"Macrophages on the move: how podosomes contribute to immune cell invasion"

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Podosomes and invadopodia: organelles of invasive cells

For successful migration, invasive cells have to overcome many barriers, in particular the dense meshwork of interconnected fibres that makes up the extracellular matrix (ECM). Two different modes of migration present themselves: amoeboid migration, where cells squeeze through the holes between ECM fibres, and mesenchymal migration, with cells proteolytically degrading local ECM obstructions (1). In consequence, cells using the mescenchymal mode have to develop methods to fine-tune the local degradation of matrix material.

Podosomes and invadopodia, collectively called "invadosomes", are cell-matrix contacts with an inherent ability to lyse extracellular matrix material (2-5). This is achieved by localized release of matrix-lytic factors, especially proteases of the matrix metalloproteinase (MMP) family (6). Podosomes are mostly formed in a physiological context and have been described for monocytic cells such as macrophages, dendritic cells and osteoclasts, but also in endothelial cells and smooth muscle cells. Invadopodia are formed by several types of cancer cells and thus seem to contribute to a more pathological scenario (2-6). Both types of structures have attracted widespread attention during the last few years, and the invadosome field is progressing significantly, as evidenced by the ever-growing amount of publications and of labs joining the field (www.invadosomes.org).

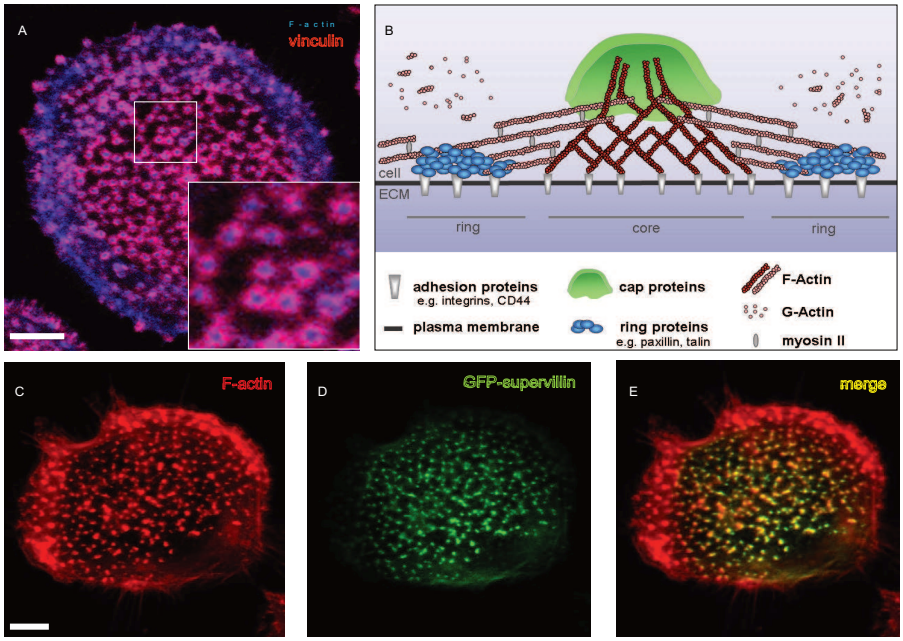

***Figure 1:* Podosome structure.** (A) Confocal micrograph of a primary human macrophage stained for F-actin (blue) and vinculin (red). Note that each actin-rich podosome core is surrounded by a vinculin-containing ring structure. Image by Petra Kopp. (B) Podosome model: a core of branched f-actin (dark red), surrounded by possibly unbranched actin filaments (light red) bundled by myosin II (dark grey), a cap structure on top of the actin core (green), and the surrounding ring structure (blue). Contact to the matrix is established by integrins and CD44 (light grey). A surrounding cloud of G- and F-actin is indicated above the podosome. Reprinted, with permission, from (5). (C-E) Podosome subpopulations show different molecular composition. Confocal micrograph of primary macrophage stained for F-actin (C), and expressing GFP-supervillin (D), with merge in (E). Note that large precursor podosomes in the cell periphery are virtually devoid of GFP-supervillin. Images by Susanne Cornfine. Bars in (A,C): 10 µm.

Podosomes in macrophages: multiple uses for the molecular toolbox

Podosomes in primary macrophages are a relevant and accessible system to study invadosome regulation for several reasons: i) primary macrophages show constitutive formation of podosomes, ii) they display high numbers of often up to 500 podosomes per cell, which allows statistical analysis, and iii) primary macrophages are unaltered, i.e. not immortalized, allowing the study of signal cascades in their practically pristine configuration.

Podosomes show a typical architecture: a core structure consisting of F-actin and actin-associated proteins such as WASP, Arp2/3, gelsolin or cortactin, and a ring structure of plaque proteins such as talin, vinculin, paxillin or zyxin (2). Recent findings also demonstrate the presence of a cap structure on top of the actin core, which contains the formin FMNL-1 (7) or the membrane-associated protein supervillin (8), and

several other proteins (P.Cervero and S. Linder, unpublished). This structure may regulate podosome growth or could function as a hub for incoming vesicels (Figure 1).

The ultrastructure of podosomes is currently under intensive investigation, and recent evidence hints at the existence of a layer of unbranched actin filaments that surround the branched network of the core. This would also be in line with the detection of myosin around the core (9,10), and the involvement of actomyosin-dependent contractility in both mechanosensing by podosomes (see below) and turnover of the podosome structure itself (11,12). Actomyosin cables also run between podosomes and thus connect individual structures into a higher-ordered group. This actomyosin-generated tug-of-war may also explain the striking regularity of the podosome pattern in cells and help to coordinate net movement of a field of podosomes.

Podosomes are multifunctional organelles that combine several key features. First, they most probably function as adhesive hotspots of cells, as they are enriched in adhesion-promoting proteins such as integrins (4,13,14) or CD44 (Chabadel et al., 2007). TIRF microscopy also revealed close contact of podosomes to the underlying substratum (15). A second key feature is the ability to locally degrade the extracellular matrix (see below). A third intriguing feature is the ability for mechanosensing. Podosomes can detect traction forces, and both spacing and lifetime of podosomes is modulated by matrix rigidity (16,17). Again, the actomyosin system seems to be critical for this ability. A fourth, and mostly speculative, function concerns the possible role of podosomes in providing adhesion points within newly established protrusions, thus supporting the directional migration of cells. Further experiments will be needed to determine whether this ability can be added to the growing repertoire of these multipurpose organelles.

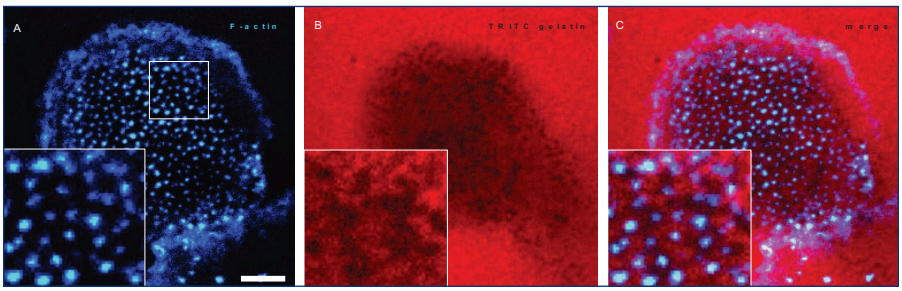

***Figure 2:* Podosomes are sites of extracellular matrix degradation.** Confocal micrographs of a primary human macrophage, stained for F-actin (A; blue), seeded on fluorescently labeled gelatin matrix (B; red). Sites of matrix degradation are visible as black defects and mostly coincide with podosomes (C). White box in (A) indicates detail images shown as insets. White bar: 10 µm. Images by Christiane Wiesner.

All podosomes are equal – but some are more equal than others

In several systems, podosomal structures can be induced by overexpression of active RhoGTPases such as $CDC42^{V12}$ (18), stimulating PKC pathways by adding phorbol esters (19), or transformation with oncogenes such as Src (20). While these are highly effective treatments, more upstream regulators that induce maturation or diversification of the structure may thus be bypassed. In fact, one of the most intriguing features of cells that show constitutive podosome formation is the existence of podosome subpopulations. In primary human macrophages, at least two subpopulations exist that show distinct characteristics such as size, lifetime, dynamics and subcellular localization. Larger podosomes at the cell periphery or the leading edge of migrating cells, called precursors, have

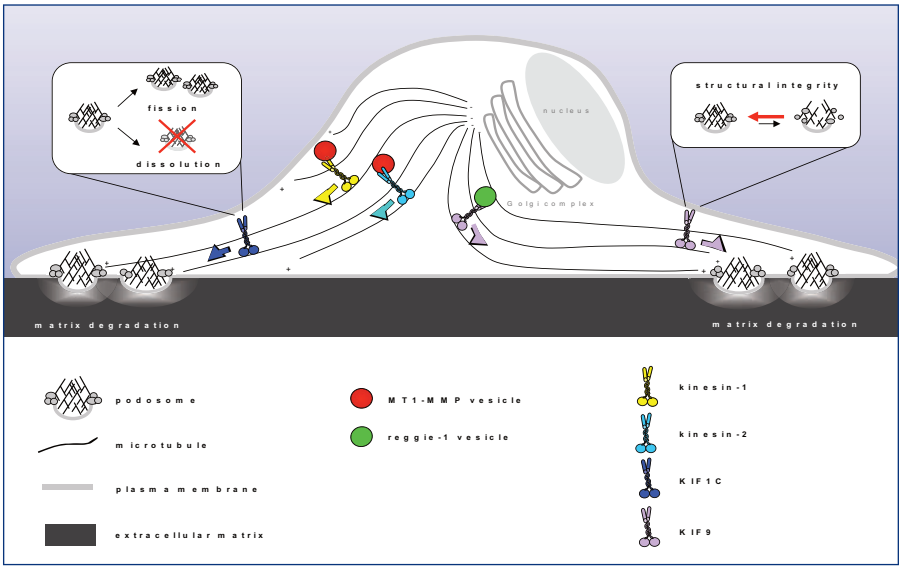

***Figure 3:* Regulation of podosomes by microtubule-dependent transport.** Microtubules contact podosomes with their plus ends. Plus end–directed kinesin motors transport cargo vesicles to podosomes, which influence podosome structure and function. Kinesin-1 and kinesin-2 transport MT1-MMP-positive vesicles that probably influence matrix degradation. KIF1C carries as yet unidentified cargo and regulates the dynamics of precursor podosomes. KIF9 transports vesicles positive for reggie-1/flotillin-2 and influences matrix degradation, while another, as yet unidentified cargo of KIF9 supports the structural integrity of podosomes. Modified from (5), and reprinted with permission.

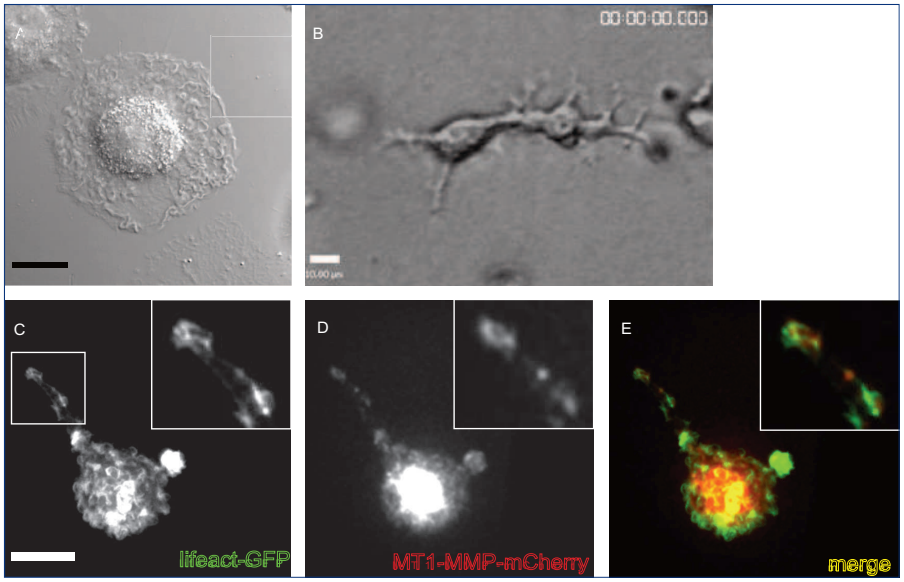

***Figure 4:* Macrophages in in 2D and 3D.** (A, B) Macrophages change their morphology in 3D. (A) Brightfield micrograph of macrophage seeded on glass coverslip. Note central dome-shaped part containing the nucleus and most of the cytoplasm, and surrounding thin layer forming multiple ruffles. Image by Mirko Himmel. (B) Still images from time lapse movie of macrophages embedded in gelled collagen I. Note numerous finger-like protrusions. (C-E) Confocal micrographs of a primary human macrophage embedded in gelled collagen I, and overexpressing lifeact-GFP, detecting F-actin (C) and MT1-MMP-mCherry (D), with merge shown in (E). Note the absence of typical podosomes, but the formation of F-actin rich clusters at cell protrusions, that also contain MT1-MMP-mCherry. White box indicates area of detail images. Bars: 10 µm. Images by Christiane Wiesner.

been shown to turn over quickly and also split off daughter podosomes that move into the more inner regions of the cell (21). This process is influenced by contact of microtubule plus ends (9). The population of smaller podosomes, called successors, do not show fission processes and are interconnected by a meshwork of contractile actomyosin cables. Local myosin contractility at podosomes is controlled by supervillin, a member of the villin family, that binds both contractile myosin and myosin light chain, which leads to further stimulation of myosin activity, and thus induces a feed-forward cycle of increasing actomyosin contraction resulting in podosome dissolution (8). Interestingly, also precursor podosomes acquire supervillin and myosin prior to their dissolution. Collectively, these findings indicate that podosome subpopulations in macrophages differ in their molecular makeup, and that their composition alters during their lifecycle. Further molecular differences between podosome subpopulations that fine-tune podosome architecture and function are to be expected.

Breaching the matrix: proteolytic degradation at podosomes

Degradation of extracellular matrix material is one of the hallmarks and defining features of podosomes (6). It also helps to discriminate between podosomes and other actin-rich structures of cells. Classically, matrix degradation is demonstrated by seeding cells on fluorescently labeled matrix. Sites of local ECM degradation then appear underneath podosomes, as the matrix is degraded and the label is lost (Figure 2). Podosomes are cellular all-purpose weapons that can attack a large variety of ECM materials such as fibronectin, collagen or gelatin. They do this by locally concentrating and releasing ECM-lytic factors, most notably proteins of the matrix metalloproteinase family (MMP) such as MT1-MMP, but also other metalloproteinases such as ADAMs (6,18,20). Accumulation of proteinases at podosomes is probably achieved through microtubule-dependent trafficking (see below). For example, MT1-MMP has been demonstrated to travel along microtubules towards podosomes in vesicles that are powered by kinesin-1 and kinesin-2 (22). However, the fine-tuning of this process, the regulatory molecules and the podosome substructures that are involved, are still to be determined. An intriguing question is also how onset and cessation of matrix degradation at podosomes is timed, and whether all podosomes are able to degrade ECM in equal measure and at all times.

Podosome traffic: a busy hub for intracellular transport

Due to their composition, podosomes depend on actin-regulatory processes. However, it is increasingly apparent that podosomes are also influenced by other parts of the cytoskeleton, and particularly by microtubules and microtubule-dependent trafficking. For example, intact microtubules are necessary for the formation of podosomes (23), and live cell imaging has demonstrated the dynamic contact of microtubule plus ends with podosomes. This contact also influences the dynamics of podosome precursors, suggesting the delivery of regulatory factors along microtubules to podosomes (9). Consistently, a variety of motor proteins have been identified that regulate different aspects of podosome dynamics and function: kinesin-1 and kinesin-2 are important for the delivery of the key metalloproteinase MT1-MMP to podosomes (22), the kinesin KIF1C regulates the fission rates of podosome precursors (9), and the kinesin KIF9 has emerged as an important regulator of both podosome stability and matrix degadration (24). It seems that podosomes act as subcellular hubs that coordinate an intricate and highly dynamic cargo delivery system (Figure 3). It will be highly interesting to determine how this system

is fine tuned to ensure correct and timely delivery of both podosome components and regulatory factors.

More than a special effect: podosomes in 3D

So far, podosomes and invadopodia have been studied mostly on 2 dimensional, artificial surfaces. This may be relevant, as also in some physiological situations flat, 2 dimensional interfaces are likely to exist between the matrix and podosome forming cells (5). These include contact of monocytic cells with vessel walls, of endothelial cells with the basement membrane and of osteoclasts with the bone surface. In these situations, cells most likely form podosome structures that are similar to their *in vitro* cousins.

However, cells embedded in 3 dimensional environments show drastic alterations of their morphology and behaviour (Figure 4). Consequently, it is to be expected that also podosomes and related structures, if they indeed exist within tissues, have altered appearances. Initial experiments with macrophages embedded in 3D gelled collagen show that cells lose their typical "fried egg" appearance and display a central body that forms multiple and highly dynamic extensions. Intriguingly, these extensions often end in dot-like, actin-rich accumulations that also contain typical podosomal proteins such as cortactin or vinculin. Moreover, they also accumulate proteases such as MT1-MMP (Figure 4), a prerequisite for local matrix degradation. Further studies are necessary to determine if these structure are indeed degrading matrix material and can thus be identified as 3D equivalents of 2D podosomes.

Conclusions

Podosomes are a, if not *the*, major feature of the actin cytoskeleton of macrophages. These cells invest considerable resources in the formation, upkeep and highly fine-tuned turnover of podosomes. Not unwisely so, as podosomes are multipurpose organelles that combine several key abilities of macrophages, including adhesion, localized matrix degradation and mechanosensing. They should thus be instrumental for macrophages in gathering information about the environment, adhering to suitable surface features, and paving the way during invasive migration. Current research activities point to the existence of equivalent structures also in 3D situations, underlining the likely in vivo relevance of podosomes. Looking back, podosomes have come a long way from their discovery in the 1980s, when they were mostly regarded as "funny curiosities". It is to the credit of pioneers like Pier Carlo Marchisio that we are now able to see and study these structures as relevant, multifunctional and integral parts of cells.

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Stefan Linder studied Biology at the Ludwig Maximilians University in Munich. He gained his PhD in the lab of Manfred Schliwa (Institute for Cell Biology, LMU), where he became fatally interested in the cytoskeleton. For a postdoc, he moved to the lab of Martin Aepfelbacher (Institute for Cardiovascular Diseases, LMU), where he stumbled upon podosomes and got hooked ever since. He is now professor for Cellular Microbiology at the University Medical Center Eppendorf (UKE). He holds positions as an editor of *European Journal of Cell Biology*, co-president of the *Invadosome Consortium* (www.invadosomes.org), and coordinator of the EU-FP7 programme *Tissue Transmigration Training Network* (T3Net; www.t3net-itn.org). His lab studies cytoskeletal regulation in primary human cells, especially macrophages and endothelial cells, in the context of adhesion, migration, invasion and phagocytosis.