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Abstract (182 words)

Invadosomes, which encompass podosomes and invadopodia, are actin rich adhesive and protrusive structures facilitating invasion and migration in various cell types. Podosomes are mostly found in normal cells, while invadopodia are hallmarks of invasive transformed cells. Despite evident structural differences, both structures mostly rely on the same pathways for their formation and their activity. While the role of actin cytoskeleton is undeniable, the involvement of microtubules (MTs) in invadosome formation/activity has recently been demonstrated but also somehow underestimated. MTs are components of the eukaryotic cytoskeleton well known for their essential roles for cell division, the maintenance of cell shape, intracellular transport and cell motility. Until now, MTs were mostly seen as railways for the delivery of various cargos required for invadosome functions but recent data suggest a more complex role. In this review, we address the specific functions of MTs on invadosome dynamics, activity, maturation and organization in light with recent data, which extended far beyond simple track delivery. Indeed, MT dynamic instability, which in turn modulates Rho GTPase signalling and likely MT post-translational modifications are playing major roles in invadosome functions.

Keywords: invadosome, podosome, invadopodia, microtubule, anchoring, kinesins, RhoA.

Introduction

Podosomes and invadopodia, collectively called invadosomes, are adhesive actin-enriched membrane structures protruding at the ventral side of cells when grown in 2D, which can have extracellular matrix (ECM) degradation properties. The term podosome is used to define the structure found in normal cells (e.g. monocytic cells, megakaryocyte, endothelial cells, smooth muscle cells) while in invasive cancer cells and in Src-transformed fibroblasts organised into rosettes it is called *invadopodium* (Fig.1). Invadosomes are essential for processes involving cells to cross tissue barriers such as cell transmigration for immune cells in physiological conditions but also for pathological processes such as dissemination of cancer cells during metastasis. Despite sharing several similarities, invadosomes present major differences. Indeed, podosomes have a diameter of around 1 µm and a height of 0.5 µm whereas invadopodia can reach 8 µm in diameter and 5 µm in depth suggesting a more aggressive matrix degradation in this latter. The dynamics of both structures as well as their abundance in cells are also significantly different. While invadopodia can last hours, podosome lifetime is usually in the range of minutes (Linder et al., 2011; Paterson and Courtneidge, 2018). Furthermore, podosomes and invadopodia rely on different signalling proteins and inputs (Hoshino et al., 2013). For example, the scaffold protein Tks5 is characteristic and essential for signalling pathway leading to invadopodia formation (Eddy et al., 2017). Finally, a ring containing adhesive molecules, such as integrins or vinculin, surrounds the F-actin core of podosomes (Fig. 1A). The presence of such a ring structure in invadopodia is apparently conserved (Branch et al., 2012; Pignatelli et al., 2012) (Fig. 1B).

Among the similarities, podosomes and invadopodia are both F-actin rich dot-like structures involving actin polymerisation, mostly mediated by the Arp2/3 complex and formins, and adhesive molecules (Linder et al., 2011; van den Dries et al., 2019) (Fig. 1). The role of actin

cytoskeleton in invadosome activity has been addressed in various excellent reviews to which readers can refer (Kedziora et al., 2016; Linder et al., 2011; van den Dries et al., 2019). Briefly, the core of podosomes is composed by branched actin filaments contacting adhesion molecules such as CD44 in osteoclasts (Chabadel et al., 2007). This branched network is surrounded by unbranched actin filaments bundled by myosin II linked to adhesion molecules such as integrins through ring proteins containing, among others, talin and vinculin (Chabadel et al., 2007; Linder et al., 2011). Finally, these actin networks are covered by cap proteins comprising for example the formin FMNL-1 (Mersich et al., 2010; van den Dries et al., 2019) (Fig. 1A). Branched and unbranched actin filaments are also found in invadopodia. Indeed, the base contains a branched network potentially linked to ring proteins and adhesion molecules (Branch et al., 2012; Pignatelli et al., 2012). A similar interaction is also possibly present at the side of invadopodia (Beaty et al., 2014; Proszynski and Sanes, 2013) (Fig. 1B). Finally, bundled actin filaments are found at the tip of the invadopodium (Schoumacher et al., 2010) (Fig. 1B). Besides the crucial role played by actin cytoskeleton, invadosomes are also influenced by microtubules (MTs) (Linder et al., 2011). For a long time according to most of the publications in the field, the function of MT on invadosome activity was restricted to tracks for the delivery of cargos. However, this implies the targeting or capture of MTs to invadosomes, a process which starts to be well documented. Furthermore, recent data demonstrated that MTs also have signalling functions regulating the formation of podosomes and potentially invadopodia.

In this review, we would like to focus on the specific functions of MTs on invadosome dynamics, activity and organisation in light with recent data.

MTs are key structural elements of invadosomes

MTs are hollow tube polymers formed by the non-covalent association of αβ-tubulin heterodimers. They are involved in a number of essential functions such as cell division, cell shape maintenance, intracellular transport, and cell motility. MTs are polarised polymers with minus ends (–ends) anchored to microtubule-organising centres, centrosome and the Golgi apparatus, where they are stabilised. At the opposite, the plus ends (+ends) radiating from those structures towards the cell periphery are highly dynamic switching between growing and shrinking phase defining the so-called dynamic instability of MTs. The flexibility of MTs to adapt to diverse functions relies on the spatiotemporal regulation of their dynamic instability and organisation, which depends on the specific recruitment of microtubule-associated proteins (MAPs) (Bodakuntla et al., 2019; Bowne-Anderson et al., 2015). Tubulin isotypes as well as posttranslational modifications (PTMs) are thought to play a major role in regulating the binding of MAPs necessary for locally adapting MT functions (Janke and Magiera, 2020; Roll-Mecak, 2019). Indeed, sequences in tubulin isotypes mainly diverge at the level of C-terminal tails of both α- and β-tubulin, which is the siege of most PTMs such as polyglutamylation and detyrosination. Furthermore, tubulin C-terminal tails, which are exposed at the surface of the polymer, are involved in the binding and/or the regulation of MAP activity (Bodakuntla et al., 2019); conversely, MAPs influence MT dynamics and molecular motors. Thus, tubulin isotypes and PTMs define the so-called "tubulin code" translated into a specific function by the spatiotemporal recruitment of MAPs. Acetylation of lysine 40 on α-tubulin represents a particular PTM. Indeed, this modification does not occur on an exposed residue but buried within the MT lumen (Janke and Magiera, 2020; Roll-Mecak, 2019). Whether MT acetylation regulates MAP binding is unclear but it protects MT against mechanical breakage (Xu et al., 2017).

In interphasic, differentiated or non-dividing cells, MTs are best known as "cellular railways" for various cargos made of vesicles, proteins or nucleic acids. Thus, MTs are essential for

intracellular vesicular trafficking between organelles and also to or from the plasma membrane. The transport of cargos on MT is mediated by a specific set of MAPs: the molecular motors kinesins and dynein (Sweeney and Holzbaur, 2018).

The interplay between actin cytoskeleton and MTs plays a major role in the regulation of invadosome activity. Such interplay between both cytoskeletons has been particularly well documented on focal adhesions. Indeed, while MT depolymerisation induces focal adhesion maturation, MT regrowth has the opposite effect and rely, in part, on the activation of small GTPases, respectively RhoA and Rac (Ren et al., 1999; Waterman-Storer et al., 1999). While invadosomes and focal adhesions are both adhesive structures, the role of MTs seems to be different. Indeed, MT depolymerisation or inhibition of MT dynamics using MT-interfering drugs, such as taxol or nocodazole, inhibits podosome formation in macrophages or podosome organisation in vascular smooth muscle cells (VSMCs) treated with phorbol ester and in osteoclasts (Biosse Duplan et al., 2014; Destaing et al., 2003; Efimova et al., 2014; Evans et al., 2003; Linder et al., 2000; Rafiq et al., 2019). Similarly, MT destabilisation also affects invadopodia activity (Revach et al., 2015; Schoumacher et al., 2010). A β-tubulin specific isotype was recently shown to be required for podosome organisation in osteoclasts likely by regulating MT dynamics (Guérit et al., 2020). Those data demonstrated the crucial role of MTs on invadosome integrity.

Regulation of invadosomes requires MT anchoring .

To be properly delivered, cargos have to be assigned to the right destination, meaning that MTs have to be targeted to invadosomes. Accordingly, MTs have been observed in close vicinity of podosomes in various cells (Akisaka et al., 2011; Linder et al., 2000) but also engaged into invadopodia (Schoumacher et al., 2010). Many aspects of invadosome behaviour are expected to be regulated by MTs considering the variety of cargos transported on MTs.

Thus, MTs are essentials for *de novo* podosome formation (Linder et al., 2000; Rafiq et al., 2019) but also for podosome dynamics (fusion and fission) and localisation in a variety of cellular models (Biosse Duplan et al., 2014; Destaing et al., 2003; Efimova et al., 2014; Evans et al., 2003; Kopp et al., 2006; McMichael et al., 2010). As a consequence, MTs regulate the ECM-degradation activity of podosomes (Linder et al., 2011). Similarly, invadopodium maturation and activity rely on an intact MT network (Revach et al., 2015; Schoumacher et al., 2010).

A subclass of MAPs, the +end tracking proteins (+TIPs) play a key role in MTs capture to the cell cortex, focal adhesions and podosomes (Noordstra and Akhmanova, 2017; Seetharaman and Etienne-Manneville, 2019). Indeed, the +TIPs, EB1 and the cytoplasmic linker associated proteins (CLASPs), are essential for the organisation and the dynamics of podosomes in osteoclasts and VSMCs (Biosse Duplan et al., 2014; Efimova et al., 2014). Through the interaction with the cortical protein LL5β, a PIP3 binding protein, CLASPs associate with the cortical MT stabilizing complexes (CMSCs) essential for MTs targeting (Noordstra and Akhmanova, 2017). The CMSCs are composed by several scaffold proteins, liprin-α1 and -β1, ELKS and KANK1. KANK1, through the binding of talin a major component of focal adhesion, and LL5β, through CLASPs interaction, allow MT capture to focal adhesion but also to podosomes (Fig. 2). CLASPs acting as an anti-catastrophe factor promote MT stabilisation around adhesive structures. This activity is likely reinforced by the recruitment of KIF21A, a member of the kinesin-4 family, through KANK1 binding, which inhibits both MT growth and catastrophe (van der Vaart et al., 2013). It is not yet known whether a similar mechanism is involved in MT capture in invadopodia. Nevertheless, talin has been observed within invadopodia (Beaty et al., 2014). Furthermore, Amotl2, a LL5β interactor, induces invodopodia disruption upon depletion (Proszynski and Sanes, 2013). Altogether these data suggest a possible recruitment of CMSCs into invadopodia. Accordingly, liprin-α1, ELKS

and LL5β were recently shown to be required for invadopodia activity and motility in human breast cancer MDA-MB-231 cells (Sala et al., 2018).

MT +end capture at invadosomes required for the delivery of cargos may also involved myosin X, an unconventional myosin, which binds actin, MT and integrins (Sousa, 2006). Indeed, myosin X is a critical regulator of podosome patterning and for sealing zone motility and activity in mouse osteoclasts (McMichael et al., 2010). For efficient bone resorption, podosomes from osteoclasts have to be organised into specific structures called podosome belt or sealing zone, when cultured on bone slices or mineral matrix, which rely on the reorganisation of podosome rings (Luxenburg et al., 2007; Ma et al., 2008; Maurin et al., 2018; Saltel et al., 2004; Takito et al., 2018).

Finally, the cytoskeletal scaffolding protein IQGAP1 might also be involved in MT +end anchoring. IQGAP1, known to link tubulin and actin cytoskeleton networks, is indeed essential for invadopodia formation and activity (Sakurai-Yageta et al., 2008). IQGAP1 is recruited to invadopodia in a β1-integrin and ILK dependent manner (Branch et al., 2012) and interacts with the +TIP protein CLIP-170 (Fukata et al., 2002), which may allow MT tethering to invadopodia.

Altogether, these proteins link MTs and invadosomes establishing the railways for cargo delivery necessary for invadosome formation, maturation, dynamics, localisation and activity as addressed into the next section.

MTs allow the vesicular trafficking of essential cargos to invadosomes.

Once anchored to invadosomes MTs are used for cargo delivery which involves molecular motors: kinesins and dynein. The first kinesin demonstrated to be involved in invadosome function was KIF1C, a member of the kinesin-3 family (Kopp et al., 2006). MT +end contacting podosomes has been shown to influence their cellular fate in primary human

macrophages (Kopp et al., 2006). KIF1C was identified as a MT +end enriched kinesin that regulates podosome turnover. Depletion of KIF1C or expression of an ATP-binding mutant induces a strong decrease in the number of podosomes (Kopp et al., 2006). KIF1C accumulates to podosomes induced by phorbol ester in VSMCs in a CLASP-dependent manner (Efimova et al., 2014). Thus, CLASPs may recruit KIF1C to growing MT +ends and promote podosome formation. The nature of the essential podosome component transported by KIF1C is unknown but integrins were identified as KIF1C cargos (Theisen et al., 2012) (Fig. 2). Finally, the CLASP/KIF1C couple is also involved in phorbol ester induced podosome dynamics and their relocation from cellular periphery to the centre in VSMCs (Zhu et al., 2016). Podosome relocation involves MT bending at the cortex in a CLASP-dependent manner and likely involving CSMCs (Zhu et al., 2016). Interestingly, bended MTs which grow above podosomes and form a dense circular network are observed in osteoclasts at the level of the podosome belt (Okumura et al., 2006). Accordingly, KIF1C was recently shown to be involved in podosome patterning and bone resorption in osteoclasts (Kobayakawa et al., 2019).

The formation and activity of invadosomes also require the local deposition of new membrane and the delivery of specific proteases involved in ECM degradation. Among the many extracellular proteases expressed in mammalian cells, matrix metalloproteinases (MMPs) have been identified as crucial enzymes, in particular MT1-MMP, involved in matrix degradation by invadosomes (Linder et al., 2011). MT1-MMP is a transmembrane protease cleaving most of ECM components but also cell-surface proteins such as CD44, αV-integrin and other MMPs (Poincloux et al., 2009). The vast majority of MT1-MMP is provided by the recruitment of intracellular storage compartments to invadosomes, which depends on an intact MT network (Poincloux et al., 2009; Remacle et al., 2005). The kinesins KIF5B and KIF3A/KIF3B as well as dynein are involved in the bidirectional movement of MT1-MMP

stored in Golgi-derived vesicles along MTs in primary human macrophages (Wiesner et al., 2010). Thus, aforementioned molecular motor allow the exposure of MT1-MMP at the cell surface and local ECM degradation in the vicinity of podosomes (Fig. 2). Similar molecular motors were shown to be involved in surface delivery of MT1-MMP to invadopodia (Castro-Castro et al., 2016; Infante et al., 2018; Marchesin et al., 2015). In addition, the local delivery of MT1-MMP also relies on the exocytosis of recycling vesicles involving the exocyst complex (Sakurai-Yageta et al., 2008). IQGAP1, which potentially tethers MTs to invadopodia through binding to CLIP-170 (Fukata et al., 2002), interacts with a component of the exocyst complex (Sec8) and promotes the focal delivery of MT1-MMP required for invadopodia formation (Sakurai-Yageta et al., 2008). Finally, MMP2 and MMP9, two other metalloproteinases involved in ECM degradation, are addressed to invadopodia in a Rab40b- and MT-dependent manner (Jacob et al., 2013; Schnaeker et al., 2004)

As mentioned, invadosome formation and potentially activity require new membrane deposition. The kinesin KIF9 has been shown to regulate podosome formation and activity in human primary macrophages. Interestingly, KIF9 interacts with flotillin proteins, which are involved in the delivery of membrane and associated proteins from an internal pool to specific sites at the plasma membrane (Cornfine et al., 2011; Otto and Nichols, 2011). Depletion of flotillins impaired the ability of podosomes to degrade the extracellular matrix (Cornfine et al., 2011), suggesting that the delivery of membrane materials is required for podosome activity (Fig. 2). The involvement of KIF9 in invadopodia formation or activity is unknown. However, flotillins were recently shown to be involved in the regulation of MT1-MMP endocytosis, which is crucial for its exocytosis at degradation sites (Planchon et al., 2018).

MTs emerge as an essential signalling platform for invadosome function.

MTs not only regulate invadosome formation, dynamics and activity through the local delivery of vesicles but are also involved in signalling pathways. As mentioned, disruption of MTs leads to an increased size and number of focal adhesions (Bershadsky et al., 1996; Enomoto, 1996) while a similar treatment promotes a rapid disassembly of podosomes in macrophages (Linder et al., 2000; Rafiq et al., 2019) and disorganisation in osteoclasts (Destaing et al., 2005). The MT capture, through KANK proteins, to those adhesive structures has recently been shown to be responsible of such differences. Indeed, KANK depletion, which promotes MTs dissociation from focal adhesions and podosomes, leads to an increase in the activity of the GTPase RhoA. Active RhoA induces the assembly of myosin IIa filaments through the activation of the kinase ROCK (Rafiq et al., 2019). While focal adhesion integrity requires acto-myosin-dependent traction forces, podosome integrity does not and is disrupted when subjected to such forces (Rafiq et al., 2019). Thus, the consequences of RhoA activation are opposite but rely on the same signalling pathway. Indeed, when MTs are anchored to integrin-mediated adhesive structures through KANK proteins, the RhoA exchange factor GEF-H1 is trapped at their surface and inactive (Rafiq et al., 2019). Upon disconnection, GEF-H1 is released from MTs and active towards RhoA, which drives the assembly of myosin IIa filaments (Rafiq et al., 2019) (Fig. 2). Therefore, MTs regulate RhoA signalling pathways depending on their anchoring to focal adhesions or podosomes.

Tight control of RhoA activity is also required for podosome patterning in osteoclasts (Destaing et al., 2005; Gil-Henn et al., 2007; McMichael et al., 2014). The GTPase RhoA negatively regulates podosome belt integrity by inhibiting a MT post-translational modification (Fig. 2). Indeed, acetylation of lysine 40 on α-tubulin increases during osteoclast differentiation and was thus proposed to be involved during this process (Destaing et al., 2005). Acetylated MTs present an increased mechanical resilience allowing their persistence

likely explaining why they are associated with MT stability (Janke and Magiera, 2020; Roll-Mecak, 2019; Xu et al., 2017). This increase of MT acetylation in osteoclasts might be a consequence of MT stabilisation due to gradual MT anchoring to podosomes. When activated, RhoA not only induces the formation of myosin IIa filaments (Rafiq et al., 2019), but also promotes α-tubulin deacetylation by HDAC6 activated by mDia2 (Destaing et al., 2005) (Fig. 2). The induction of MT deacetylation is associated with the disruption of podosome belt (Destaing et al., 2005; Guimbal et al., 2019; Matsumoto et al., 2013; Zalli et al., 2016). Therefore, MT acetylation is required for stabilising podosomes within the belt in osteoclasts. This is consistent with the accumulation of acetylated MTs in the vicinity of the podosome belt in osteoclasts (Akisaka et al., 2011). Thereby in osteoclasts, proteins, which negatively regulate RhoA activity, have the potential to be involved in resorption by stabilising the podosome belt. These include for example the tyrosine kinase Pyk2 (Gil-Henn et al., 2007) and the Rho GAPs Myosin 9b (McMichael et al., 2014) and Arap1 (Segeletz et al., 2018). The role of RhoA in invadopodium function is not clear but available data suggest the requirement of a tight regulation of this GTPase (Sedgwick et al., 2015; Yan et al., 2018).

Acetylated MTs were also shown to regulate podosome formation in macrophages. In this model, increased MT acetylation reduces podosome number, correlated with a reduction of MT targeted to podosomes and compromised KIF1C motility (Bhuwania et al., 2014). This latter suggests that MT acetylation may influence trafficking of vesicles or protein cargos by regulating the association of MTs with molecular motors. In agreement, the modulation of acetylation regulates the movement of MT1-MMP containing vesicles and also matrix degradation by invadopodia in breast tumour cells (Castro-Castro et al., 2012). However, an involvement of cortactin acetylation in those processes cannot be excluded (Castro-Castro et al., 2012). Similarly, acetylated cortactin could be involved in podosome belt formation in osteoclasts (Biosse Duplan et al., 2014; Zalli et al., 2016).

Future prospects

As mentioned earlier alternative mechanisms have been proposed to anchor MT to invadosomes, such as CLIP170/IQGAP1 (Fukata et al., 2002). However their impact on RhoA activity, potentially through the regulation of GEF-H1, and its targets are unknown and might deserve further investigations. Furthermore, in addition to the acetylation of lysine 40 on α-tubulin, MTs are subjected to various other PTMs such as detyrosination or polyglutamylation known to regulate MAP binding or molecular motor activities (Janke and Magiera, 2020; Roll-Mecak, 2019). It would thus be of interest to investigate the involvement of such PTMs in invadosome formation, organisation and activity. Finally, in addition to PTMs, MT functions may be regulated by their tubulin composition. Tubb6 was recently shown to be required for the formation/stabilisation of the podosome belt in osteoclast likely through the regulation of MT dynamics and/or the binding of MAPs (Guérit et al., 2020). Interestingly, Tubb3 expression is correlated with invasion in some cancer cells (Parker et al., 2017). Similarly to Tubb6, Tubb3 could positively regulate invadopodia activity.

Conclusion

It is now becoming obvious that MTs do not only act as railways for cargos regulating invadosome formation and dynamics. MT anchoring to adhesive structures is crucial for this latter and relies on the regulation of MT dynamic instability, which in turn modulates Rho GTPase signalling and likely MT post-translational modifications. MT capture at the level of invadosome through KANK proteins negatively regulates GEF-H1 activity towards RhoA and thus promoting podosome formation. Preventing RhoA activation likely favours the acetylation of lysine 40 on α-tubulin. The level of acetylated MTs has to be finely tuned either favouring or preventing podosome formation/organisation depending on the flexibility required.

The roles of MTs in invadosome biology are only emerging and are thus likely to increase in the near future.

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References

Akisaka, T., Yoshida, H., Takigawa, T., 2011. Differential distribution of posttranslationally modified microtubules in osteoclasts. J. Histochem. Cytochem. Off. J. Histochem. Soc. 59, 630–638. https://doi.org/10.1369/0022155411405334

Beaty, B.T., Wang, Y., Bravo-Cordero, J.J., Sharma, V.P., Miskolci, V., Hodgson, L., Condeelis, J., 2014. Talin regulates moesin-NHE-1 recruitment to invadopodia and promotes mammary tumor metastasis. J. Cell Biol. 205, 737–751. https://doi.org/10.1083/jcb.201312046

Bershadsky, A., Chausovsky, A., Becker, E., Lyubimova, A., Geiger, B., 1996. Involvement of microtubules in the control of adhesion-dependent signal transduction. Curr. Biol. CB 6, 1279–1289. https://doi.org/10.1016/s0960-9822(02)70714-8

Bhuwania, R., Castro-Castro, A., Linder, S., 2014. Microtubule acetylation regulates dynamics of KIF1C-powered vesicles and contact of microtubule plus ends with podosomes. Eur. J. Cell Biol. 93, 424–437. https://doi.org/10.1016/j.ejcb.2014.07.006

Biosse Duplan, M., Zalli, D., Stephens, S., Zenger, S., Neff, L., Oelkers, J.M., Lai, F.P., Horne, W., Rottner, K., Baron, R., 2014. Microtubule dynamic instability controls podosome patterning in osteoclasts through EB1, cortactin, and Src. Mol Cell Biol 34, 16–29. https://doi.org/10.1128/MCB.00578-13

Bodakuntla, S., Jijumon, A.S., Villablanca, C., Gonzalez-Billault, C., Janke, C., 2019. Microtubule-Associated Proteins: Structuring the Cytoskeleton. Trends Cell Biol. 29, 804–819. https://doi.org/10.1016/j.tcb.2019.07.004

Bowne-Anderson, H., Hibbel, A., Howard, J., 2015. Regulation of Microtubule Growth and Catastrophe: Unifying Theory and Experiment. Trends Cell Biol 25, 769–79. https://doi.org/10.1016/j.tcb.2015.08.009

Branch, K.M., Hoshino, D., Weaver, A.M., 2012. Adhesion rings surround invadopodia and

promote maturation. Biol. Open 1, 711–722. https://doi.org/10.1242/bio.20121867

Castro-Castro, A., Janke, C., Montagnac, G., Paul-Gilloteaux, P., Chavrier, P., 2012. ATAT1/MEC-17 acetyltransferase and HDAC6 deacetylase control a balance of acetylation of alpha-tubulin and cortactin and regulate MT1-MMP trafficking and breast tumor cell invasion. Eur. J. Cell Biol. 91, 950–960. https://doi.org/10.1016/j.ejcb.2012.07.001

Castro-Castro, A., Marchesin, V., Monteiro, P., Lodillinsky, C., Rossé, C., Chavrier, P., 2016. Cellular and Molecular Mechanisms of MT1-MMP-Dependent Cancer Cell Invasion. Annu. Rev. Cell Dev. Biol. 32, 555–576. https://doi.org/10.1146/annurev-cellbio-111315-125227

Chabadel, A., Bañon-Rodríguez, I., Cluet, D., Rudkin, B.B., Wehrle-Haller, B., Genot, E., Jurdic, P., Anton, I.M., Saltel, F., 2007. CD44 and beta3 integrin organize two functionally distinct actin-based domains in osteoclasts. Mol. Biol. Cell 18, 4899–4910. https://doi.org/10.1091/mbc.e07-04-0378

Cornfine, S., Himmel, M., Kopp, P., El Azzouzi, K., Wiesner, C., Krüger, M., Rudel, T., Linder, S., 2011. The kinesin KIF9 and reggie/flotillin proteins regulate matrix degradation by macrophage podosomes. Mol. Biol. Cell 22, 202–215. https://doi.org/10.1091/mbc.E10-05-0394

Destaing, O., Saltel, F., Géminard, J.-C., Jurdic, P., Bard, F., 2003. Podosomes display actin turnover and dynamic self-organization in osteoclasts expressing actin-green fluorescent protein. Mol. Biol. Cell 14, 407–416. https://doi.org/10.1091/mbc.e02-07-0389

Destaing, O., Saltel, F., Gilquin, B., Chabadel, A., Khochbin, S., Ory, S., Jurdic, P., 2005. A novel Rho-mDia2-HDAC6 pathway controls podosome patterning through microtubule acetylation in osteoclasts. J Cell Sci 118, 2901–11. https://doi.org/10.1242/jcs.02425

Eddy, R.J., Weidmann, M.D., Sharma, V.P., Condeelis, J.S., 2017. Tumor Cell Invadopodia: Invasive Protrusions that Orchestrate Metastasis. Trends Cell Biol. 27, 595–607. https://doi.org/10.1016/j.tcb.2017.03.003

Efimova, N., Grimaldi, A., Bachmann, A., Frye, K., Zhu, X., Feoktistov, A., Straube, A., Kaverina, I., 2014. Podosome-regulating kinesin KIF1C translocates to the cell periphery in a CLASP-dependent manner. J. Cell Sci. 127, 5179–5188. https://doi.org/10.1242/jcs.149633

Enomoto, T., 1996. Microtubule disruption induces the formation of actin stress fibers and focal adhesions in cultured cells: possible involvement of the rho signal cascade. Cell Struct. Funct. 21, 317–326. https://doi.org/10.1247/csf.21.317

Evans, J.G., Correia, I., Krasavina, O., Watson, N., Matsudaira, P., 2003. Macrophage podosomes assemble at the leading lamella by growth and fragmentation. J. Cell Biol. 161, 697–705. https://doi.org/10.1083/jcb.200212037

Fukata, M., Watanabe, T., Noritake, J., Nakagawa, M., Yamaga, M., Kuroda, S., Matsuura, Y., Iwamatsu, A., Perez, F., Kaibuchi, K., 2002. Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell 109, 873–885. https://doi.org/10.1016/s0092-8674(02)00800-0

Gil-Henn, H., Destaing, O., Sims, N.A., Aoki, K., Alles, N., Neff, L., Sanjay, A., Bruzzaniti, A., De Camilli, P., Baron, R., Schlessinger, J., 2007a. Defective microtubule-dependent podosome organization in osteoclasts leads to increased bone density in Pyk2(-/-) mice. J. Cell Biol. 178, 1053–1064. https://doi.org/10.1083/jcb.200701148

Gil-Henn, H., Destaing, O., Sims, N.A., Aoki, K., Alles, N., Neff, L., Sanjay, A., Bruzzaniti, A., De Camilli, P., Baron, R., Schlessinger, J., 2007b. Defective microtubule-dependent podosome organization in osteoclasts leads to increased bone density in Pyk2(-/-) mice. J. Cell Biol. 178, 1053–1064. https://doi.org/10.1083/jcb.200701148

Guérit, D., Marie, P., Morel, A., Maurin, J., Verollet, C., Raynaud-Messina, B., Urbach, S., Blangy, A., 2020. Primary myeloid cell proteomics and transcriptomics: importance of ß tubulin isotypes for osteoclast function. J. Cell Sci. jcs.239772. https://doi.org/10.1242/jcs.239772

Guimbal, S., Morel, A., Guérit, D., Chardon, M., Blangy, A., Vives, V., 2019. Dock5 is a new regulator of microtubule dynamic instability in osteoclasts. Biol. Cell 111, 271–283. https://doi.org/10.1111/boc.201900014

Hoshino, D., Branch, K.M., Weaver, A.M., 2013. Signaling inputs to invadopodia and podosomes. J. Cell Sci. 126, 2979–2989. https://doi.org/10.1242/jcs.079475

Infante, E., Castagnino, A., Ferrari, R., Monteiro, P., Agüera-González, S., Paul-Gilloteaux, P., Domingues, M.J., Maiuri, P., Raab, M., Shanahan, C.M., Baffet, A., Piel, M., Gomes, E.R., Chavrier, P., 2018. LINC complex-Lis1 interplay controls MT1-MMP matrix digest-on-demand response for confined tumor cell migration. Nat. Commun. 9, 2443. https://doi.org/10.1038/s41467-018-04865-7

Jacob, A., Jing, J., Lee, J., Schedin, P., Gilbert, S.M., Peden, A.A., Junutula, J.R., Prekeris, R., 2013. Rab40b regulates trafficking of MMP2 and MMP9 during invadopodia formation and invasion of breast cancer cells. J. Cell Sci. 126, 4647–4658. https://doi.org/10.1242/jcs.126573

Janke, C., Magiera, M.M., 2020. The tubulin code and its role in controlling microtubule properties and functions. Nat. Rev. Mol. Cell Biol. https://doi.org/10.1038/s41580-020-0214-3

Kedziora, K.M., Isogai, T., Jalink, K., Innocenti, M., 2016. Invadosomes - shaping actin networks to follow mechanical cues. Front. Biosci. Landmark Ed. 21, 1092–1117. https://doi.org/10.2741/4444

Kobayakawa, M., Matsubara, T., Mizokami, A., Hiura, F., Takakura, N., Kokabu, S., Matsuda, M., Yasuda, H., Nakamura, I., Takei, Y., Honda, H., Hosokawa, R., Jimi, E., 2019. Kif1c regulates osteoclastic bone resorption as a downstream molecule of p130Cas. Cell Biochem. Funct. https://doi.org/10.1002/cbf.3476

Kopp, P., Lammers, R., Aepfelbacher, M., Woehlke, G., Rudel, T., Machuy, N., Steffen, W.,

Linder, S., 2006. The kinesin KIF1C and microtubule plus ends regulate podosome dynamics in macrophages. Mol. Biol. Cell 17, 2811–2823. https://doi.org/10.1091/mbc.e05-11-1010

Linder, S., Hüfner, K., Wintergerst, U., Aepfelbacher, M., 2000. Microtubule-dependent formation of podosomal adhesion structures in primary human macrophages. J. Cell Sci. 113 Pt 23, 4165–4176.

Linder, S., Wiesner, C., Himmel, M., 2011. Degrading devices: invadosomes in proteolytic cell invasion. Annu. Rev. Cell Dev. Biol. 27, 185–211. https://doi.org/10.1146/annurev-cellbio-092910-154216

Luxenburg, C., Geblinger, D., Klein, E., Anderson, K., Hanein, D., Geiger, B., Addadi, L., 2007. The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly. PloS One 2, e179. https://doi.org/10.1371/journal.pone.0000179

Ma, T., Samanna, V., Chellaiah, M.A., 2008. Dramatic inhibition of osteoclast sealing ring formation and bone resorption in vitro by a WASP-peptide containing pTyr294 amino acid. J. Mol. Signal. 3, 4. https://doi.org/10.1186/1750-2187-3-4

Marchesin, V., Castro-Castro, A., Lodillinsky, C., Castagnino, A., Cyrta, J., Bonsang-Kitzis, H., Fuhrmann, L., Irondelle, M., Infante, E., Montagnac, G., Reyal, F., Vincent-Salomon, A., Chavrier, P., 2015. ARF6-JIP3/4 regulate endosomal tubules for MT1-MMP exocytosis in cancer invasion. J. Cell Biol. 211, 339–358. https://doi.org/10.1083/jcb.201506002

Matsumoto, T., Nagase, Y., Hirose, J., Tokuyama, N., Yasui, T., Kadono, Y., Ueki, K., Kadowaki, T., Nakamura, K., Tanaka, S., 2013. Regulation of bone resorption and sealing zone formation in osteoclasts occurs through protein kinase B-mediated microtubule stabilization. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 28, 1191–1202. https://doi.org/10.1002/jbmr.1844

Maurin, J., Morel, A., Hassen-Khodja, C., Vives, V., Jurdic, P., Machuca-Gayet, I., Blangy,

A., 2018. Combined strategy of siRNA and osteoclast actin cytoskeleton automated imaging to identify novel regulators of bone resorption shows a non-mitotic function for anillin. Eur. J. Cell Biol. 97, 568–579. https://doi.org/10.1016/j.ejcb.2018.10.002

McMichael, B.K., Cheney, R.E., Lee, B.S., 2010. Myosin X regulates sealing zone patterning in osteoclasts through linkage of podosomes and microtubules. J. Biol. Chem. 285, 9506–9515. https://doi.org/10.1074/jbc.M109.017269

McMichael, B.K., Scherer, K.F., Franklin, N.C., Lee, B.S., 2014. The RhoGAP activity of myosin IXB is critical for osteoclast podosome patterning, motility, and resorptive capacity. PloS One 9, e87402. https://doi.org/10.1371/journal.pone.0087402

Mersich, A.T., Miller, M.R., Chkourko, H., Blystone, S.D., 2010. The formin FRL1 (FMNL1) is an essential component of macrophage podosomes. Cytoskelet. Hoboken NJ 67, 573–585. https://doi.org/10.1002/cm.20468

Noordstra, I., Akhmanova, A., 2017. Linking cortical microtubule attachment and exocytosis. F1000Research 6, 469. https://doi.org/10.12688/f1000research.10729.1

Okumura, S., Mizoguchi, T., Sato, N., Yamaki, M., Kobayashi, Y., Yamauchi, H., Ozawa, H., Udagawa, N., Takahashi, N., 2006. Coordination of microtubules and the actin cytoskeleton is important in osteoclast function, but calcitonin disrupts sealing zones without affecting microtubule networks. Bone 39, 684–693. https://doi.org/10.1016/j.bone.2006.04.010

Otto, G.P., Nichols, B.J., 2011. The roles of flotillin microdomains - endocytosis and beyond. J. Cell Sci. 124, 3933–3940. https://doi.org/10.1242/jcs.092015

Parker, A.L., Teo, W.S., McCarroll, J.A., Kavallaris, M., 2017. An Emerging Role for Tubulin Isotypes in Modulating Cancer Biology and Chemotherapy Resistance. Int. J. Mol. Sci. 18. https://doi.org/10.3390/ijms18071434

Paterson, E.K., Courtneidge, S.A., 2018. Invadosomes are coming: new insights into function and disease relevance. FEBS J. 285, 8–27. https://doi.org/10.1111/febs.14123

Pignatelli, J., Tumbarello, D.A., Schmidt, R.P., Turner, C.E., 2012. Hic-5 promotes invadopodia formation and invasion during TGF-β-induced epithelial-mesenchymal transition. J. Cell Biol. 197, 421–437. https://doi.org/10.1083/jcb.201108143

Planchon, D., Rios Morris, E., Genest, M., Comunale, F., Vacher, S., Bièche, I., Denisov, E.V., Tashireva, L.A., Perelmuter, V.M., Linder, S., Chavrier, P., Bodin, S., Gauthier-Rouvière, C., 2018. MT1-MMP targeting to endolysosomes is mediated by upregulation of flotillins. J. Cell Sci. 131. https://doi.org/10.1242/jcs.218925

Poincloux, R., Lizárraga, F., Chavrier, P., 2009. Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia. J. Cell Sci. 122, 3015–3024. https://doi.org/10.1242/jcs.034561

Proszynski, T.J., Sanes, J.R., 2013. Amotl2 interacts with LL5β, localizes to podosomes and regulates postsynaptic differentiation in muscle. J. Cell Sci. 126, 2225–2235. https://doi.org/10.1242/jcs.121327

Rafiq, N.B.M., Nishimura, Y., Plotnikov, S.V., Thiagarajan, V., Zhang, Z., Shi, S., Natarajan, M., Viasnoff, V., Kanchanawong, P., Jones, G.E., Bershadsky, A.D., 2019. A mechano-signalling network linking microtubules, myosin IIA filaments and integrin-based adhesions. Nat. Mater. 18, 638–649. https://doi.org/10.1038/s41563-019-0371-y

Remacle, A.G., Rozanov, D.V., Baciu, P.C., Chekanov, A.V., Golubkov, V.S., Strongin, A.Y., 2005. The transmembrane domain is essential for the microtubular trafficking of membrane type-1 matrix metalloproteinase (MT1-MMP). J. Cell Sci. 118, 4975–4984. https://doi.org/10.1242/jcs.02610

Ren, X.D., Kiosses, W.B., Schwartz, M.A., 1999. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO J. 18, 578–585. https://doi.org/10.1093/emboj/18.3.578

Revach, O.-Y., Weiner, A., Rechav, K., Sabanay, I., Livne, A., Geiger, B., 2015. Mechanical

interplay between invadopodia and the nucleus in cultured cancer cells. Sci. Rep. 5, 9466. https://doi.org/10.1038/srep09466

Roll-Mecak, A., 2019. How cells exploit tubulin diversity to build functional cellular microtubule mosaics. Curr. Opin. Cell Biol. 56, 102–108. https://doi.org/10.1016/j.ceb.2018.10.009

Sakurai-Yageta, M., Recchi, C., Le Dez, G., Sibarita, J.-B., Daviet, L., Camonis, J., D'Souza-Schorey, C., 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. https://doi.org/10.1083/jcb.200709076

Sala, K., Raimondi, A., Tonoli, D., Tacchetti, C., de Curtis, I., 2018. Identification of a membrane-less compartment regulating invadosome function and motility. Sci. Rep. 8, 1164. https://doi.org/10.1038/s41598-018-19447-2

Saltel, F., Destaing, O., Bard, F., Eichert, D., Jurdic, P., 2004. Apatite-mediated actin dynamics in resorbing osteoclasts. Mol. Biol. Cell 15, 5231–5241. https://doi.org/10.1091/mbc.e04-06-0522

Schnaeker, E.-M., Ossig, R., Ludwig, T., Dreier, R., Oberleithner, H., Wilhelmi, M., Schneider, S.W., 2004. Microtubule-Dependent Matrix Metalloproteinase-2/Matrix Metalloproteinase-9 Exocytosis: Prerequisite in Human Melanoma Cell Invasion. Cancer Res. 64, 8924–8931. https://doi.org/10.1158/0008-5472.CAN-04-0324

Schoumacher, M., Goldman, R.D., Louvard, D., Vignjevic, D.M., 2010. Actin, microtubules, and vimentin intermediate filaments cooperate for elongation of invadopodia. J. Cell Biol. 189, 541–556. https://doi.org/10.1083/jcb.200909113

Sedgwick, A.E., Clancy, J.W., Olivia Balmert, M., D'Souza-Schorey, C., 2015. Extracellular microvesicles and invadopodia mediate non-overlapping modes of tumor cell invasion. Sci. Rep. 5, 14748. https://doi.org/10.1038/srep14748

Seetharaman, S., Etienne-Manneville, S., 2019. Microtubules at focal adhesions - a double-edged sword. J. Cell Sci. 132. https://doi.org/10.1242/jcs.232843

Segeletz, S., Danglot, L., Galli, T., Hoflack, B., 2018. ARAP1 Bridges Actin Dynamics and AP-3-Dependent Membrane Traffic in Bone-Digesting Osteoclasts. iScience 6, 199–211. https://doi.org/10.1016/j.isci.2018.07.019

Sousa, A.D., 2006. Myo10 in brain: developmental regulation, identification of a headless isoform and dynamics in neurons. J. Cell Sci. 119, 184–194. https://doi.org/10.1242/jcs.02726

Sweeney, H.L., Holzbaur, E.L.F., 2018. Motor Proteins. Cold Spring Harb. Perspect. Biol. 10. https://doi.org/10.1101/cshperspect.a021931

Takito, J., Inoue, S., Nakamura, M., 2018. The Sealing Zone in Osteoclasts: A Self-Organized Structure on the Bone. Int. J. Mol. Sci. 19. https://doi.org/10.3390/ijms19040984

Theisen, U., Straube, E., Straube, A., 2012. Directional persistence of migrating cells requires Kif1C-mediated stabilization of trailing adhesions. Dev. Cell 23, 1153–1166. https://doi.org/10.1016/j.devcel.2012.11.005

van den Dries, K., Linder, S., Maridonneau-Parini, I., Poincloux, R., 2019. Probing the mechanical landscape - new insights into podosome architecture and mechanics. J. Cell Sci. 132. https://doi.org/10.1242/jcs.236828

van der Vaart, B., van Riel, W.E., Doodhi, H., Kevenaar, J.T., Katrukha, E.A., Gumy, L., Bouchet, B.P., Grigoriev, I., Spangler, S.A., Yu, K.L., Wulf, P.S., Wu, J., Lansbergen, G., van Battum, E.Y., Pasterkamp, R.J., Mimori-Kiyosue, Y., Demmers, J., Olieric, N., Maly, I.V., Hoogenraad, C.C., Akhmanova, A., 2013. CFEOM1-associated kinesin KIF21A is a cortical microtubule growth inhibitor. Dev. Cell 27, 145–160. https://doi.org/10.1016/j.devcel.2013.09.010

Waterman-Storer, C.M., Worthylake, R.A., Liu, B.P., Burridge, K., Salmon, E.D., 1999. Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. Nat.

Cell Biol. 1, 45–50. https://doi.org/10.1038/9018

Wiesner, C., Faix, J., Himmel, M., Bentzien, F., Linder, S., 2010. KIF5B and KIF3A/KIF3B kinesins drive MT1-MMP surface exposure, CD44 shedding, and extracellular matrix degradation in primary macrophages. Blood 116, 1559–1569. https://doi.org/10.1182/blood-2009-12-257089

Xu, Z., Schaedel, L., Portran, D., Aguilar, A., Gaillard, J., Marinkovich, M.P., Théry, M., Nachury, M.V., 2017. Microtubules acquire resistance from mechanical breakage through intralumenal acetylation. Science 356, 328–332. https://doi.org/10.1126/science.aai8764

Yan, T., Zhang, A., Shi, F., Chang, F., Mei, J., Liu, Y., Zhu, Y., 2018. Integrin αvβ3-associated DAAM1 is essential for collagen-induced invadopodia extension and cell haptotaxis in breast cancer cells. J. Biol. Chem. 293, 10172–10185. https://doi.org/10.1074/jbc.RA117.000327

Zalli, D., Neff, L., Nagano, K., Shin, N.Y., Witke, W., Gori, F., Baron, R., 2016. The Actin-Binding Protein Cofilin and Its Interaction With Cortactin Are Required for Podosome Patterning in Osteoclasts and Bone Resorption In Vivo and In Vitro. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 31, 1701–1712. https://doi.org/10.1002/jbmr.2851

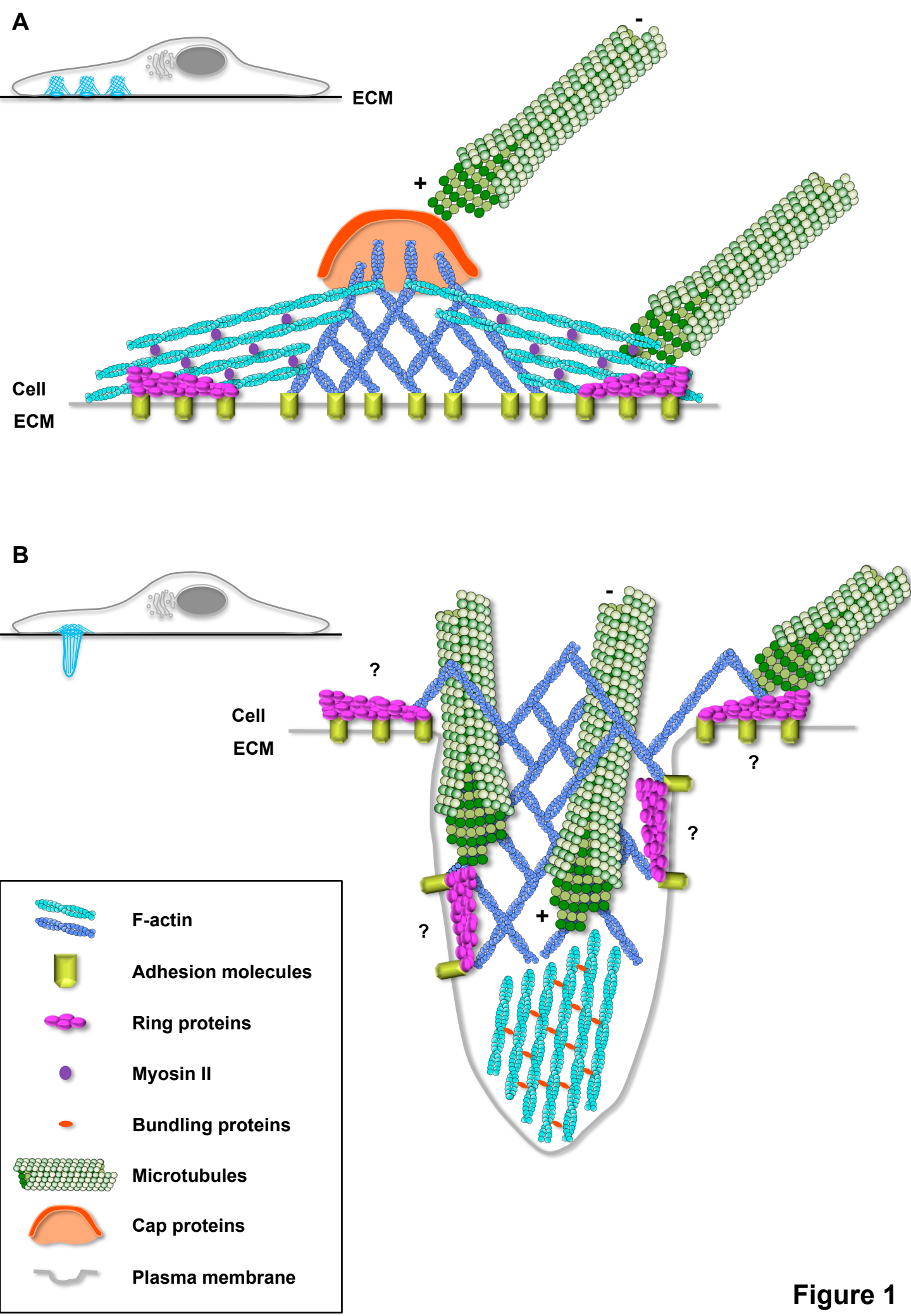
Zhu, X., Efimova, N., Arnette, C., Hanks, S.K., Kaverina, I., 2016. Podosome dynamics and location in vascular smooth muscle cells require CLASP-dependent microtubule bending. Cytoskelet. Hoboken NJ 73, 300–315. https://doi.org/10.1002/cm.21302

Figure legends

Figure 1: Structure and ultrastructure of invadosomes. (A) Podosomes appear as an actin cylinder in normal cells. Branched actin filaments (dark blue) compose the core of the podosome surrounded by unbranched actin filaments (vivid blue) bundled by myosin II (purple). Cap proteins (orange) are located on top. Actin filaments interact through ring proteins (pink: such as talin and vinculin) to adhesion molecules (dark yellow: among others αβ-integrin and CD44) which are connected to extra-cellular matrix (ECM) or different adaptors not depicted. MTs (dark and light green) are connected to podosomes at the level of the cap and the ring proteins. (B) Invadopodia are protruding structures deeply engaged through the ECM upon degradation and are found only in invasive cancer cells. Invadopodia are filled with actin filaments mostly branched except at the tip where F-actin is bundle through bundling proteins (red: e.g. fascin). MTs extend throughout the invadopodium. Adhesion proteins might be organised as a ring at the basis of invadopodia (e.g. paxillin) and might be found at the side recruiting ring proteins (e.g. talin). Similarly to podosomes, actin filaments and MTs could potentially interact with those structures as indicated by question marks. + and – indicate MT + and –ends.

Figure 2: MTs regulate invadosome formation/activity through cargo delivery and RhoA. For proper cargo delivery MTs need to be anchored at adhesion sites. MTs capture to integrins involved talin, KANK, CMDC and CLASP proteins. In addition, KIF21A bound to KANK allows MT +end stabilisation. As a consequence, anchorage increases MT stabilisation and potentially (question mark) acetylation (dashed red box) and also GEF-H1 sequestration. Captured MTs allow the local delivery of MTI-MMP through KIF5B or KIF3A/3B, integrin through KIF1C and membrane through KIF9-Flotillin. When MTs detach from adhesion sites through depolymerisation, GEF-H1 is released and prone to activate the small GTPase RhoA. RhoA activation leads to MT deacetylation through mDia2/HDAC6

activation and to the formation of myosin II filaments through the activation of ROCK. Altogether, it should alter cargo delivery and invadosome ultrastructure leading to change in invadosome formation/activity. + and – indicate MT + and –ends.


A
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Myosin II
Bundling proteins
Microtubules
Cap proteins
Plasma membrane

Figure 1

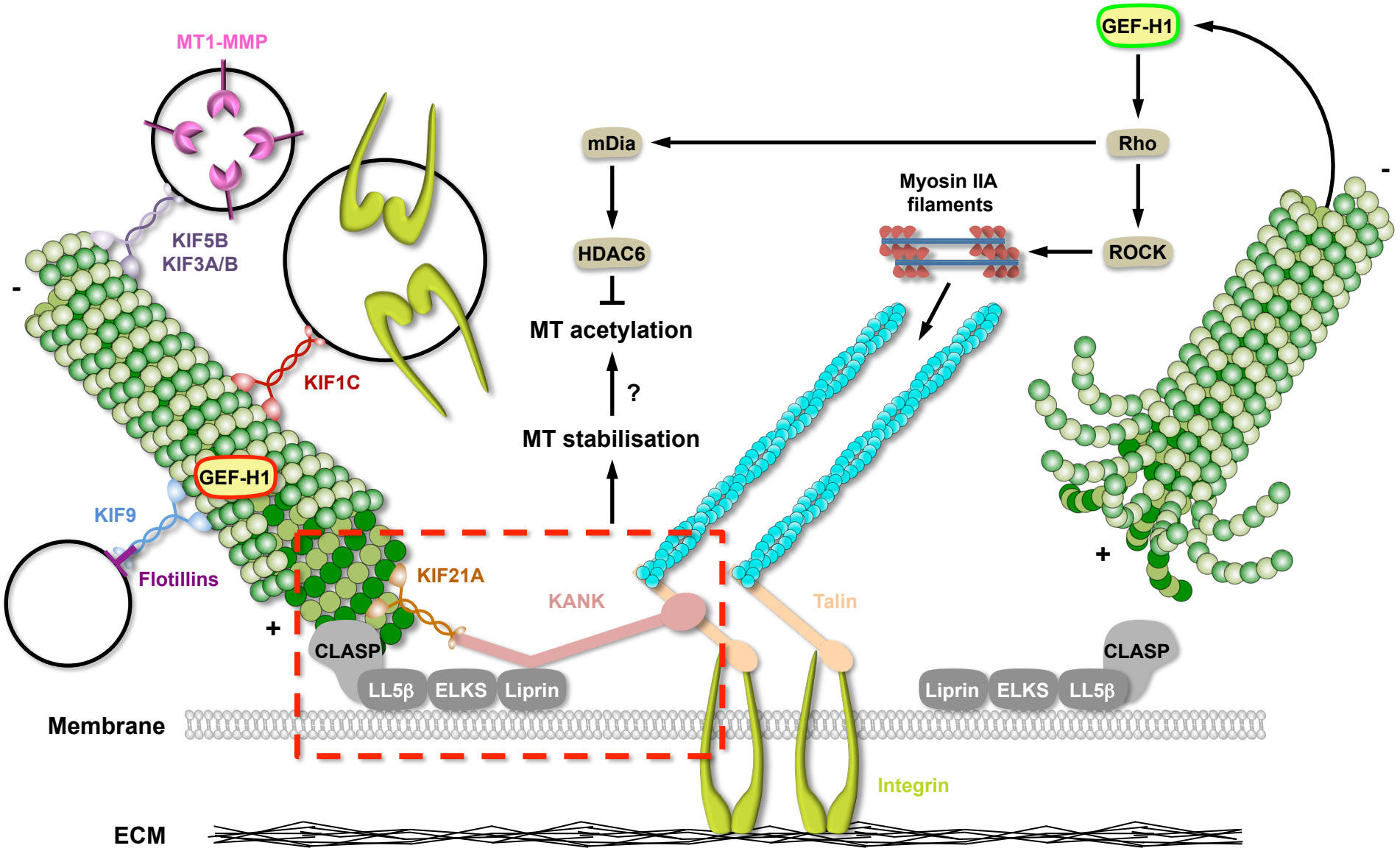
MT1-MMP
KIF5B
KIF3A/B
KIF1C
GEF-H1
KIF9
Flotillins
KIF21A
CLASP
LL5β
ELKS
Liprin
KANK
Talin
Membrane
ECM
Integrin
mDia
HDAC6
MT acetylation
?
MT stabilisation
Rho
ROCK
Myosin IIA
filaments
GEF-H1
CLASP
Liprin
ELKS
LL5β
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Figure 2