Invadosomes at a glance

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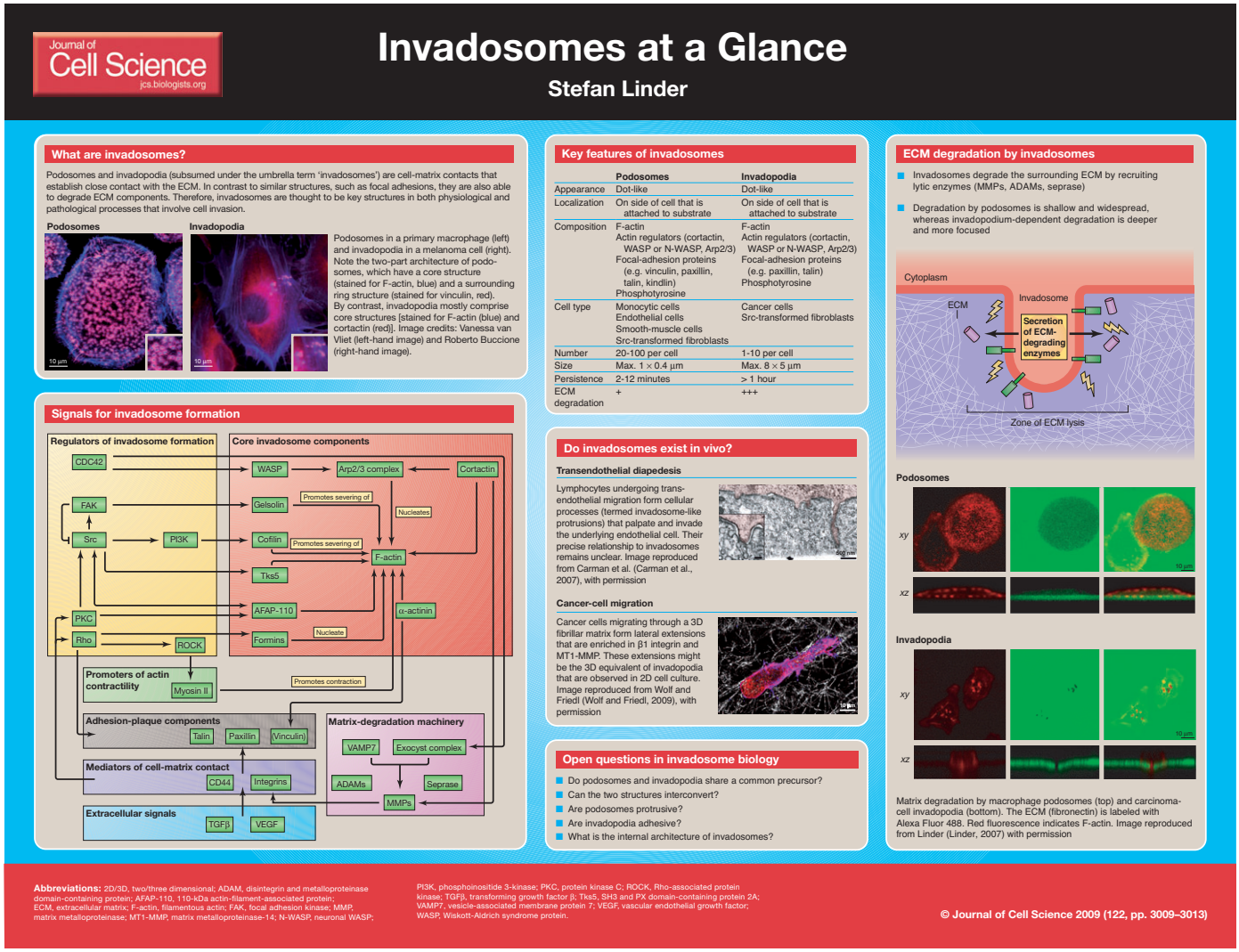
This article is part of a Minifocus on invadopodia and podosomes. For further reading, please see related articles: 'Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia' by Renaud Poincloux et al. (*J. Cell Sci.* **122**, 3015-3024), 'Mechanisms for transcellular diapedesis: probing and pathfinding by 'invadosome-like protrusions'' by Christopher V. Carman (*J. Cell Sci.* **122**, 3025-3035) and 'Actin machinery and mechanosensitivity in invadopodia, podosomes and focal adhesions' by Corinne Albiges-Rizo et al. (*J. Cell Sci.* **122**, 3037-3049).

Podosomes and invadopodia (which can be subsumed under the umbrella term 'invadosomes') are cellular structures that establish close contact with the extracellular matrix (ECM). In contrast to similar structures such as focal adhesions, they are also able to degrade components of the ECM [for a comparison between podosomes and focal adhesions, see Block et al. (Block et al., 2008)]. Invadosomes are, therefore, thought to be key structures of cell invasion. Accordingly, much effort is currently focused on their potential roles in both physiological and pathological invasive processes, such as transendothelial diapedesis and inflammation, and atherosclerosis and metastasis. This poster article provides an introduction to the field, discusses currently investigated topics in invadosome regulation and points out future challenges for invadosome-related research.

Identification

Podosomes and invadopodia share common features that can be used to distinguish them from other cell-matrix contacts or superficially similar structures. For example, both present as dot-like accumulations of filamentous actin (F-actin) at the substrate-contacting side of the cell. Typical markers include actin-regulatory proteins such as the Arp2/3 complex, cortactin and WASP or N-WASP (Linder and Aepfelbacher, 2003; Buccione et al., 2004; Gimona and Buccione, 2006; Weaver, 2006; Linder, 2007; Buccione et al., 2009), which colocalize with the actin-rich core of both structures. Moreover, many components of invadosomes are regulated by tyrosine kinase signaling, resulting in a high local enrichment of phosphotyrosine residues (Linder and Aepfelbacher, 2003; Luxenburg et al., 2006).



(See poster insert)

Despite their obvious similarities, podosomes and invadopodia differ from each other in several respects. Podosomes feature a prominent ring structure of adhesion-plaque proteins (such as talin, paxillin and vinculin) that surrounds the actin-rich core. Some of these proteins, such as paxillin, are also present at invadopodia (Bowden et al., 1999) but, because invadopodia lack a ring structure, they mostly colocalize with the core. Vinculin, in particular, is not enriched at invadopodia, and its usefulness as a marker to distinguish between podosomes and invadopodia is being discussed (Gimona et al., 2008).

Podosomes and invadopodia also differ in other regards. Podosomes are typically formed in monocytic cells (monocytes, macrophages, dendritic cells and osteoclasts), endothelial cells and smooth muscle cells, whereas invadopodia are mostly found in cancer cells (Buccione et al., 2004; Linder and Kopp, 2005; Ayala et al., 2006; Gimona and Buccione, 2006; Weaver, 2006; Linder, 2007). Invadosomes in fibroblasts that have been transformed with tyrosine kinases such as Src show features of both podosomes and invadopodia, and their exact identity is currently unclear. In these cells, numerous individual units are arranged in ring-like superstructures called 'rosettes' (reviewed by Linder, 2007). A distinction between classical podosomes and invadopodia is possible because of their distinct size and abundance: typically, podosomes have a diameter of approximately 1 μm and a height of about 0.4 μm, whereas invadopodia typically present as larger arrays of up to 8 μm × 5 μm in size. Cells form numerous (often more than 100) podosomes but, in many cases, only a few invadopodia (between one and ten). Podosomes and invadopodia also differ markedly in their dynamics: podosomes are highly dynamic, with a lifetime of several minutes, whereas invadopodia are more stable and can persist for over 1 hour (Linder, 2007) (see also 'Matrix degradation' below).

As a rule of thumb, dot-like actin accumulations at the substrate-contacting cell side that are enriched in the Arp2/3 complex, cortactin and phosphotyrosine are good candidates for invadosomes. Following this tentative identification, one should confirm the ability of the observed structures to degrade the matrix. Furthermore, if these structures are surrounded by a ring of vinculin, and are numerous and short-lived, they meet the criteria for podosomes. If they lack distinctive vinculin rings, and are only few in number and persistent, they probably correspond to invadopodia.

The adhesive apparatus

It is currently unclear whether the F-actin-rich core structure of invadosomes is preformed and subsequently recruits ECM-binding proteins, or whether clustering of matrix-binding receptors such as integrins precedes actin nucleation and core formation. The exact sequence of these events might also be cell-type specific [for virus-transformed cells, see Badowski et al. (Badowski et al., 2008)]. In any case, invadosomes are only formed upon contact with the substratum, underscoring the importance of signaling events between the matrix and the cellular adhesive apparatus.

Integrins, which bind to ECM components such as fibronectin or vitronectin, are enriched at podosomes in endothelial (α6β4 integrin) and monocytic (αvβ1, α2β1 and αvβ3 integrins) cells, and also at invadopodia from melanoma (α6β1, α3β1 and a5β1 integrins) and carcinoma (αvβ3 integrin) cells (Gimona et al., 2008). In addition to their function as bridging molecules between the matrix and cytoskeleton, integrins also serve as docking stations for ECM-degrading enzymes such as seprase [α3β1 integrin (Mueller et al., 1999)] or a complex of matrix metalloproteinase-14 (MT1-MMP, MMP-14) and MMP-2 [αvβ3 integrin (Deryugina et al., 2001)] (see also 'Matrix degradation' below). However, contact between the cell and matrix can also be established through other proteins such as CD44, a receptor for hyaluronic acid. CD44 localizes beneath the core structure of the densely packed podosomes that constitute the building blocks of osteoclast sealing zones (Chabadel et al., 2007; Saltel et al., 2008).

As mentioned above, integrin-activating proteins such as talin and kindlin are enriched at the ring structure of podosomes (Zambonin-Zallone et al., 1989; Ussar et al., 2006) and at the actin-rich core of invadosomes in Src-transformed fibroblasts [talin only (Mueller et al., 1992); see also 'Identification' above]. Other adhesion-plaque proteins such as vinculin (Linder et al., 1999) and paxillin (Bowden et al., 1999; Pfaff and Jurdic, 2001) probably act as linkers between matrix-contacting components and the cytoskeleton. The apparent absence of vinculin from invadopodia, however, has raised questions about the adhesive function of these structures (Gimona et al., 2008).

The cytoskeletal backbone

Signaling downstream of integrins is mediated by various cytoskeleton-associated kinases such as protein kinase C (PKC), phosphoinositide 3-kinase (PI3K), Src or focal adhesion kinase (FAK) (reviewed by Linder and Kopp, 2005). Src, in particular, seems to be a master switch for invadosome formation (Cortesio et al., 2008; Destaing et al., 2008; Oikawa et al., 2008), and inhibition of Src-family proteins using inhibitors such as PP2 (Linder et al., 2000a) has proven to be a useful tool for their manipulation. FAK and its hematopoietic homolog Pyk2 bind to both β1 and β3 integrins as well as to Src, and have been shown to localize to invadopodia (Alexander et al., 2008) and the ring structure of podosomes (Pfaff and Jurdic, 2001). Interestingly, FAK was found to function upstream of Src as a negative regulator of invadopodium formation in adenocarcinoma cells (Chan et al., 2009).

The actin-rich core structure of invadosomes is essential for their maintenance, as addition of drugs that interfere with actin polymerization or F-actin stability leads to their disappearance (Linder et al., 2000a; Destaing et al., 2003). Core structures contain a variety of actin-regulatory molecules, which are involved in actin polymerization [the Arp2/3 complex, WASP or N-WASP, and WASP-interacting protein (WIP)], crosslinking (α-actinin and caldesmon), and filament turnover and/or stability (cofilin, tropomyosin and coronin). In particular, the pathway involving the Rho GTPase CDC42, WASP or N-WASP and the Arp2/3 complex, and the importance of this pathway in invadosomal F-actin polymerization, has been well documented (Linder et al., 1999; Linder et al., 2000b; Burns et al., 2001). A role for formins (which are actin-nucleating proteins) has been speculated upon and is now gradually being confirmed for both podosomes [S. Blystone, personal communication; for formin-binding FBP17, see Tsuboi et al. (Tsuboi et al., 2009)] and invadopodia (Lizárraga et al., 2009).

The high degree of interconnectedness in invadosomal actin regulation can be glimpsed through the requirement for several adaptor proteins (particularly WASP, cortactin, AFAP-110 and Tks5), which seem to function as integrative 'hubs' within the core structure. An important adaptor is WASP (or N-WASP), which binds to the Arp2/3 complex, profilin, kinases such as Src and Fyn, and further adaptors such as Grb2, Nck and cortactin (Takenawa and Suetsugu, 2007). Cortactin has long been known to be a crucial component of invadosomes, and its colocalization with phosphotyrosine has been used to identify matrix-degrading invadopodia (Bowden et al., 2006). AFAP-110 can bind to and activate Src, and this is probably a central event in invadosome genesis (Walker et al., 2007). Finally, the multidomain protein Tks5 seems to be crucial for membrane-associated clustering of invadosome components in Src-transformed cells (Seals et al., 2005; Gimona et al., 2008; Oikawa et al., 2008). Interestingly, the closely related protein Tks4 was found to be non-redundant and required for matrix degradation in these cells (Buschman et al., 2009). Tks-

family proteins thus seem to play multiple and central roles in invadosome regulation.

Dynamics and turnover

Several regulators have been identified that influence invadosome dynamics. For instance, knockdown of cofilin by short interfering RNA (siRNA) led to a decreased life span of invadopodia in carcinoma cells (Yamaguchi et al., 2005), and expression of a mutant of AFAP-110 that is defective in phosphorylation by PKC resulted in an increased number of stable podosomes in smooth-muscle cells (Dorfleutner et al., 2008). Cleavage of WASP by the protease calpain is crucial for proper turnover of podosomes, and blockage of calpain activity leads to enhanced lifetimes of podosomes in dendritic cells (Calle et al., 2006). Finally, Src-dependent phosphorylation of paxillin is required for the expansion of rosettes (i.e. formation of new podosomes at the outer rim and dissolution of older podosomes at the inner rim of rosettes) in Rous-sarcoma-virus-transformed kidney cells (Badowski et al., 2008).

Besides their other crucial functions in invadosome regulation (see above and below), Rho GTPases also have a role in the subcellular positioning of invadosomes – for example, CDC42 is involved in the recruitment of podosomes to the leading edge of migrating dendritic cells (Burns et al., 2001), and microtubule-dependent positioning of peripheral arrays of podosomes (so-called podosome belts) in osteoclasts is regulated by a pathway that is controlled by RhoA and histone deacetylase 6 (Destaing et al., 2005; Ory et al., 2008). Finally, microtubules and microtubule-based motor proteins such as the kinesin KIF1C also play a role in the fission (the formation of new podosomes by splitting off from larger precursors) and dissolution processes of podosomes in human macrophages (Kopp et al., 2006).

Local contractility

Actomyosin-based contractility plays a major role in podosome formation and turnover. Accordingly, myosin II has been localized to podosomes in osteoclasts (Krits et al., 2002) and dendritic cells (van Helden et al., 2008), in which it surrounds the core structure. Conflicting data exist about the exact role of myosin (Burgstaller and Gimona, 2005; Clark et al., 2006; Kopp et al., 2006; Collin et al., 2008), but a possibly unifying hypothesis proposes that basal myosin-II activity is required for the formation and maintenance of podosomes, whereas sudden increases in myosin-II activity trigger podosome dissolution (van Helden et al., 2008). Myosin-generated contractility can, depending on substrate stiffness, also be translated into traction forces beneath podosomes, which would enable them to function as mechanosensors (Collin et al., 2008).

Not surprisingly, RhoA–Rho-kinase (ROCK) signaling has emerged as a major pathway in the regulation of actomyosin-dependent podosome dynamics. For example, prostaglandin-induced actomyosin contraction and podosome dissolution in dendritic cells is mediated by RhoA and ROCK (van Helden et al., 2008), whereas phosphorylation of β1 integrin is required to suppress Rho-mediated contractility in order to assemble podosomes in Src-transformed epithelial cells (Huveneers et al., 2008).

In contrast to the situation in podosomes, the role of contractility-inducing proteins in invadopodium regulation is just beginning to be explored. Myosin II has been detected around some active invadopodia, but myosin is mostly absent from these structures. Still, invadopodial localization of other proteins involved in mechanotransduction, such as Cas or FAK, is sensitive to myosin inhibition (Alexander et al., 2008). Clearly, this is an area that needs further exploration.

Matrix degradation

Both podosomes (Burgstaller and Gimona, 2005; Osiak et al., 2005) and invadopodia (Yamaguchi et al., 2005; Bowden et al., 2006) can degrade components of the ECM. However, matrix degradation by podosomes tends to be shallow and widespread, whereas invadopodia show deeper and more focused degradation (see poster), probably as a result of the short lifetime and high abundance of podosomes, which contrasts with the long lifetime and low abundance of invadopodia per cell (Linder, 2007). ECM-degrading ability is conferred by the recruitment of lytic enzymes such as metalloproteinases or serine proteinases to invadosomes. Prominent members of these groups include the matrix metalloproteinases (MMPs) MT1-MMP, MMP-2 and MMP-9, which seem to be central to both podosome- (Sato et al., 1997; Delaissé et al., 2000; Guegan et al., 2008) and invadopodium- (Monsky et al., 1993; Nakahara et al., 1997; Redondo-Muñoz et al., 2006) based matrix degradation (for details, see Linder, 2007). ADAMs (disintegrin and metalloproteinase domain-containing proteins), which are members of another family of metalloproteinases, have also been localized to invadosomes, where they interact with β1 integrins (Seals and Courtneidge, 2003) or the adaptor Tks5 (Abram et al., 2003), although their exact role is currently unclear. So far, serine proteinases such as seprase or DPP4 have been observed only at invadopodia of cancer cells (Artym et al., 2006) or invadosomal structures in transformed fibroblasts (Ghersi et al., 2002), where they seem to be involved in ECM degradation. It remains to be determined whether this family of proteinases is also present at podosomes.

The pathways leading to enrichment of proteinases, and particularly of MMPs, at invadosomes are not well understood. However, they are most likely to involve microtubule-dependent trafficking of vesicles from the Golgi. Indeed, the Golgi is always observed in close proximity to invadopodia (Buccione et al., 2004; Ayala et al., 2006), and parts of the machinery for vesicle docking and fusion, such as the v-SNARE VAMP7 (Steffen et al., 2008) or the exocyst complex (Sakurai-Yageta et al., 2008), are required for MT1-MMP accumulation at invadopodia. In addition, MMP recruitment at invadosomes seems to involve parts of the actin cytoskeleton, most notably cortactin (Clark and Weaver, 2008).

Signaling between the matrix-degrading cell and the substratum might be quite complex. For example, blockage or knockdown of MMPs results in longer lifetimes of podosomes in osteoclasts (Goto et al., 2002), and leads to enhanced numbers of podosome-containing endothelial cells (Tatin et al., 2006) and reduced numbers of invadopodia in cancer cells (Steffen et al., 2008); all of these data point to a potential interdependence of matrix degradation and invadosome formation and/or maturation. Furthermore, podosome formation can also be induced by external cues such as the growth factors transforming growth factor-β (TGFβ) (Varon et al., 2006) and vascular endothelial growth factor (VEGF) (Osiak et al., 2005). Considering that growth factors can be bound to and locally released from the matrix (van Hinsbergh et al., 2006), the existence of highly localized feedback loops at matrix-degrading invadosomes seems likely.

Invadosomes in vivo?

Whether podosomes and invadopodia form in vivo, and what form they might take, are crucial unsolved issues. Osteoclast podosomes that form on a planar bone surface might closely resemble podosomes that are formed experimentally on glass or bone slices. By contrast, it might be that invadopodia of cancer cells, which move through a fibrillar matrix, more closely resemble filopodia or lamellipodia (Gimona et al., 2008).

The first hints that cells can form invadosomes in three-dimensional culture have come from the observation that smooth-muscle cells embedded in matrix form invadopodia-like protrusions over the whole cell surface

(Burgstaller and Gimona, 2005). Also, leukocytes undergoing transendothelial diapedesis have been shown to form protrusions that resemble invadosomes (Carman et al., 2007; Carman, 2009), although their relationship to podosomes and invadopodia is unclear. Finally, fibrosarcoma cells migrating through a fibrillar matrix form numerous lateral spikes, which contact matrix fibers and are enriched in MT1-MMP–GFP (Wolf and Friedl, 2009), making them good candidates for in vivo counterparts of invadopodia.

Obviously, these important issues can be best addressed by a concerted effort of various specialists. To this end, the Invadosome Consortium, a free and open network of laboratories that are interested in invadosomes, matrix degradation and tissue invasion, has now been established. For more information on this network and its activities, please visit the website (www.invadosomes.org).

Open questions, future challenges

During the last few years, the invadosome field has progressed substantially. However, there are still many open questions, several of which are surprisingly basic (Gimona et al., 2008). The genesis of invadosomes, for example, is still not well understood, and the signals that specify the exact subcellular position for their formation need to be explored further. Similarly, the relationship between podosomes in monocytic cells, invadosomes in transformed fibroblasts and invadopodia in carcinoma cells is still unclear. Are these structures distinct forms of cell-matrix contacts, or do they represent parts of a continuum of possible cellular responses to the ECM? Do podosomes and invadopodia share a common precursor, a kind of cellular ‘missing link’? Can they transform into each other upon proper stimulation? Contrary to common assumptions, it is also still unclear whether podosomes are indeed protrusive. The internal architecture of invadosomal structures is likely to depend on Arp2/3-induced actin filaments, but careful ultrastructural analysis is still missing. Finally, the influence of aspects of the ECM, such as substrate rigidity or release of growth factors, is just beginning to be explored. Moreover, the regulation [and eventual possibility of therapeutic modulation (Stylli et al., 2008)] of invadosome-dependent matrix degradation also needs to be studied in more detail. All of these points clearly illustrate the fact that, nearly 30 years after their initial description (David-Pfeuty and Singer, 1980), invadosomes still hold many challenges and surprises in store. The lively and still-growing field seems more than ready to meet them.

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References

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

Alexander, N. R., Branch, K. M., Parekh, A., Clark, E. S., Iwueke, I. C., Guelcher, S. A. and Weaver, A. M. (2008). Extracellular matrix rigidity promotes invadopodia activity. *Curr. Biol.* **18**, 1295-1299.

Artym, V. V., Zhang, Y., Seillier-Moiseiwitsch, F., Yamada, K. M. and Mueller, S. C. (2006). Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: defining the stages of invadopodia formation and function. *Cancer Res.* **66**, 3034-3043.

Ayala, I., Baldassarre, M., Caldieri, G. and Buccione, R. (2006). Invadopodia: a guided tour. *Eur. J. Cell Biol.* **85**, 159-164.

Badowski, C., Pawlak, G., Grichine, A., Chabadel, A., Oddou, C., Jurdic, P., Pfaff, M., Albiges-Rizo, C. and Block, M. R. (2008). Paxillin phosphorylation controls invadopodia/podosomes spatiotemporal organization. *Mol. Biol. Cell* **19**, 633-645.

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

Bowden, E. T., Barth, M., Thomas, D., Glazer, R. I. and Mueller, S. C. (1999). An invasion-related complex of cortactin, paxillin and PKCmu associates with invadopodia at sites of extracellular matrix degradation. *Oncogene* **18**, 4440-4449.

Bowden, E. T., Onikoyi, E., Slack, R., Myoui, A., Yoneda, T., Yamada, K. M. and Mueller, S. C. (2006). Co-localization of cortactin and phosphotyrosine identifies active invadopodia in human breast cancer cells. *Exp. Cell Res.* **312**, 1240-1253.

Buccione, R., Orth, J. D. and McNiven, M. A. (2004). Foot and mouth: podosomes, invadopodia and circular dorsal ruffles. *Nat. Rev. Mol. Cell Biol.* **5**, 647-657.

Buccione, R., Caldieri, G. and Ayala, I. (2009). Invadopodia: specialized tumor cell structures for the focal degradation of the extracellular matrix. *Cancer Metastasis Rev.* **28**, 137-149.

Burgstaller, G. and Gimona, M. (2005). Podosome-mediated matrix resorption and cell motility in vascular smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* **288**, H3001-H3005.

Burns, S., Thrasher, A. J., Blundell, M. P., Machesky, L. and Jones, G. E. (2001). Configuration of human dendritic cell cytoskeleton by Rho GTPases, the WAS protein, and differentiation. *Blood* **98**, 1142-1149.

Buschman, M. D., Bromann, P. A., Cejudo-Martin, P., Wen, F., Pass, I. and Courtneidge, S. A. (2009). The novel adaptor protein Tks4 (SH3PXD2B) is required for functional podosome formation. *Mol. Biol. Cell* **20**, 1302-1311.

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

Carman, C. V. (2009). Mechanisms for transcellular diapedesis: probing and pathfinding by ‘invadosome-like protrusions’. *J. Cell Sci.* **122**, 3025-3035.

Carman, C. V., Sage, P. T., Sciuto, T. E., de la Fuente, M. A., Geha, R. S., Ochs, H. D., Dvorak, H. F., Dvorak, A. M. and Springer, T. A. (2007). Transcellular diapedesis is initiated by invasive podosomes. *Immunity* **26**, 784-797.

Chabadel, A., Banon-Rodriguez, I., Cluet, D., Rudkin, B. B., Wehrle-Haller, B., Genot, E., Jurdic, P., Anton, I. M. and Saltel, F. (2007). CD44 and beta3 integrin organize two functionally distinct actin-based domains in osteoclasts. *Mol. Biol. Cell* **18**, 4899-4910.

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

Clark, E. S. and Weaver, A. M. (2008). A new role for cortactin in invadopodia: regulation of protease secretion. *Eur. J. Cell Biol.* **87**, 581-590.

Clark, K., Langeslag, M., van Leeuwen, B., Ran, L., Ryazanov, A. G., Figdor, C. G., Moolenaar, W. H., Jalink, K. and van Leeuwen, F. N. (2006). TRPM7, a novel regulator of actomyosin contractility and cell adhesion. *EMBO J.* **25**, 290-301.

Collin, O., Na, S., Chowdhury, F., Hong, M., Shin, M. E., Wang, F. and Wang, N. (2008). Self-organized podosomes are dynamic mechanosensors. *Curr. Biol.* **18**, 1288-1294.

Cortesio, C. L., Chan, K. T., Perrin, B. J., Burton, N. O., Zhang, S., Zhang, Z. Y. and Huttenlocher, A. (2008). Calpain 2 and PTP1B function in a novel pathway with Src to regulate invadopodia dynamics and breast cancer cell invasion. *J. Cell Biol.* **180**, 957-971.

David-Pfeuty, T. and Singer, S. J. (1980). Altered distribution of the cytoskeletal proteins vinculin and α-actinin in cultured fibroblasts transformed by Rous sarcoma virus. *Proc. Natl. Acad. Sci. USA* **7**, 6687-6691.

Delaisse, J. M., Engsig, M. T., Everts, V., del Carmen, O. M., Ferreras, M., Lund, L., Vu, T. H., Werb, Z., Winding, B., Lochter, A. et al. (2000). Proteinases in bone resorption: obvious and less obvious roles. *Clin. Chim. Acta* **291**, 223-234.

Deryugina, E. I., Ratnikov, B., Monosov, E., Postnova, T. I., DiScipio, R., Smith, J. W. and Strongin, A. Y. (2001). MT1-MMP initiates activation of pro-MMP-2 and integrin alphavbeta3 promotes maturation of MMP-2 in breast carcinoma cells. *Exp. Cell Res.* **263**, 209-223.

Destaing, O., Saltel, F., Geminard, J. C., Jurdic, P. and Bard, F. (2003). Podosomes display actin turnover and dynamic self-organization in osteoclasts expressing actin-green fluorescent protein. *Mol. Biol. Cell* **14**, 407-416.

Destaing, O., Saltel, F., Gilquin, B., Chabadel, A., Khochbin, S., Ory, S. and Jurdic, P. (2005). A novel Rho-mDia2-HDAC6 pathway controls podosome patterning through microtubule acetylation in osteoclasts. *J. Cell Sci.* **118**, 2901-2911.

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

Dorfleutner, A., Cho, Y., Vincent, D., Cunnick, J., Lin, H., Weed, S. A., Stehlik, C. and Flynn, D. C. (2008). Phosphorylation of AFAP-110 affects podosome lifespan in A7r5 cells. *J. Cell Sci.* **121**, 2394-2405.

Ghersi, G., Dong, H., Goldstein, L. A., Yeh, Y., Hakkinen, L., Larjava, H. S. and Chen, W. T. (2002). Regulation of fibroblast migration on collagenous matrix by a cell surface peptidase complex. *J. Biol. Chem.* **277**, 29231-29241.

Gimona, M. and Buccione, R. (2006). Adhesions that mediate invasion. *Int. J. Biochem. Cell Biol.* **38**, 1875-1892.

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

Goto, T., Maeda, H. and Tanaka, T. (2002). A selective inhibitor of matrix metalloproteinases inhibits the migration of isolated osteoclasts by increasing the life span of podosomes. *J. Bone Miner. Metab.* **20**, 98-105.

Guegan, F., Tatin, F., Leste-Lasserre, T., Drutel, G., Genot, E. and Moreau, V. (2008). p190B RhoGAP regulates endothelial-cell-associated proteolysis through MT1-MMP and MMP2. *J. Cell Sci.* **121**, 2054-2061.

Huveneers, S., Arslan, S., van de Water, B., Sonnenberg, A. and Danen, E. H. (2008). Integrins uncouple Src-induced morphological and oncogenic transformation. *J. Biol. Chem.* **283**, 13243-13251.

Kopp, P., Lammers, R., Aepfelbacher, M., Woehlke, G., Rudel, T., Machuy, N., Steffen, W. and Linder, S. (2006). The kinesin KIF1C and microtubule plus ends regulate podosome dynamics in macrophages. *Mol. Biol. Cell* **17**, 2811-2823.

Krits, I., Wysolmerski, R. B., Holliday, L. S. and Lee, B. S. (2002). Differential localization of myosin II isoforms in resting and activated osteoclasts. *Calcif. Tissue Int.* **71**, 530-538.

Linder, S. (2007). The matrix corroded: podosomes and invadopodia in extracellular matrix degradation. *Trends Cell Biol.* **17**, 107-117.
Linder, S. and Aepfelbacher, M. (2003). Podosomes: adhesion hot-spots of invasive cells. *Trends Cell Biol.* **13**, 376-385.
Linder, S. and Kopp, P. (2005). Podosomes at a glance. *J. Cell Sci.* **118**, 2079-2082.
Linder, S., Nelson, D., Weiss, M. and Aepfelbacher, M. (1999). Wiskott-Aldrich syndrome protein regulates podosomes in primary human macrophages. *Proc. Natl. Acad. Sci. USA* **96**, 9648-9653.
Linder, S., Hufner, K., Wintergerst, U. and Aepfelbacher, M. (2000a). Microtubule-dependent formation of podosomal adhesion structures in primary human macrophages. *J. Cell Sci.* **113**, 4165-4176.
Linder, S., Higgs, H., Hufner, K., Schwarz, K., Pannicke, U. and Aepfelbacher, M. (2000b). The polarization defect of Wiskott-Aldrich syndrome macrophages is linked to dislocalization of the Arp2/3 complex. *J. Immunol.* **165**, 221-225.
Lizárraga, F., Poincloux, R., Romao, M., Montagnac, G., Le Dez, G., Bonne, I., Rigaill, G., Raposo, G. and Chavrier, P. (2009). Diaphanous-related formins are required for invadopodia formation and invasion of breast tumor cells. *Cancer Res.* **69**, 2792-2800.
Luxenburg, C., Addadi, L. and Geiger, B. (2006). The molecular dynamics of osteoclast adhesions. *Eur. J. Cell Biol.* **85**, 203-211.
Monsky, W. L., Kelly, T., Lin, C. Y., Yeh, Y., Stetler-Stevenson, W. G., Mueller, S. C. and Chen, W. T. (1993). Binding and localization of M(r) 72,000 matrix metalloproteinase at cell surface invadopodia. *Cancer Res.* **53**, 3159-3164.
Mueller, S. C., Yeh, Y. and Chen, W. T. (1992). Tyrosine phosphorylation of membrane proteins mediates cellular invasion by transformed cells. *J. Cell Biol.* **119**, 1309-1325.
Mueller, S. C., Ghersi, G., Akiyama, S. K., Sang, Q. X., Howard, L., Pineiro-Sanchez, M., Nakahara, H., Yeh, Y. and Chen, W. T. (1999). A novel protease-docking function of integrin at invadopodia. *J. Biol. Chem.* **274**, 24947-24952.
Nakahara, H., Howard, L., Thompson, E. W., Sato, H., Seiki, M., Yeh, Y. and Chen, W. T. (1997). Transmembrane/cytoplasmic domain-mediated membrane type 1-matrix metalloprotease docking to invadopodia is required for cell invasion. *Proc. Natl. Acad. Sci. USA* **94**, 7959-7964.
Oikawa, T., Itoh, T. and Takenawa, T. (2008). Sequential signals toward podosome formation in NIH-src cells. *J. Cell Biol.* **182**, 157-169.
Ory, S., Brazier, H., Pawlak, G. and Blangy, A. (2008). Rho GTPases in osteoclasts: orchestrators of podosome arrangement. *Eur. J. Cell Biol.* **87**, 469-477.
Osiak, A. E., Zenner, G. and Linder, S. (2005). Subconfluent endothelial cells form podosomes downstream of cytokine and RhoGTPase signaling. *Exp. Cell Res.* **307**, 342-353.
Pfaff, M. and Jurdic, P. (2001). Podosomes in osteoclast-like cells: structural analysis and cooperative roles of paxillin, proline-rich tyrosine kinase 2 (Pyk2) and integrin alphaVbeta3. *J. Cell Sci.* **114**, 2775-2786.
Redondo-Muñoz, J., Escobar-Diaz, E., Samaniego, R., Terol, M. J., Garcia-Marco, J. A. and Garcia-Pardo, A. (2006). MMP-9 in B-cell chronic lymphocytic leukemia is upregulated by {alpha}4{beta}1 integrin or CXCR4 engagement via distinct signaling pathways, localizes to podosomes, and is involved in cell invasion and migration. *Blood* **108**, 3143-3151.
Sakurai-Yageta, M., Recchi, C., Le Dez, G., Sibarita, J. B., Daviet, L., Camonis, J., D'Souza-Schorey, C. and Chavrier, P. (2008). The interaction of IQGAP1 with the exocyst complex is required for tumor cell invasion downstream of Cdc42 and RhoA. *J. Cell Biol.* **181**, 985-998.
Saltel, F., Chabadel, A., Bonnelye, E. and Jurdic, P. (2008). Actin cytoskeletal organisation in osteoclasts: a model to decipher transmigration and matrix degradation. *Eur. J. Cell Biol.* **87**, 459-468.
Sato, T., del Carmen, O. M., Hou, P., Heegaard, A. M., Kumegawa, M., Foged, N. T. and Delaisse, J. M. (1997). Identification of the membrane-type matrix metalloproteinase MT1-MMP in osteoclasts. *J. Cell Sci.* **110**, 589-596.
Seals, D. F. and Courtneidge, S. A. (2003). The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* **17**, 7-30.
Seals, D. F., Azucena, E. F., Jr, Pass, I., Tesfay, L., Gordon, R., Woodrow, M., Resau, J. H. and Courtneidge, S. A. (2005). The adaptor protein Tks5/Fish is required for podosome formation and function, and for the protease-driven invasion of cancer cells. *Cancer Cell* **7**, 155-165.
Steffen, A., Le Dez, G., Poincloux, R., Recchi, C., Nassoy, P., Rottner, K., Galli, T. and Chavrier, P. (2008). MT1-MMP-dependent invasion is regulated by TI-VAMP/VAMP7. *Curr. Biol.* **18**, 926-931.
Stylli, S. S., Kaye, A. H. and Lock, P. (2008). Invadopodia: at the cutting edge of tumour invasion. *J. Clin. Neurosci.* **15**, 725-737.
Takenawa, T. and Suetsugu, S. (2007). The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* **8**, 37-48.
Tatin, F., Varon, C., Genot, E. and Moreau, V. (2006). A signalling cascade involving PKC, Src and Cdc42 regulates podosome assembly in cultured endothelial cells in response to phorbol ester. *J. Cell Sci.* **119**, 769-781.
Tsuboi, S., Takada, H., Hara, T., Mochizukj, N., Funyu, T., Saitoh, H., Terayama, Y., Yamaya, K., Ohyama, C., Nonoyama, S. et al. (2009). FBP17 mediates a common molecular step in the formation of podosomes and phagocytic cups in macrophages. *J. Biol. Chem.* **284**, 8548-8556.
Ussar, S., Wang, H. V., Linder, S., Fassler, R. and Moser, M. (2006). The Kindlins: subcellular localization and expression during murine development. *Exp. Cell Res.* **312**, 3142-3151.
van Helden, S. F., Oud, M. M., Joosten, B., Peterse, N., Figdor, C. G. and van Leeuwen, F. N. (2008). PGE2-mediated podosome loss in dendritic cells is dependent on actomyosin contraction downstream of the RhoA-Rho-kinase axis. *J. Cell Sci.* **121**, 1096-1106.
van Hinsbergh, V. W., Engelse, M. A. and Quax, P. H. (2006). Pericellular proteases in angiogenesis and vasculogenesis. *Arterioscler. Thromb. Vasc. Biol.* **26**, 716-728.
Varon, C., Tatin, F., Moreau, V., Obberghen-Schilling, E., Fernandez-Sauze, S., Reuzeau, E., Kramer, I. and Genot, E. (2006). Transforming growth factor beta induces rosettes of podosomes in primary aortic endothelial cells. *Mol. Cell. Biol.* **26**, 3582-3594.
Walker, V. G., Ammer, A., Cao, Z., Clump, A. C., Jiang, B. H., Kelley, L. C., Weed, S. A., Zot, H. and Flynn, D. C. (2007). PI3K activation is required for PMA-directed activation of cSrc by AFAP-110. *Am. J. Physiol. Cell Physiol.* **293**, C119-C132.
Weaver, A. M. (2006). Invadopodia: specialized cell structures for cancer invasion. *Clin. Exp. Metastasis* **23**, 97-105.
Wolf, K. and Friedl, P. (2009). Mapping proteolytic cancer cell-extracellular matrix interfaces. *Clin. Exp. Metastasis* **26**, 289-298.
Yamaguchi, H., Lorenz, M., Kempiak, S., Sarmiento, C., Coniglio, S., Symons, M., Segall, J., Eddy, R., Miki, H., Takenawa, T. et al. (2005). Molecular mechanisms of invadopodium formation: the role of the N-WASP-Arp2/3 complex pathway and cofilin. *J. Cell Biol.* **168**, 441-452.
Zambonin-Zallone, A., Teti, A., Grano, M., Rubinacci, A., Abbadini, M., Gaboli, M. and Marchisio, P. C. (1989). 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.* **182**, 645-652.

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