is molecularly similar to other types of integrin-mediated adhesions (e.g., focal adhesions; see also **Fig. 2**, white arrow). It is suggested that activation of integrins, upon binding to ECM ligands in invadopodia sites, induces the local recruitment of adhesion plaque proteins such as vinculin, paxillin, and Hic-5. IQGAP also localizes to the adhesion domain, and possibly interacts with the plaque proteins and facilitates the exocytosis of MT1-MMP (see "proteolytic domain"). (**B**) Organization of the different functional domains of invadopodia. A schematic representation of the structural organization of invadopodia domains (cross-section) is shown. Each domain is denoted in different color. The proteolytic domain (in orange) includes the ER and Golgi systems, which produce protease-containing vesicles and transfer them to the invadopod. Anchoring and secretion of MMP-containing vesicles are apparent in close proximity to the membrane (see "proteolytic domain," **Fig. 1A**), as are recycling and exocytosis of the membrane-bound MT1-MMP. The invasive domain (in black) is localized inside invadopodial protrusions into the ECM. This domain is composed of actin and actin-associated proteins (see invasive domain **Fig. 1A**). The protein interactions within this domain lead to actin polymerization in a branched and parallel fashion. The polymerizing actin bundles generate forces that are applied on the ECM and push the ventral cell membrane toward it. Around the core, at the protrusion's edges the adhesive domain is localized, anchored to the ECM via integrins, and associated adhesome cytoplasmic plaque proteins. This adhesion can mechanically support the invasive process. Most of the cross-talk between the three domains occurs upon recruitment of the various components to the plasma membrane, at the site of forming invadopodia. This cross-talk orchestrates the force generation mechanism which is applied to the matrix, together with mechanical stabilization by adhesion and matrix degradation, ultimately driving the invasion and migration of the cell into the matrix.

proteins presented in 1A are bolded in the text), and discuss the cross-talk between them during the invasion process.

The Proteolytic Domain

(**Fig. 1A [middle panel] B**). The matrix degradation capacity of invadopodia is attributed to a variety of proteases, belonging to the metalloproteinase (MMPs), ADAM, and serine protease families.[1,5,33,34] Among these, the MMPs were shown to be the most functionally significant proteases associated with invadopodia activity in cancer cells. MMPs comprise a large family of zinc-dependent enzymes, both membrane-bound and soluble,[1] which are capable of cleaving multiple ECM proteins[35]. The membrane-bound form, **MT1-MMP** (or **MMP14**), was shown to be not just an invasion-promoting component, but also a master regulator of invadopodia development,[33,36] whose recruitment promotes invadopodia maturation.[2] MT1-MMP displays intrinsic collagenolytic activity, and can also cleave and activate some of the soluble MMPs.[34,37] Soluble MMP9 and MMP2 are secreted around invadopodia, and promote local ECM degradation.[16,35-41] Blocking MMP activity by inhibitors, antibodies, or siRNA impairs invadopodia function and matrix degradation.[1,5] Furthermore, MMP inhibition also inhibits invadopodia formation, suggesting that MMPs regulate and fine-tune invadopodia function, at early stages of their formation.[2,5]

The mechanisms where by proteases are recruited to sites of invadopodia remain unclear, and likely involve several recruitment pathways (**Fig. 1A and B**; proteolytic domain). These include vesicle trafficking along microtubules, which was demonstrated for **MMP2** and **MMP9** in melanoma cells.[42] MT1-MMP is also regulated by clathrin-mediated and caveolar endocytosis;[40] its recycling is suggested as a means of generating a membrane-active enzyme.[43] Polarized exocytosis and transport of vesicles of MT1-MMP to sites of invadopodia is regulated by Rab8 GTPase in breast cell carcinoma.[44] In macrophages Rab5a, Rab8a, and Rab14 were shown to be important for MT1-MMP polarized transport.[45] MT1-MMP can be phosphorylated in a src-depended manner;[46] and such phosphorylation was shown to promote clathrin-mediated endocytosis.[40] Exocytosis was also shown to be involved in MT1-MMP regulation via **IQGAP,** a key regulator of cell polarity, which connects the actin cytoskeleton to microtubules.[47] Upon activation of **RhoA** and **CDC42**, IQGAP is localized to invadopodia together with sec8, a component of the exocytotic machinery; together, they regulate the docking of MT1-MMP vesicles to the plasma membrane.[48] Furthermore, the actin polymerization regulator **cortactin** was shown to participate in the recruitment of MMPs to invadopodia, and regulate

their secretion.[36,39,49] The adhesion machinery is also thought to play a role in invadopodia maturation and matrix degradation: β1 integrin co-clusters with MT1-MMP at sites of interaction with collagen fibers along the cell's leading edge.[50,51] In addition, inhibition of integrin-mediated adhesion reduces recruitment of IQGAP to invadopodia, as well as matrix degradation.[52] A possible mechanism for protease recruitment by β1 integrin involves Arg kinase, which can phosphorylate cortactin on tyrosin 421 and promote matrix degradation.[53] Finally, **dynamin 2** was shown to regulate the local matrix degradation in invadopodia, through proper localization and its GTPase activity.[54,55]

Cancer cells are commonly associated with elevated levels of MMPs, whose expression is correlated with cancer aggressiveness and poor prognosis.[40,56] Thus, studies of their activation and of mechanisms that could inhibit their activity are of great medical interest. Treating cancer with MMP inhibitors was considered to be a very promising approach and was studied in a variety of clinical trials as therapy for various types of cancers.[57-59] Unfortunately, those trials were largely unsuccessful, most likely due to the development of drug resistance by the tumor cells,[60] secretion of MMP from stroma cells around the tumor,[61] lack of sufficient specificity of the inhibitors, and changes in the cancer cell migration and invasion mechanism, capable of switching from proteolysis-dependent migration to amoeboid migration (a so-called "mesenchymal–amoeboid transition").[50,62]

The failure of MMP inhibition to block cancer invasion indicates that the remodeling of the ECM per se is not sufficient for preventing cancer invasion and metastasis, and suggests that cell invasion into the matrix requires an active migratory process, whereby cancer cells "force their way" into the matrix. This mechanical penetration is primarily attributed to the invasive domain of invadopodia.

The Invasive Domain

General overview

(**Fig. 1A [right panel] and B**). Local matrix invasion, driven by invadopodia, is a highly coordinated process, whereby enzymatic ECM degradation and mechanical cytoskeletal "pushing" occur at approximately the same time and in the same area, ultimately leading to effective penetration of the cell protrusion into the matrix. Considerable efforts were invested in characterizing the mechanical component of this process, which is believed to be driven primarily by actin polymerization, in a manner similar to the protrusive phase observed in the lamellipodia of migratory cells.[1-3,5] In this section, we will introduce the overall structure and assembly of the cytoskeletal machinery of invadopodia, and then discuss the specific contributions of each component to the overall process.

The temporal sequence of events that eventually leads to invadopodia formation is not fully understood; yet it has been proposed that this process is initiated by ligand-induced activation of receptor tyrosine kinases, such as the **EGF receptor** [28,63] (**Fig. 1A and B**). This signaling event triggers the activation of **c-src**,[7,64-66] **PKC** isoforms,[67-70] and eventually, the adaptor protein **TKS5**,[71] which plays a key role in early stages of the assembly of the actin machinery, TKS5 can then bind to **Ptdlns(3,4)P_2** at the plasma membrane, promoting the recruitment of actin regulators such as the SH2/SH3-containing, signaling adaptor protein **NCK1** to the membrane.[2]

The exact temporal sequence of these events is still not elucidated. A new approach of high-resolution spatiotemporal live cell imaging, published recently, shows that cortactin, N-WASP, cofilin, and actin arrive simultaneously to form the invadopodia precursor, while TKS5 is recruited later and is required for the structure stabilization.[72]

Actin polymerization in the core of invadopodia is regulated by small Rho-family GTPases, mostly, **CDC42**,[1,28] which can directly activate the **N-WASP-WIP** complex, which, in turn, drives actin polymerization by the **Arp2/3 complex**.[73-76] Arp 2/3 actin nucleation is promoted by the severing activity of **cofillin**.[77-79] Additional reports indicate that actin polymerization in invadopodia of certain cancer cells (e.g., MDA-231 breast cancer cell line) can also be induced by specific Diaphanous-related formins, whose cell type specificity, and mode of action and regulation, are still unclear.[80] The actin bundle, thus formed, is further stabilized and mechanically reinforced by **cortactin**[5] and **fascin**,[73,81,82] producing a stable "invasive protrusion" that pushes against the ventral cell membrane, promoting its penetration into the ECM.[5,27,28,36,39,68,83]

To get a closer look at the constituents of the invasive domain (**Fig. 1A and B**; invasive domain) we summarized below the modes of action of the major proteins that are believed to participate in invadopodia nanomechanics:

(1) The adaptor protein N-WASP and its regulators were previously shown to be an essential component of invadopodia.[28,84] N-WASP elimination reduced the ability of mammary adenocarcinoma cells to form invadopodia, and metastasize.[26] N-WASP, together with WASP in hematopoietic cells and WAVE 1–3, are major regulators of actin cytoskeleton dynamics.[26] Mutations in WASP are known to cause an X-linked inherited disease, Wiskott–Aldrich syndrome, characterized by immunodeficiency. WASP is negatively regulated by WASP-interacting protein (WIP), which is removed upon stimulation by upstream regulators such as NCK1.[85] In invadopodia, N-WASP is a direct effector of CDC42,[5] and is regulated by upstream signals from NCK, src, and phosphoinositides.[26] N-WASP is characterized by a C-terminal catalytic verprolin homology and a central acidic (VCA) domain, which mediate activation of the Arp2/3 complex. Activation of Arp 2/3 leads to actin polymerization, which generates the mechanical force that enables invadopodia to penetrate the ECM.[86,87] N-WASP was recently suggested to bind to barbed ends and accelerate actin assembly and polymerization in an Arp 2/3-independent manner.[88]

(2) The Arp2/3 complex drives the nucleation of a new actin filament that branches off an existing filament.[5] Arp2/3 is a 7-subunit complex composed of Arp2, Arp3, and five additional subunits (ARPC1–5[89]). Arp2/3 is associated with invadopodia, and its knockdown by siRNA, or preventing N-WASP from binding to it, inhibits invadopodia formation.[27,28] The Arp2/3 complex, together with N-WASP, is upregulated in tumors and invading

cells.[90,91] Stabilization of the actin polymerization capacity of the Arp 2/3 complex is attributed to the binding of Arp 2/3 to both N-WASP and cortactin, the latter also binds to actin, and stabilizes the filament.[76,92,93]

(3) Cortactin is a cytoskeletal, multi-domain protein that is important for the induction of actin polymerization.[30,63,94] Cortactin is ubiquitously expressed, and can be phosphorylated by src kinase.[95,96] Cortactin phosphorylation was shown to be an Arg kinase-dependent process, triggered by EGFR activation, and leading to invadopodia maturation.[97] Tyrosin phosphorylation by src facilitates the response of cortactin to upstream signaling, including dynamic changes in the actin cytoskeleton.[98] Phosphorylation of Y421, Y466, and Y482 in the proline-rich domain is necessary for metastatic dissemination of breast carcinoma cells,[99] while Y421 and Y466, but not Y482, are required for the generation of free barbed ends of actin filaments of invadopodia.[100] When phosphorylated, cortactin releases cofilin, and enables it to sever actin filaments, in order to form barbed ends that promote Arp 2/3 actin polymerization.[77,92,101] Cortactin can also bridge between the Arp 2/3 complex and the actin bundle, due to its Arp 2/3 binding N-terminal acidic domain, and thus, serves as an actin stabilizer.[102] Blocking cortactin activity by means of inhibitory antibodies, inhibition of src phosphorylation, or cortactin knockdown by siRNA,[68] block both the formation of invadopodia and matrix degradation. Interestingly, besides its role in the regulation of the actin machinery, cortactin is believed to directly connect the cytoskeletal components of invadopodia to the proteolytic machinery.[36,39,103]

(4) Cofilin was shown to promote actin polymerization by its severing activity, which generates free barbed ends that induce the nucleation of actin polymerization by the Arp 2/3 complex.[5,77-79] Cofilin was shown to be important for invadopodia stabilization, most likely due to its interaction with cortactin (see above) and in its absence invadopodia become small and short-lived.[28] Cofilin severing activity is regulated and confined to invadopodia by RhoC and its upstream regulators p190RhoGEF and p190RhoGAP.[104] RhoC regulation on cofilin is mediated by regulation on cofilin phosphorylation through Rho-associated protein kinase and LIM-domain kinase.[79,104]

(5) Fascin is an actin-bundling protein that cross-links actin filaments into a tightly packed bundle.[105,106] In humans, there are three members of the fascin family (1–3), of which fascin 1 is the most widely expressed in tissues.[107] The actin bundling capacity of fascin is attributed to the actin-binding sites at its N- and C-termini,[108,109] and the bundling activity in invadopodia is specifically attributed to a PKC phosphrylation site at serine 39.[81] Fascin is upregulated in cells localized at the invasive front of various invasive cancers.[110,111] Fascin is important for invadopodia elongation and actin filament stability, and its depletion was shown to reduce the ability of invadopodia to degrade the ECM.[81,83]

(6) Other cytoskeletal systems, besides the actin network, namely, microtubules, and intermediate filaments, are believed to play either direct or indirect roles in invadopodia formation and function.

Little is known about the specific mechanisms underlying the roles of microtubules and intermediate filaments in invadosomes in general, and particularly, in invadopodia of cancer cells. Nevertheless, intact microtubules are required for the function of other invadosomes, such as podosomes of monocytes, macrophages, and osteoclasts[1,112,113]. In breast cancer cells, intact microtubules are not necessary for invadopodia formation,[114,115] but their destruction affects invadopodia elongation and maturation.[83] In melanoma cells, disruption of microtubules by inhibitory drugs causes invadopodia disassembly, inhibits their de novo formation, and consequently, reduces matrix degradation.[116] Previous studies also show that transport of proteins toward the leading edge of migrating fibroblasts requires intact, functioning microtubules,[117] a process that is thought to be important for the polarized transport of MMPs to invadopodia.[116]

Intermediate filaments were shown to interact with actin filaments via fimbrin during the formation of podosomes in macrophages.[118] Vimentin intermediate filaments are localized to newly formed podosomes of vascular smooth muscle cells, but are not directly necessary for their formation. In invadopodia, vimentin, but not cytokeratin, was shown to play a role in the invadopodia elongation.[83]

To summarize, various proteins of the actin machinery work together in order to form a dense web of a dendritic actin network and long parallel bundles, which, together, apply mechanical force that pushes the plasma membrane toward and into the ECM. Other cytoskeletal elements; namely, microtubules and intermediate filaments, are believed to affect the elongation stage of invadopodia; but their specific roles are yet to be explored.

The mechanical force generated by actin polymerization plays a key role in invadopodia protrusive action, but several mechanistic questions still remain open: Is inavdopodia protrusive activity sufficient for driving the entire process of cell invasion? What prevents a protrusion from retracting? What mechanisms coordinate, spatially, tumor cell "proteolytic invasion" and migration? What keeps the actin network from extending deep into the cytoplasm rather than pushing the plasma membrane?

The Adhesive Domain

(**Fig. 1A [left panel] and B**). Tissue cells grow and function in tight association with diverse components of the ECM. These adhesive interactions play a central role in tissue morphogenesis and remodeling, as well as in physiological and pathological cell migration. Cells connect their cytoskeleton to ECM components via a variety of specialized transmembranal receptors, the most common of which are the heterodimeric integrins.[119] Integrins interact with the ECM components via their extracellular domains, and are connected to the actin cytoskeleton via their cytoplasmic tails, and a multi-protein complex of adaptor, scaffold, and signaling proteins, known collectively as the integrin adhesome.[120,121] In addition to their mechanical role, integrin adhesions to the ECM act as "multi-sensory" sites, enabling cells to "scan" the ECM, and respond to its chemical-molecular

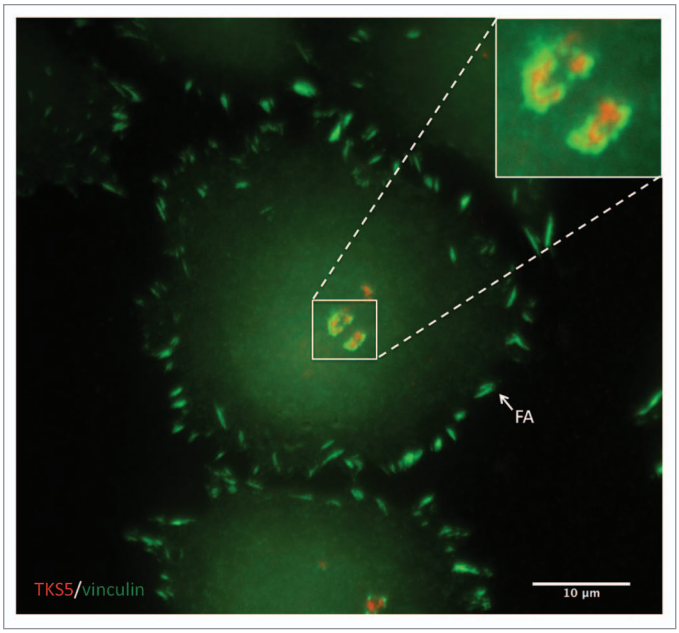

Figure 2. The actin core and adhesion ring of invadopodia: A375 cells were cultured on gelatin-coated glass bottomed dishes for 2h. Cells were fixed and stained for TKS5, an invadopodia core marker, and vinculin, a marker for integrin adhesions. The adhesion ring (shown enlarged in the upper-right corner) is associated with the invadopodia core at early stages (within minutes) of invadopodia formation, and often disappears at later stages (hours). Stable focal adhesions (FAs), located mainly at the cell's periphery, are also formed in these cells.

properties, as well its physical characteristics, including rigidity, stretching,[122-125] and topography of the ECM.[126-130]

It is commonly accepted that the spatiotemporally regulated coupling between protrusive processes and the establishment of nearby adhesions is essential for cell locomotion and invasion.[63,131,132] During the migration process, for example, the forward extension of lamellipodia or filopodia, driven by local actin polymerization, is guaranteed by the presence of focal adhesions or focal complexes at the posterior aspects of these protrusions.[131,133]

Do matrix adhesions play an essential role in ECM invasion by invadopodia, or is this process primarily driven by MMPs that locally degrade the matrix? Over the years, researchers have addressed this question, yet information about the adhesive domain of invadopodia, its molecular composition, and its structure, remains scarce. Previous studies suggest that invadopodia of cancer cells lack a distinct adhesive capacity, unlike podosomes, which contain a conspicuous adhesive ring.[3,4] It is noteworthy that podosomes, have typical lifetimes on the order of a few minutes, while invadopodia can be stable for hours.[1] More recently, these claims were challenged by the detection of vinculin, paxillin, and Hic-5 in rings located at the periphery of newly formed invadopodia in various cultured cancer cell lines[52,134] (and our unpublished data **Fig. 2**). It is likely that this apparent discrepancy is attributable to differences between the cancer cell lines, or to variations in the persistence of the adhesion rings during the lifespan of the invadopodia. In fact, it was reported that the adhesive domain of invadopodia is formed soon after the formation of the core F-actin bundle, showing an oscillatory behavior, and occasionally, disappears at later stages, after stabilization of the structure[52,72] (and our unpublished data). The mechanisms underlying the interplay between the core and the adhesion ring, and their significance for ECM remodeling, are still poorly understood.

Nevertheless, it was shown that integrin-mediated adhesion regulates invadopodia formation by activation of Rho GTPase family members and various kinases, together with recruitment of adhesion molecules[53,135,136] (**Fig. 1A and B**; adhesive domain). Several integrins were shown to be associated with invadopodia, including α6β1 in melanoma[135,136] and α3β1 in breast cancer cells,[53,137] suggesting that the association of integrins with invadopodia is tissue-specific. Within the adhesion sites, integrins are believed to bind and activate metalloproteases, besides their "classical" signaling and scaffolding functions. Blocking of integrin-mediated adhesion by soluble RGD peptides or inhibitory antibodies results in reduced matrix degradation, and less localization of MT1–MMP to invadopodia sites.[52]

Integrins are not the only players in the adhesive domain of invadopodia. In order to form stable adhesions with the ECM and link them to the cytoskeleton, various components of the integrin adhesome, including scaffolding and signaling molecules, are recruited to the adhesion site.[138-141] For example, the adaptor protein paxillin[142] is tyrosine-phosphorylated, and can form a complex with PKCμ and cortactin in invadopodia.[68] Hic-5, a member of the paxillin family, localizes to invadopodia rings, and upon TGFβ stimulation, promotes ECM degradation in a src phosphorylation-dependent manner.[134] The role of Focal Adhesion Kinase in invadopodia regulation is rather controversial; it was shown to function as a negative regulator of invadopodia through the spatial control of src in MTLn3 mammary adenocarcinoma cells,[143] While other reports indicate that it is localized to invadopodia, and its increased expression promotes the formation of src-induced invadopodia.[144] VASP, another adhesome component, accumulates at the degradation sites of invadopodia in MDA-231 breast cancer cells[83] and its phosphorylation on Ser239 was reported to suppress invadopodia formation, and inhibit the formation of cancer metastasis.[145]

Like other adhesion structures, invadopodia are highly regulated by the chemical and physical properties of the ECM. Matrix rigidity directly increases both the number and activity of invadopodia. This mechanosensing is regulated by the contractile machinery of the cells (e.g, myosin II and Rho kinase).[146] Matrix organization can also affect invadopodia shape. For example, tumor cells cultured on a fibrillar collagen I matrix form linear F-actin structures, which share features with conventional invadosomes, and are capable of degrading the matrix.[147]

To date, it is not clear which adhesion proteins participate in formation of the ring in periphery of the invadopodia core. Are they all co localized in the ring, both spatially and temporally, or are there differential kinetics and localization for each protein, as reported for focal adhesions?[148,149] Blocking of integrin-mediated adhesion didn't block invadopodia formation; yet, it reduced the extent of matrix degradation.[52] These and other results raise many

questions about the role of the adhesive domain in recruitment of the degradation machinery, and the cross-talk between them. Which of the adhesion components are important for invadopodia formation and function? Why do they appear primarily at the early stages of the invadopodia formation? Are there other roles for the adhesion molecules in the invasion process? Does adhesion to the ECM stabilize invadopodia mechanically? Are there differences between the adhesion mechanism, structure, and dynamics in 2D vs. 3D matrices? What is the interplay between the adhesive domain, and other cytoskeletal components?

An Integrated View of Invadopodia's Functional Architecture

In this article, we attempted to review the structure–function relationships in invadopodia, focusing on structures that carry out specific functions in driving cell invasion, which distinguish them from other types of cellular protrusions (e.g., filopodia or lamellipodia) or other adhesion sites (e.g., focal adhesions or podosomes). Unique to invadopodia is the spatial and temporal coordination of invasion, adhesion, and matrix degradation. Each of these three processes is regulated and executed by a set of multiple proteins that can be define as a "functional domain," the spatial organization of which is schematically depicted in **Figure 1B**.

The overall "mission" of invadopodia is the promotion of cell migration across connective tissue barriers (commonly practiced by metastatic cancer cells), as opposed to locomotion along specific, pre-existing migration tracks (as physiologically occurs during organogenesis and embryogenesis). The special property of these three functional domains of invadopodia is their spatial organization and temporal coordination, which enables them to act in concert, in what might be described as a coherent "grip-soften-push" mechanism. Such tactics require fine-tuned cross-talk between the proteolytic (enzymatic), invasive (cytoskeletal), and adhesive domains.

According to this view, the cell utilizes its integrin adhesion machinery to probe the ECM and bind to it (the "grip" step). Upon appropriate stimulation, global or local, actin nucleation is initiated at the cytoplasmic aspects of these adhesions, forming an initial actin core. Subsequently, the proteolytic domain is activated, presenting MT1-MMP on the ventral cell surface, and releasing MMP2 and MMP9. Focal degradation of the matrix by MT1-MMP, and broader degradation by the soluble proteases, reduces matrix barriers under and around invadopodia (the "softening" step), conditioning these areas for the penetration step. Naturally, ECM degradation can also affect nearby adhesion to the matrix by remodeling the ECM, which may explain the observation that not all invadopodia are associated with adhesion rings. Next, rigorous actin polymerization in the core of the invasive domain pushes against the plasma membrane, driving the penetration into the "softened" matrix (the "push" step). Repeated cycles of this "grip-soften-push" by multiple invadopodia could effectively constitute the mechanism underlying invadopodia-mediated cell invasion. Recent evidence for repeating cycles of protrusion and retraction of invadopodia was indeed described.[150]

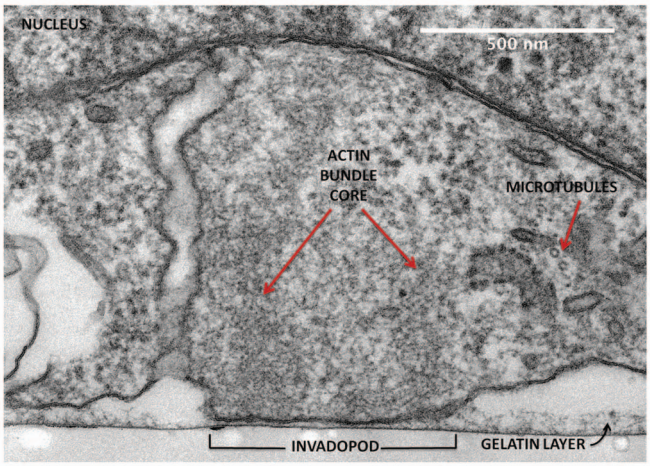

Figure 3. Imaging of invadopodia by electron microscopy: A375, human metastatic melanoma cells were cultured for 2h on glass-bottom dishes, coated with cross-linked gelatin. Cells were fixed (3% paraformaldehyde, 2% glutaraldehyde, 5 mM $CaCl_2$) in 0.1 M cacodylate buffer (pH 7.4) for 1h, washed, and post-fixed with 1% OsO_4. Samples were washed again with 0.1 M cacodylate buffer and stained with 2% aqueous uranyl acetate (EMS) for 1h, dehydrated, and embedded in Epon. Ultrathin sections were then cut and examined in an FEI Tecnai™ Spirit T12 transmission electron microscope. In this electron micrograph actin filaments are apparent throughout the core of the invadopodial protrusion, excluding other cytoplasmic structures (e.g., ribosomes) from the core area. The ventral membrane at the invadopode area is in close contact with the glass and the gelatin layer is cleared.

The three functional domains of invadopodia, which are discussed above, are in fact, packed into one coherent structure presented in the transmission electron micrograph, shown in **Figure 3**. Transmission electron micrographs of invadopodia were shown previously in several cell types.[33,54,83] In the ultrathin section shown here, cut perpendicular to the plane of the gelatin matrix, a single invadopod is seen, pushing against the gelatin layer and apparently compressing or degrading it. The invadopod itself is packed with F-actin fibers, and is devoid of cytoplasmic organelles. Microtubules are often detected at the periphery of the core actin bundle (indicated by the arrow in **Fig. 3**). Interestingly, the gelatin layer at the peripheral aspect of the invadopod is slightly thicker, in line with the possibility that this region correspond to the adhesion ring. Naturally, invadopodia formed by cultured cells, growing on thin ECM, are rather shallow structures compared with their in vivo counterparts, yet the general distributions of their functional/structural domains might be quite similar.

It should be acknowledged that despite our vast information about invadopodia, their structure, and their physiological roles, we are still left with many basic, as yet unanswered questions concerning their coordinated action. To mention a few: are there special features of the ECM (composition, rigidity, topography) that contribute to the assembly and activation of the different domains of invadopodia? How does MMP enzymatic activity affect invadopodia formation, adhesion, and mechanics? How is the timing of the different phases in the "invasion cycle"

controlled and coordinated? Answers to these and other open questions may provide a clearer sense of the mechanisms underlying cancer invasion and metastasis and, hopefully, pave the way toward novel and effective therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. LinderS. The matrix corroded: podosomes and invadopodia in extracellular matrix degradation.Trends Cell Biol2007; 17:107-17; PMID:17275303; http://dx.doi.org/10.1016/j.tcb.2007.01.002
2. MurphyDA, CourtneidgeSA. The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function.Nat Rev Mol Cell Biol2011; 12:413-26; PMID:21697900; http://dx.doi.org/10.1038/nrm3141
3. GimonaM, BuccioneR, CourtneidgeSA, LinderS. Assembly and biological role of podosomes and invadopodia.Curr Opin Cell Biol2008; 20:235-41; PMID:18337078; http://dx.doi.org/10.1016/j.ceb.2008.01.005
4. LinderS, WiesnerC, HimmelM. Degrading devices: invadosomes in proteolytic cell invasion.Annu Rev Cell Dev Biol2011; 27:185-211; PMID:21801014; http://dx.doi.org/10.1146/annurev-cellbio-092910-154216
5. CaldieriG, AyalaI, AttanasioF, BuccioneR. Cell and molecular biology of invadopodia.Int Rev Cell Mol Biol2009; 275:1-34; PMID:19491051; http://dx.doi.org/10.1016/S1937-6448(09)75001-4
6. David-PfeutyT, SingerSJ. Altered distributions of the cytoskeletal proteins vinculin and alpha-actinin in cultured fibroblasts transformed by Rous sarcoma virus.Proc Natl Acad Sci U S A1980; 77:6687-91; PMID:6256755; http://dx.doi.org/10.1073/pnas.77.11.6687
7. TaroneG, CirilloD, GiancottiFG, ComoglioPM, MarchisioPC. Rous sarcoma virus-transformed fibroblasts adhere primarily at discrete protrusions of the ventral membrane called podosomes.Exp Cell Res1985; 159:141-57; PMID:2411576; http://dx.doi.org/10.1016/S0014-4827(85)80044-6
8. CarleyWW, BarakLS, WebbWW. F-actin aggregates in transformed cells.J Cell Biol1981; 90:797-802; PMID:6270163; http://dx.doi.org/10.1083/jcb.90.3.797
9. CarleyWW, BretscherA, WebbWW. F-actin aggregates in transformed cells contain alpha-actinin and fimbrin but apparently lack tropomyosin.Eur J Cell Biol1986; 39:313-20; PMID:3007147
10. ShriverK, RohrschneiderL. Organization of pp60src and selected cytoskeletal proteins within adhesion plaques and junctions of Rous sarcoma virus-transformed rat cells.J Cell Biol1981; 89:525-35; PMID:6265469; http://dx.doi.org/10.1083/jcb.89.3.525
11. ChenWT, ChenJM, ParsonsSJ, ParsonsJT. Local degradation of fibronectin at sites of expression of the transforming gene product pp60src.Nature1985; 316:156-8; PMID:2989711; http://dx.doi.org/10.1038/316156a0
12. ChenWT. Proteolytic activity of specialized surface protrusions formed at rosette contact sites of transformed cells.J Exp Zool1989; 251:167-85; PMID:2549171; http://dx.doi.org/10.1002/jez.1402510206
13. MuellerSC, YehY, ChenWT. Tyrosine phosphorylation of membrane proteins mediates cellular invasion by transformed cells.J Cell Biol1992; 119:1309-25; PMID:1447304; http://dx.doi.org/10.1083/jcb.119.5.1309
14. Zambonin-ZalloneA, TetiA, CaranoA, MarchisioPC. The distribution of podosomes in osteoclasts cultured on bone laminae: effect of retinol.J Bone Miner Res1988; 3:517-23; PMID:3195364; http://dx.doi.org/10.1002/jbmr.5650030507
15. KellyT, MuellerSC, YehY, ChenWT. Invadopodia promote proteolysis of a wide variety of extracellular matrix proteins.J Cell Physiol1994; 158:299-308; PMID:8106567; http://dx.doi.org/10.1002/jcp.1041580212
16. MonskyWL, LinCY, AoyamaA, KellyT, AkiyamaSK, MuellerSC, ChenWT. A potential marker protease of invasiveness, seprase, is localized on invadopodia of human malignant melanoma cells.Cancer Res1994; 54:5702-10; PMID:7923219
17. DaviesWA, StosselTP. Peripheral hyaline blebs (podosomes) of macrophages.J Cell Biol1977; 75:941-55; PMID:925088; http://dx.doi.org/10.1083/jcb.75.3.941
18. KanehisaJ, YamanakaT, DoiS, TurksenK, HeerscheJN, AubinJE, TakeuchiH. A band of F-actin containing podosomes is involved in bone resorption by osteoclasts.Bone1990; 11:287-93; PMID:2242294; http://dx.doi.org/10.1016/8756-3282(90)90082-A
19. CalleY, BurnsS, ThrasherAJ, JonesGE. The leukocyte podosome.Eur J Cell Biol2006; 85:151-7; PMID:16546557; http://dx.doi.org/10.1016/j.ejcb.2005.09.003
20. HaiCM, HahneP, HarringtonEO, GimonaM. Conventional protein kinase C mediates phorboldibutyrate-induced cytoskeletal remodeling in a7r5 smooth muscle cells.Exp Cell Res2002; 280:64-74; PMID:12372340; http://dx.doi.org/10.1006/excr.2002.5592
21. GénotE. [The endothelial podosome]. Med Sci (Paris)2009; 25:168-74; PMID:19239849
22. ChenWT, LeeCC, GoldsteinL, BernierS, LiuCH, LinCY, YehY, MonskyWL, KellyT, DaiM, et al. Membrane proteases as potential diagnostic and therapeutic targets for breast malignancy.Breast Cancer Res Treat1994; 31:217-26; PMID:7881100; http://dx.doi.org/10.1007/BF00666155
23. SutohM, HashimotoY, YoneyamaT, YamamotoH, HatakeyamaS, KoieT, OkamotoA, YamayaK, SaitohH, FunyuT, et al.Invadopodia formation by bladder tumor cells.Oncol Res2010; 19:85-92; PMID:21302809; http://dx.doi.org/10.3727/096504010X12875107808008
24. HwangYS, ParkKK, ChungWY. Invadopodia formation in oral squamous cell carcinoma: the role of epidermal growth factor receptor signalling.Arch Oral Biol2012; 57:335-43; PMID:21920495; http://dx.doi.org/10.1016/j.archoralbio.2011.08.019
25. DesaiB, MaT, ChellaiahMA. Invadopodia and matrix degradation, a new property of prostate cancer cells during migration and invasion.J Biol Chem2008; 283:13856-66; PMID:18337256; http://dx.doi.org/10.1074/jbc.M709401200
26. GligorijevicB, WyckoffJ, YamaguchiH, WangY, RoussosET, CondeelisJ. N-WASP-mediated invadopodium formation is involved in intravasation and lung metastasis of mammary tumors.J Cell Sci2012; 125:724-34; PMID:22389406; http://dx.doi.org/10.1242/jcs.092726
27. BaldassarreM, AyalaI, BeznoussenkoG, GiacchettiG, MacheskyLM, LuiniA, BuccioneR. Actin dynamics at sites of extracellular matrix degradation.Eur J Cell Biol2006; 85:1217-31; PMID:17010475; http://dx.doi.org/10.1016/j.ejcb.2006.08.003
28. YamaguchiH, LorenzM, KempiakS, SarmientoC, ConiglioS, SymonsM, SegallJ, EddyR, MikiH, TakenawaT, et al.Molecular mechanisms of invadopodium formation: the role of the N-WASP-Arp2/3 complex pathway and cofilin.J Cell Biol2005; 168:441-52; PMID:15684033; http://dx.doi.org/10.1083/jcb.200407076
29. LinderS, AepfelbacherM. Podosomes: adhesion hotspots of invasive cells.Trends Cell Biol2003; 13:376-85; PMID:12837608; http://dx.doi.org/10.1016/S0962-8924(03)00128-4
30. AyalaI, BaldassarreM, CaldieriG, BuccioneR. Invadopodia: a guided tour.Eur J Cell Biol2006; 85:159-64; PMID:16546558; http://dx.doi.org/10.1016/j.ejcb.2005.09.005
31. ArtymVV, MatsumotoK, MuellerSC, YamadaKM. Dynamic membrane remodeling at invadopodia differentiates invadopodia from podosomes.Eur J Cell Biol2011; 90:172-80; PMID:20656375; http://dx.doi.org/10.1016/j.ejcb.2010.06.006
32. OserM, DovasA, CoxD, CondeelisJ. Nck1 and Grb2 localization patterns can distinguish invadopodia from podosomes.Eur J Cell Biol2011; 90:181-8; PMID:20850195; http://dx.doi.org/10.1016/j.ejcb.2010.08.006
33. BuccioneR, CaldieriG, AyalaI. Invadopodia: specialized tumor cell structures for the focal degradation of the extracellular matrix.Cancer Metastasis Rev2009; 28:137-49; PMID:19153671; http://dx.doi.org/10.1007/s10555-008-9176-1
34. EckertMA, LwinTM, ChangAT, KimJ, DanisE, Ohno-MachadoL, YangJ. Twist1-induced invadopodia formation promotes tumor metastasis.Cancer Cell2011; 19:372-86; PMID:21397860; http://dx.doi.org/10.1016/j.ccr.2011.01.036
35. JacobA, JingJ, LeeJ, SchedinP, GilbertSM, PedenAA, JunutulaJR, PrekerisR. Rab40b regulates trafficking of MMP2 and MMP9 during invadopodia formation and invasion of breast cancer cells.J Cell Sci2013; 126:4647-58; PMID:23902685; http://dx.doi.org/10.1242/jcs.126573
36. ArtymVV, ZhangY, Seillier-MoiseiwitschF, YamadaKM, MuellerSC. Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: defining the stages of invadopodia formation and function.Cancer Res2006; 66:3034-43; PMID:16540652; http://dx.doi.org/10.1158/0008-5472.CAN-05-2177
37. NakaharaH, HowardL, ThompsonEW, SatoH, SeikiM, YehY, ChenWT. Transmembrane/cytoplasmic domain-mediated membrane type 1-matrix metalloprotease docking to invadopodia is required for cell invasion.Proc Natl Acad Sci U S A1997; 94:7959-64; PMID:9223295; http://dx.doi.org/10.1073/pnas.94.15.7959
38. BourguignonLY, Gunja-SmithZ, IidaN, ZhuHB, YoungLJ, MullerWJ, CardiffRD. CD44v(3,8-10) is involved in cytoskeleton-mediated tumor cell migration and matrix metalloproteinase (MMP-9) association in metastatic breast cancer cells.J Cell Physiol1998; 176:206-15; PMID:9618160; http://dx.doi.org/10.1002/(SICI)1097-4652(199807)176:1<206::AID-JCP22>3.0.CO;2-3

39. ClarkES, WhighamAS, YarbroughWG, WeaverAM. Cortactin is an essential regulator of matrix metalloproteinase secretion and extracellular matrix degradation in invadopodia.Cancer Res2007; 67:4227-35; PMID:17483334; http://dx.doi.org/10.1158/0008-5472.CAN-06-3928
40. PoinclouxR, LizárragaF, ChavrierP. Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia.J Cell Sci2009; 122:3015-24; PMID:19692588; http://dx.doi.org/10.1242/jcs.034561
41. MonskyWL, KellyT, LinCY, YehY, Stetler-StevensonWG, MuellerSC, ChenWT. Binding and localization of M(r) 72,000 matrix metalloproteinase at cell surface invadopodia.Cancer Res1993; 53:3159-64; PMID:8391388
42. SchnaekerEM, OssigR, LudwigT, DreierR, OberleithnerH, WilhelmiM, SchneiderSW. Microtubule-dependent matrix metalloproteinase-2/matrix metalloproteinase-9 exocytosis: prerequisite in human melanoma cell invasion.Cancer Res2004; 64:8924-31; PMID:15604254; http://dx.doi.org/10.1158/0008-5472.CAN-04-0324
43. ItohY, SeikiM. MT1-MMP: a potent modifier of pericellular microenvironment.J Cell Physiol2006; 206:1-8; PMID:15920734; http://dx.doi.org/10.1002/jcp.20431
44. Bravo-CorderoJJ, Marrero-DiazR, MegíasD, GenísL, García-GrandeA, GarcíaMA, ArroyoAG, MontoyaMC. MT1-MMP proinvasive activity is regulated by a novel Rab8-dependent exocytic pathway.EMBO J2007; 26:1499-510; PMID:17332756; http://dx.doi.org/10.1038/sj.emboj.7601606
45. WiesnerC, El AzzouziK, LinderS. A specific subset of RabGTPases controls cell surface exposure of MT1-MMP, extracellular matrix degradation and three-dimensional invasion of macrophages.J Cell Sci2013; 126:2820-33; PMID:23606746; http://dx.doi.org/10.1242/jcs.122358
46. NyalendoC, MichaudM, BeaulieuE, RoghiC, MurphyG, GingrasD, BéliveauR. Src-dependent phosphorylation of membrane type I matrix metalloproteinase on cytoplasmic tyrosine 573: role in endothelial and tumor cell migration.J Biol Chem2007; 282:15690-9; PMID:17389600; http://dx.doi.org/10.1074/jbc.M608045200
47. BrownMD, SacksDB. IQGAP1 in cellular signaling: bridging the GAP.Trends Cell Biol2006; 16:242-9; PMID:16595175; http://dx.doi.org/10.1016/j.tcb.2006.03.002
48. Sakurai-YagetaM, RecchiC, Le DezG, SibaritaJB, DavietL, CamonisJ, D'Souza-SchoreyC, ChavrierP. The interaction of IQGAP1 with the exocyst complex is required for tumor cell invasion downstream of Cdc42 and RhoA.J Cell Biol2008; 181:985-98; PMID:18541705; http://dx.doi.org/10.1083/jcb.200709076
49. ClarkES, WeaverAM. A new role for cortactin in invadopodia: regulation of protease secretion.Eur J Cell Biol2008; 87:581-90; PMID:18342393; http://dx.doi.org/10.1016/j.ejcb.2008.01.008
50. WolfK, MazoI, LeungH, EngelkeK, von AndrianUH, DeryuginaEI, StronginAY, BröckerEB, FriedlP. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis.J Cell Biol2003; 160:267-77; PMID:12527751; http://dx.doi.org/10.1083/jcb.200209006
51. WolfK, WuYI, LiuY, GeigerJ, TamE, OverallC, StackMS, FriedlP. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion.Nat Cell Biol2007; 9:893-904; PMID:17618273; http://dx.doi.org/10.1038/ncb1616
52. BranchKM, HoshinoD, WeaverAM. Adhesion rings surround invadopodia and promote maturation.Biol Open2012; 1:711-22; PMID:23213464; http://dx.doi.org/10.1242/bio.20121867
53. BeatyBT, SharmaVP, Bravo-CorderoJJ, SimpsonMA, EddyRJ, KoleskeAJ, CondeelisJ. β1 integrin regulates Arg to promote invadopodial maturation and matrix degradation.Mol Biol Cell2013; 24:1661-75, S1-11; PMID:23552693; http://dx.doi.org/10.1091/mbc.E12-12-0908
54. BaldassarreM, PompeoA, BeznoussenkoG, CastaldiC, CortellinoS, McNivenMA, LuiniA, BuccioneR. Dynamin participates in focal extracellular matrix degradation by invasive cells.Mol Biol Cell2003; 14:1074-84; PMID:12631724; http://dx.doi.org/10.1091/mbc.E02-05-0308
55. McNivenMA, BaldassarreM, BuccioneR. The role of dynamin in the assembly and function of podosomes and invadopodia.Front Biosci2004; 9:1944-53; PMID:14977600; http://dx.doi.org/10.2741/1348
56. DeryuginaEI, QuigleyJP. Matrix metalloproteinases and tumor metastasis.Cancer Metastasis Rev2006; 25:9-34; PMID:16680569; http://dx.doi.org/10.1007/s10555-006-7886-9
57. TurkB. Targeting proteases: successes, failures and future prospects.Nat Rev Drug Discov2006; 5:785-99; PMID:16955069; http://dx.doi.org/10.1038/nrd2092
58. AbbenanteG, FairlieDP. Protease inhibitors in the clinic.Med Chem2005; 1:71-104; PMID:16789888; http://dx.doi.org/10.2174/1573406053402569
59. KonstantinopoulosPA, KaramouzisMV, PapatsorisAG, PapavassiliouAG. Matrix metalloproteinase inhibitors as anticancer agents.Int J Biochem Cell Biol2008; 40:1156-68; PMID:18164645; http://dx.doi.org/10.1016/j.biocel.2007.11.007
60. SparanoJA, BernardoP, StephensonP, GradisharWJ, IngleJN, ZuckerS, DavidsonNE. Randomized phase III trial of marimastat versus placebo in patients with metastatic breast cancer who have responding or stable disease after first-line chemotherapy: Eastern Cooperative Oncology Group trial E2196.J Clin Oncol2004; 22:4683-90; PMID:15570070; http://dx.doi.org/10.1200/JCO.2004.08.054
61. ZuckerS, CaoJ. Selective matrix metalloproteinase (MMP) inhibitors in cancer therapy: ready for prime time?Cancer Biol Ther2009; 8:2371-3; PMID:19959934; http://dx.doi.org/10.4161/cbt.8.24.10353
62. FriedlP, WolfK. Tumour-cell invasion and migration: diversity and escape mechanisms.Nat Rev Cancer2003; 3:362-74; PMID:12724734; http://dx.doi.org/10.1038/nrc1075
63. YamaguchiH, CondeelisJ. Regulation of the actin cytoskeleton in cancer cell migration and invasion.Biochim Biophys Acta2007; 1773:642-52; PMID:16926057; http://dx.doi.org/10.1016/j.bbamcr.2006.07.001
64. BromannPA, KorkayaH, CourtneidgeSA. The interplay between Src family kinases and receptor tyrosine kinases.Oncogene2004; 23:7957-68; PMID:15489913; http://dx.doi.org/10.1038/sj.onc.1208079
65. ObergfellA, EtoK, MocsaiA, BuensucesoC, MooresSL, BruggeJS, LowellCA, ShattilSJ. Coordinate interactions of Csk, Src, and Syk kinases with [alpha]IIb[beta]3 initiate integrin signaling to the cytoskeleton.J Cell Biol2002; 157:265-75; PMID:11940607; http://dx.doi.org/10.1083/jcb.200112113
66. KelleyLC, AmmerAG, HayesKE, MartinKH, MachidaK, JiaL, MayerBJ, WeedSA. Oncogenic Src requires a wild-type counterpart to regulate invadopodia maturation.J Cell Sci2010; 123:3923-32; PMID:20980387; http://dx.doi.org/10.1242/jcs.075200
67. GatesmanA, WalkerVG, BaisdenJM, WeedSA, FlynnDC. Protein kinase Calpha activates c-Src and induces podosome formation via AFAP-110.Mol Cell Biol2004; 24:7578-97; PMID:15314167; http://dx.doi.org/10.1128/MCB.24.17.7578-7597.2004
68. BowdenET, BarthM, ThomasD, GlazerRI, MuellerSC. An invasion-related complex of cortactin, paxillin and PKCmu associates with invadopodia at sites of extracellular matrix degradation. Oncogene1999; 18:4440-9; PMID:10442635; http://dx.doi.org/10.1038/sj.onc.1202827
69. DestaingO, BlockMR, PlanusE, Albiges-RizoC. Invadosome regulation by adhesion signaling.Curr Opin Cell Biol2011; 23:597-606; PMID:21550788; http://dx.doi.org/10.1016/j.ceb.2011.04.002
70. RodriguezEM, DunhamEE, MartinGS. Atypical protein kinase C activity is required for extracellular matrix degradation and invasion by Src-transformed cells.J Cell Physiol2009; 221:171-82; PMID:19492416; http://dx.doi.org/10.1002/jcp.21841
71. CourtneidgeSA. Cell migration and invasion in human disease: the Tks adaptor proteins.Biochem Soc Trans2012; 40:129-32; PMID:22260678; http://dx.doi.org/10.1042/BST20110685
72. SharmaVP, EddyR, EntenbergD, KaiM, GertlerFB, CondeelisJ. Tks5 and SHIP2 regulate invadopodium maturation, but not initiation, in breast carcinoma cells.Curr Biol2013; 23:2079-89; PMID:24206842; http://dx.doi.org/10.1016/j.cub.2013.08.044
73. GrossSR. Actin binding proteins: their ups and downs in metastatic life.Cell Adh Migr2013; 7:199-213; PMID:23302954; http://dx.doi.org/10.4161/cam.23176
74. Martinez-QuilesN, RohatgiR, AntónIM, MedinaM, SavilleSP, MikiH, YamaguchiH, TakenawaT, HartwigJH, GehaRS, et al.WIP regulates N-WASP-mediated actin polymerization and filopodium formation.Nat Cell Biol2001; 3:484-91; PMID:11331876; http://dx.doi.org/10.1038/35074551
75. HoshinoD, BranchKM, WeaverAM. Signaling inputs to invadopodia and podosomes.J Cell Sci2013; 126:2979-89; PMID:23843616; http://dx.doi.org/10.1242/jcs.079475
76. Albiges-RizoC, DestaingO, FourcadeB, PlanusE, BlockMR. Actin machinery and mechanosensitivity in invadopodia, podosomes and focal adhesions.J Cell Sci2009; 122:3037-49; PMID:19692590; http://dx.doi.org/10.1242/jcs.052704
77. OserM, YamaguchiH, MaderCC, Bravo-CorderoJJ, AriasM, ChenX, DesmaraisV, van RheenenJ, KoleskeAJ, CondeelisJ. Cortactin regulates cofilin and N-WASp activities to control the stages of invadopodium assembly and maturation.J Cell Biol2009; 186:571-87; PMID:19704022; http://dx.doi.org/10.1083/jcb.200812176
78. Sibony-BenyaminiH, Gil-HennH. Invadopodia: the leading force.Eur J Cell Biol2012; 91:896-901; PMID:22633185; http://dx.doi.org/10.1016/j.ejcb.2012.04.001
79. Bravo-CorderoJJ, MagalhaesMA, EddyRJ, HodgsonL, CondeelisJ. Functions of cofilin in cell locomotion and invasion.Nat Rev Mol Cell Biol2013; 14:405-15; PMID:23778968; http://dx.doi.org/10.1038/nrm3609
80. LizárragaF, PoinclouxR, RomaoM, MontagnacG, Le DezG, BonneI, RigaillG, RaposoG, ChavrierP. Diaphanous-related formins are required for invadopodia formation and invasion of breast tumor cells. Cancer Res2009; 69:2792-800; PMID:19276357; http://dx.doi.org/10.1158/0008-5472.CAN-08-3709
81. LiA, DawsonJC, Forero-VargasM, SpenceHJ, YuX, KönigI, AndersonK, MacheskyLM. The actin-bundling protein fascin stabilizes actin in invadopodia and potentiates protrusive invasion.Curr Biol2010; 20:339-45; PMID:20137952; http://dx.doi.org/10.1016/j.cub.2009.12.035
82. JayoA, ParsonsM. Fascin: a key regulator of cytoskeletal dynamics.Int J Biochem Cell Biol2010; 42:1614-7; PMID:20601080; http://dx.doi.org/10.1016/j.biocel.2010.06.019

83. SchoumacherM, GoldmanRD, LouvardD, VignjevicDM. Actin, microtubules, and vimentin intermediate filaments cooperate for elongation of invadopodia.J Cell Biol2010; 189:541-56; PMID:20421424; http://dx.doi.org/10.1083/jcb.200909113
84. LorenzM, YamaguchiH, WangY, SingerRH, CondeelisJ. Imaging sites of N-wasp activity in lamellipodia and invadopodia of carcinoma cells.Curr Biol2004; 14:697-703; PMID:15084285; http://dx.doi.org/10.1016/j.cub.2004.04.008
85. DonnellySK, WeisswangeI, ZettlM, WayM. WIP provides an essential link between Nck and N-WASP during Arp2/3-dependent actin polymerization.Curr Biol2013; 23:999-1006; PMID:23707428; http://dx.doi.org/10.1016/j.cub.2013.04.051
86. WelchMD, RosenblattJ, SkobleJ, PortnoyDA, MitchisonTJ. Interaction of human Arp2/3 complex and the Listeria monocytogenes ActA protein in actin filament nucleation.Science1998; 281:105-8; PMID:9651243; http://dx.doi.org/10.1126/science.281.5373.105
87. MullinsRD, HeuserJA, PollardTD. 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 A1998; 95:6181-6; PMID:9600938; http://dx.doi.org/10.1073/pnas.95.11.6181
88. KhandujaN, KuhnJR. Processive acceleration of actin barbed-end assembly by N-WASP.Mol Biol Cell2014; 25:55-65; PMID:24227886; http://dx.doi.org/10.1091/mbc.E12-11-0781
89. GoleyED, WelchMD. The ARP2/3 complex: an actin nucleator comes of age.Nat Rev Mol Cell Biol2006; 7:713-26; PMID:16990851; http://dx.doi.org/10.1038/nrm2026
90. OtsuboT, IwayaK, MukaiY, MizokamiY, SerizawaH, MatsuokaT, MukaiK. Involvement of Arp2/3 complex in the process of colorectal carcinogenesis.Mod Pathol2004; 17:461-7; PMID:14990971; http://dx.doi.org/10.1038/modpathol.3800062
91. SembaS, IwayaK, MatsubayashiJ, SerizawaH, KatabaH, HiranoT, KatoH, MatsuokaT, MukaiK. Coexpression of actin-related protein 2 and Wiskott-Aldrich syndrome family verproline-homologous protein 2 in adenocarcinoma of the lung.Clin Cancer Res2006; 12:2449-54; PMID:16638851; http://dx.doi.org/10.1158/1078-0432.CCR-05-2566
92. WeaverAM, KarginovAV, KinleyAW, WeedSA, LiY, ParsonsJT, CooperJA. Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation.Curr Biol2001; 11:370-4; PMID:11267876; http://dx.doi.org/10.1016/S0960-9822(01)00098-7
93. WeaverAM, HeuserJE, KarginovAV, LeeWL, ParsonsJT, CooperJA. Interaction of cortactin and N-WASp with Arp2/3 complex.Curr Biol2002; 12:1270-8; PMID:12176354; http://dx.doi.org/10.1016/S0960-9822(02)01035-7
94. PazH, PathakN, YangJ. Invading one step at a time: the role of invadopodia in tumor metastasis. Oncogene2013; (Forthcoming); PMID:24077283; http://dx.doi.org/10.1038/onc.2013.393
95. KannerSB, ReynoldsAB, VinesRR, ParsonsJT. Monoclonal antibodies to individual tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases.Proc Natl Acad Sci U S A1990; 87:3328-32; PMID:2110361; http://dx.doi.org/10.1073/pnas.87.9.3328
96. WuH, ReynoldsAB, KannerSB, VinesRR, ParsonsJT. Identification and characterization of a novel cytoskeleton-associated pp60src substrate.Mol Cell Biol1991; 11:5113-24; PMID:1922035
97. MaderCC, OserM, MagalhaesMA, Bravo-CorderoJJ, CondeelisJ, KoleskeAJ, Gil-HennH. An EGFR-Src-Arg-cortactin pathway mediates functional maturation of invadopodia and breast cancer cell invasion. Cancer Res2011; 71:1730-41; PMID:21257711; http://dx.doi.org/10.1158/0008-5472.CAN-10-1432
98. TehraniS, TomasevicN, WeedS, SakowiczR, CooperJA. Src phosphorylation of cortactin enhances actin assembly.Proc Natl Acad Sci U S A2007; 104:11933-8; PMID:17606906; http://dx.doi.org/10.1073/pnas.0701077104
99. LiY, TondraviM, LiuJ, SmithE, HaudenschildCC, KaczmarekM, ZhanX. Cortactin potentiates bone metastasis of breast cancer cells.Cancer Res2001; 61:6906-11; PMID:11559568
100. OserM, MaderCC, Gil-HennH, MagalhaesM, Bravo-CorderoJJ, KoleskeAJ, CondeelisJ. Specific tyrosine phosphorylation sites on cortactin regulate Nck1-dependent actin polymerization in invadopodia.J Cell Sci2010; 123:3662-73; PMID:20971703; http://dx.doi.org/10.1242/jcs.068163
101. WeaverAM, YoungME, LeeWL, CooperJA. Integration of signals to the Arp2/3 complex.Curr Opin Cell Biol2003; 15:23-30; PMID:12517700; http://dx.doi.org/10.1016/S0955-0674(02)00015-7
102. UrunoT, LiuJ, ZhangP, Fan Yx, EgileC, LiR, MuellerSC, ZhanX. Activation of Arp2/3 complex-mediated actin polymerization by cortactin.Nat Cell Biol2001; 3:259-66; PMID:11231575; http://dx.doi.org/10.1038/35060051
103. AyalaI, BaldassarreM, GiacchettiG, CaldieriG, TetèS, LuiniA, BuccioneR. Multiple regulatory inputs converge on cortactin to control invadopodia biogenesis and extracellular matrix degradation.J Cell Sci2008; 121:369-78; PMID:18198194; http://dx.doi.org/10.1242/jcs.008037
104. Bravo-CorderoJJ, OserM, ChenX, EddyR, HodgsonL, CondeelisJ. A novel spatiotemporal RhoC activation pathway locally regulates cofilin activity at invadopodia.Curr Biol2011; 21:635-44; PMID:21474314; http://dx.doi.org/10.1016/j.cub.2011.03.039
105. Yamashiro-MatsumuraS, MatsumuraF. Purification and characterization of an F-actin-bundling 55-kilodalton protein from HeLa cells.J Biol Chem1985; 260:5087-97; PMID:3886649
106. SvitkinaTM, BulanovaEA, ChagaOY, VignjevicDM, KojimaS, VasilievJM, BorisyGG. Mechanism of filopodia initiation by reorganization of a dendritic network.J Cell Biol2003; 160:409-21; PMID:12566431; http://dx.doi.org/10.1083/jcb.200210174
107. AdamsJC. Roles of fascin in cell adhesion and motility.Curr Opin Cell Biol2004; 16:590-6; PMID:15363811; http://dx.doi.org/10.1016/j.ceb.2004.07.009
108. SedehRS, FedorovAA, FedorovEV, OnoS, MatsumuraF, AlmoSC, BatheM. Structure, evolutionary conservation, and conformational dynamics of Homo sapiens fascin-1, an F-actin crosslinking protein.J Mol Biol2010; 400:589-604; PMID:20434460; http://dx.doi.org/10.1016/j.jmb.2010.04.043
109. AnilkumarN, ParsonsM, MonkR, NgT, AdamsJC. Interaction of fascin and protein kinase Calpha: a novel intersection in cell adhesion and motility. EMBO J2003; 22:5390-402; PMID:14532112; http://dx.doi.org/10.1093/emboj/cdg521
110. HashimotoY, SkacelM, AdamsJC. Roles of fascin in human carcinoma motility and signaling: prospects for a novel biomarker?Int J Biochem Cell Biol2005; 37:1787-804; PMID:16002322; http://dx.doi.org/10.1016/j.biocel.2005.05.004
111. VignjevicD, SchoumacherM, GavertN, JanssenKP, JihG, LaéM, LouvardD, Ben-Ze'evA, RobineS. Fascin, a novel target of beta-catenin-TCF signaling, is expressed at the invasive front of human colon cancer.Cancer Res2007; 67:6844-53; PMID:17638895; http://dx.doi.org/10.1158/0008-5472.CAN-07-0929
112. LinderS, HüfnerK, WintergerstU, AepfelbacherM. Microtubule-dependent formation of podosomal adhesion structures in primary human macrophages.J Cell Sci2000; 113:4165-76; PMID:11069762
113. KoppP, LammersR, AepfelbacherM, WoehlkeG, RudelT, MachuyN, SteffenW, LinderS. The kinesin KIF1C and microtubule plus ends regulate podosome dynamics in macrophages.Mol Biol Cell2006; 17:2811-23; PMID:16554367; http://dx.doi.org/10.1091/mbc.E05-11-1010
114. KikuchiK, TakahashiK. WAVE2- and microtubule-dependent formation of long protrusions and invasion of cancer cells cultured on three-dimensional extracellular matrices.Cancer Sci2008; 99:2252-9; PMID:18795939; http://dx.doi.org/10.1111/j.1349-7006.2008.00927.x
115. DesmaraisV, YamaguchiH, OserM, SoonL, MouneimneG, SarmientoC, EddyR, CondeelisJ. N-WASP and cortactin are involved in invadopodium-dependent chemotaxis to EGF in breast tumor cells.Cell Motil Cytoskeleton2009; 66:303-16; PMID:19373774; http://dx.doi.org/10.1002/cm.20361
116. CaldieriG, CapestranoM, BicanovaK, BeznoussenkoG, BaldassarreM, BuccioneR. Polarised apical-like intracellular sorting and trafficking regulates invadopodia formation and degradation of the extracellular matrix in cancer cells.Eur J Cell Biol2012; 91:961-8; PMID:22564726; http://dx.doi.org/10.1016/j.ejcb.2012.03.005
117. SchmoranzerJ, KreitzerG, SimonSM. Migrating fibroblasts perform polarized, microtubule-dependent exocytosis towards the leading edge.J Cell Sci2003; 116:4513-9; PMID:14576345; http://dx.doi.org/10.1242/jcs.00748
118. CorreiaI, ChuD, ChouYH, GoldmanRD, MatsudairaP. Integrating the actin and vimentin cytoskeletons. adhesion-dependent formation of fimbrin-vimentin complexes in macrophages.J Cell Biol1999; 146:831-42; PMID:10459017; http://dx.doi.org/10.1083/jcb.146.4.831
119. TakadaY, YeX, SimonS. The integrins.Genome Biol2007; 8:215; PMID:17543136; http://dx.doi.org/10.1186/gb-2007-8-5-215
120. BrakebuschC, FässlerR. The integrin-actin connection, an eternal love affair.EMBO J2003; 22:2324-33; PMID:12743027; http://dx.doi.org/10.1093/emboj/cdg245
121. Zaidel-BarR, ItzkovitzS, Ma'ayanA, IyengarR, GeigerB. Functional atlas of the integrin adhesome. Nat Cell Biol2007; 9:858-67; PMID:17671451; http://dx.doi.org/10.1038/ncb0807-858
122. GeigerB, BershadskyA. Exploring the neighborhood: adhesion-coupled cell mechanosensors.Cell2002; 110:139-42; PMID:12150922; http://dx.doi.org/10.1016/S0092-8674(02)00831-0
123. BershadskyAD, BalabanNQ, GeigerB. Adhesion-dependent cell mechanosensitivity.Annu Rev Cell Dev Biol2003; 19:677-95; PMID:14570586; http://dx.doi.org/10.1146/annurev.cellbio.19.111301.153011
124. ChenCS. Mechanotransduction - a field pulling together?J Cell Sci2008; 121:3285-92; PMID:18843115; http://dx.doi.org/10.1242/jcs.023507
125. GeigerB, SpatzJP, BershadskyAD. Environmental sensing through focal adhesions.Nat Rev Mol Cell Biol2009; 10:21-33; PMID:19197329; http://dx.doi.org/10.1038/nrm2593
126. CurtisA, RiehleM. Tissue engineering: the biophysical background.Phys Med Biol2001; 46:R47-65; PMID:11324976; http://dx.doi.org/10.1088/0031-9155/46/4/201
127. SpatzJP, GeigerB. Molecular engineering of cellular environments: cell adhesion to nano-digital surfaces. Methods Cell Biol2007; 83:89-111; PMID:17613306; http://dx.doi.org/10.1016/S0091-679X(07)83005-6
128. VogelV, SheetzM. Local force and geometry sensing regulate cell functions.Nat Rev Mol Cell Biol2006; 7:265-75; PMID:16607289; http://dx.doi.org/10.1038/nrm1890

129. EnglerAJ, SenS, SweeneyHL, DischerDE. Matrix elasticity directs stem cell lineage specification. Cell2006; 126:677-89; PMID:16923388; http://dx.doi.org/10.1016/j.cell.2006.06.044
130. DischerDE, JanmeyP, WangYL. Tissue cells feel and respond to the stiffness of their substrate. Science2005; 310:1139-43; PMID:16293750; http://dx.doi.org/10.1126/science.1116995
131. ParsonsJT, HorwitzAR, SchwartzMA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension.Nat Rev Mol Cell Biol2010; 11:633-43; PMID:20729930; http://dx.doi.org/10.1038/nrm2957
132. RidleyAJ. Life at the leading edge.Cell2011; 145:1012-22; PMID:21703446; http://dx.doi.org/10.1016/j.cell.2011.06.010
133. KirfelG, RigortA, BormB, HerzogV. Cell migration: mechanisms of rear detachment and the formation of migration tracks.Eur J Cell Biol2004; 83:717-24; PMID:15679116; http://dx.doi.org/10.1078/0171-9335-00421
134. PignatelliJ, TumbarelloDA, SchmidtRP, TurnerCE. Hic-5 promotes invadopodia formation and invasion during TGF-β-induced epithelial-mesenchymal transition.J Cell Biol2012; 197:421-37; PMID:22529104; http://dx.doi.org/10.1083/jcb.201108143
135. NakaharaH, MuellerSC, NomizuM, YamadaY, YehY, ChenWT. Activation of beta1 integrin signaling stimulates tyrosine phosphorylation of p190RhoGAP and membrane-protrusive activities at invadopodia.J Biol Chem1998; 273:9-12; PMID:9417037; http://dx.doi.org/10.1074/jbc.273.1.9
136. NakaharaH, NomizuM, AkiyamaSK, YamadaY, YehY, ChenWT. A mechanism for regulation of melanoma invasion. Ligation of alpha6beta1 integrin by laminin G peptides.J Biol Chem1996; 271:27221-4; PMID:8910291; http://dx.doi.org/10.1074/jbc.271.44.27221
137. MuellerSC, GhersiG, AkiyamaSK, SangQX, HowardL, Pineiro-SanchezM, NakaharaH, YehY, ChenWT. A novel protease-docking function of integrin at invadopodia.J Biol Chem1999; 274:24947-52; PMID:10455171; http://dx.doi.org/10.1074/jbc.274.35.24947
138. GeigerB, YamadaKM. Molecular architecture and function of matrix adhesions.Cold Spring Harb Perspect Biol2011; 3:a005033; PMID:21441590; http://dx.doi.org/10.1101/cshperspect.a005033
139. WolfensonH, LavelinI, GeigerB. Dynamic regulation of the structure and functions of integrin adhesions. Dev Cell2013; 24:447-58; PMID:23484852; http://dx.doi.org/10.1016/j.devcel.2013.02.012
140. Zaidel-BarR, GeigerB. The switchable integrin adhesome.J Cell Sci2010; 123:1385-8; PMID:20410370; http://dx.doi.org/10.1242/jcs.066183
141. Zaidel-BarR, CohenM, AddadiL, GeigerB. Hierarchical assembly of cell-matrix adhesion complexes.Biochem Soc Trans2004; 32:416-20; PMID:15157150; http://dx.doi.org/10.1042/BST0320416
142. SchallerMD. Paxillin: a focal adhesion-associated adaptor protein.Oncogene2001; 20:6459-72; PMID:11607845; http://dx.doi.org/10.1038/sj.onc.1204786
143. ChanKT, CortesioCL, HuttenlocherA. FAK alters invadopodia and focal adhesion composition and dynamics to regulate breast cancer invasion.J Cell Biol2009; 185:357-70; PMID:19364917; http://dx.doi.org/10.1083/jcb.200809110
144. HauckCR, HsiaDA, IlicD, SchlaepferDD. v-Src SH3-enhanced interaction with focal adhesion kinase at beta 1 integrin-containing invadopodia promotes cell invasion.J Biol Chem2002; 277:12487-90; PMID:11839732; http://dx.doi.org/10.1074/jbc.C100760200
145. ZuzgaDS, Pelta-HellerJ, LiP, BombonatiA, WaldmanSA, PitariGM. Phosphorylation of vasodilator-stimulated phosphoprotein Ser239 suppresses filopodia and invadopodia in colon cancer. Int J Cancer2012; 130:2539-48; PMID:21702043; http://dx.doi.org/10.1002/ijc.26257
146. AlexanderNR, BranchKM, ParekhA, ClarkES, IwuekeIC, GuelcherSA, WeaverAM. Extracellular matrix rigidity promotes invadopodia activity.Curr Biol2008; 18:1295-9; PMID:18718759; http://dx.doi.org/10.1016/j.cub.2008.07.090
147. JuinA, BillottetC, MoreauV, DestaingO, Albiges-RizoC, RosenbaumJ, GénotE, SaltelF. Physiological type I collagen organization induces the formation of a novel class of linear invadosomes.Mol Biol Cell2012; 23:297-309; PMID:22114353; http://dx.doi.org/10.1091/mbc.E11-07-0594
148. KanchanawongP, ShtengelG, PasaperaAM, RamkoEB, DavidsonMW, HessHF, WatermanCM. Nanoscale architecture of integrin-based cell adhesions.Nature2010; 468:580-4; PMID:21107430; http://dx.doi.org/10.1038/nature09621
149. LavelinI, WolfensonH, PatlaI, HenisYI, MedaliaO, VolbergT, LivneA, KamZ, GeigerB. Differential effect of actomyosin relaxation on the dynamic properties of focal adhesion proteins.PLoS One2013; 8:e73549; PMID:24039980; http://dx.doi.org/10.1371/journal.pone.0073549
150. MagalhaesMA, LarsonDR, MaderCC, Bravo-CorderoJJ, Gil-HennH, OserM, ChenX, KoleskeAJ, CondeelisJ. Cortactin phosphorylation regulates cell invasion through a pH-dependent pathway.J Cell Biol2011; 195:903-20; PMID:22105349; http://dx.doi.org/10.1083/jcb.201103045