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HYPOTHESIS

SUBJECT COLLECTION: ADHESION, INVADOPODIA AND PODOSOMES

Biomechanical regulation of focal adhesion and invadopodia formation

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ABSTRACT

Integrin adhesions are a structurally and functionally diverse family of transmembrane, multi-protein complexes that link the intracellular cytoskeleton to the extracellular matrix (ECM). The different members of this family, including focal adhesions (FAs), focal complexes, fibrillar adhesions, podosomes and invadopodia, contain many shared scaffolding and signaling 'adhesome' components, as well as distinct molecules that perform specific functions, unique to each adhesion form. In this Hypothesis, we address the pivotal roles of mechanical forces, generated by local actin polymerization or actomyosin-based contractility, in the formation, maturation and functionality of two members of the integrin adhesions family, namely FAs and invadopodia, which display distinct structures and functional properties. FAs are robust and stable ECM contacts, associated with contractile stress fibers, while invadopodia are invasive adhesions that degrade the underlying matrix and penetrate into it. We discuss here the mechanisms, whereby these two types of adhesion utilize a similar molecular machinery to drive very different – often opposing cellular activities, and hypothesize that early stages of FAs and invadopodia assembly use similar biomechanical principles, whereas maturation of the two structures, and their 'adhesive' and 'invasive' functionalities require distinct sources of biomechanical reinforcement.

KEY WORDS: Cell adhesion, Cell invasion, Integrin adhesions, Mechanosensing, Focal adhesions, Invadopodia

Introduction

Cell-matrix interactions are essential for the regulation of cell behavior and fate in multiple developmental and homeostatic processes, among them tissue and organ formation, remodeling, and repair (Berrier and Yamada, 2007; Geiger et al., 2009; Maziveyi and Alahari, 2017). Consequently, the deregulation of adhesive interactions, often leads to pathological states, such as cancer metastasis, immune disorders and skin blistering diseases (Hegde and Raghavan, 2013; Huveneers et al., 2007; Winograd-Katz et al., 2014). To perform their diverse functions, cell adhesions assemble large transmembrane multi-protein complexes that provide mechanical coupling between the external microenvironment and the intracellular cytoskeleton (Jansen et al., 2017; Sun et al., 2016). The forces acting on these complexes are both generated and regulated by specific multi-protein scaffolding and signaling networks that are associated with specific adhesion sites. These multi-protein complexes can sense the molecular and physical properties of the microenvironment, integrate this information and eventually drive multiple cellular processes, including proliferation, survival, differentiation and motility. This process is guided both by the specificity of the adhesion receptors at the cell surface for the local molecular composition of the ECM, and by the physical properties of the matrix, its topography and rigidity (Geiger et al., 2009; Malik-Sheriff et al., 2018; Prager-Khoutorsky et al., 2011; Riveline et al., 2001). This crosstalk between cells and the environment is further complicated by the physiological heterogeneity of the ECM, with its wide variety of adhesion 'ligands' that are recognized by multiple adhesion receptors, which might interact with each other, either positively or negatively (De Arcangelis and Georges-Labouesse, 2000; Plow et al., 2000). To illustrate this complexity – the repertoire of ECM constituents, collectively known as the matrisome, is rather wide and includes over 1000 'core' and 'associated' components (Naba et al., 2016). These molecules can be recognized by specific adhesion receptors that, upon engagement with the corresponding ECM, can activate diverse signaling networks, each with its particular physiological assignments (Bökel and Brown, 2002; Huttenlocher and Horwitz, 2011; Huveneers et al., 2007). While appreciating the overwhelming diversity of the ECM and the corresponding receptors, we chose to focus here on a specific question related to the integrin family of adhesions. Namely, how do two distinct forms of integrin adhesion – focal adhesions (FAs) and invadopodia – utilize a mostly similar molecular arsenal to perform very different functions, i.e. the assembly of stable matrix adhesions and the development of invasive protrusions that degrade the ECM, respectivley (Barczyk et al., 2010; Bökel and Brown, 2002; Campbell and Humphries, 2011; Horton et al., 2016; Huttenlocher and Horwitz, 2011; Miyamoto et al., 1995; Zaidel-Bar and Geiger, 2010). Given the wealth of information on integrin adhesions, we will refrain from discussing the structure and function of these adhesions in detail but, rather briefly, highlight their overall molecular organization, mechanosensitivity and functional diversity.

Integrins are heterodimeric transmembrane adhesion receptors, consisting of different α and β chains (Campbell and Humphries, 2011). Diverse cell-type-specific combinations of these chains yield over 20 different integrin heterodimers that mediate differential cell interactions with specific ECM components, such as, fibronectin, vitronectin, collagen or fibrinogen, as well as with complementary surface components of other cells, such as intracellular adhesion molecule 1 (ICAM1), and vascular cell adhesion molecule 1 (VCAM1) (Barczyk et al., 2010; Frantz et al., 2010; De Arcangelis and Georges-Labouesse, 2000; Kim et al., 2011; Sánchez-Cortés and Mrksich, 2009). Through their short intracellular tails, integrins interact with multiple adaptor proteins and signaling enzymes that are referred to, collectively, as the integrin adhesome (Horton et al., 2016; Zaidel-Bar et al., 2007; Zaidel-Bar and Geiger, 2010) and, either directly or indirectly, bind to the actin

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cytoskeleton and regulate its organization and mechanics (Barczyk et al., 2010; Campbell and Humphries, 2011). These scaffolding links are regulated through adhesome-associated signaling networks that drive pivotal mechanosensitive processes in adhering cells (Chorev et al., 2018; Hytönen and Vogel, 2008; Lee et al., 2007; Parsons et al., 2010; Riveline et al., 2001; Zaidel-Bar and Geiger, 2010). It is important to mention that different types of integrin-mediated adhesion are associated with distinct cytoskeletal assemblies that are made primarily of polymerized actin (F-actin). For example, nascent FAs—also known as 'focal complexes' – are formed underneath a retrograde flowing branched network of F-actin that pushes forward the leading edge of migratory cells, while applying shear stress to the underlying nascent adhesions and, through them, to the underlying ECM (Alexandrova et al., 2008). By contrast, mature FAs are linked to contractile bundles made of F-actin and the motor protein myosin II (known as 'stress fibers'), which apply considerable tensile force to the adhesion sites.

Extensive studies carried out during the past four decades have addressed the mechanisms underlying the structure–function relationships in the 'prototypic' form of integrin adhesions in cultured cells, namely FAs (Balaban et al., 2001; Burridge and Guilluy, 2016; Wehrle-Haller, 2012; Wozniak et al., 2004; Zaidel-Bar et al., 2004). These studies addressed the development of FAs from their precursors, the focal complexes (Nagano et al., 2012; Wehrle-Haller, 2012; Zaidel-Bar et al., 2003), highlighted the detailed ligand specificity of distinct integrin adhesions and addressed their respective mechanosensing properties, as well as their physiological functions (see Fig. 1 upper panel) (Chen et al., 2004; Jansen et al., 2017; Riveline et al., 2001; Sun et al., 2016). Other forms and types of integrin adhesion assume distinct morphologies and mechanical properties, and probably also have

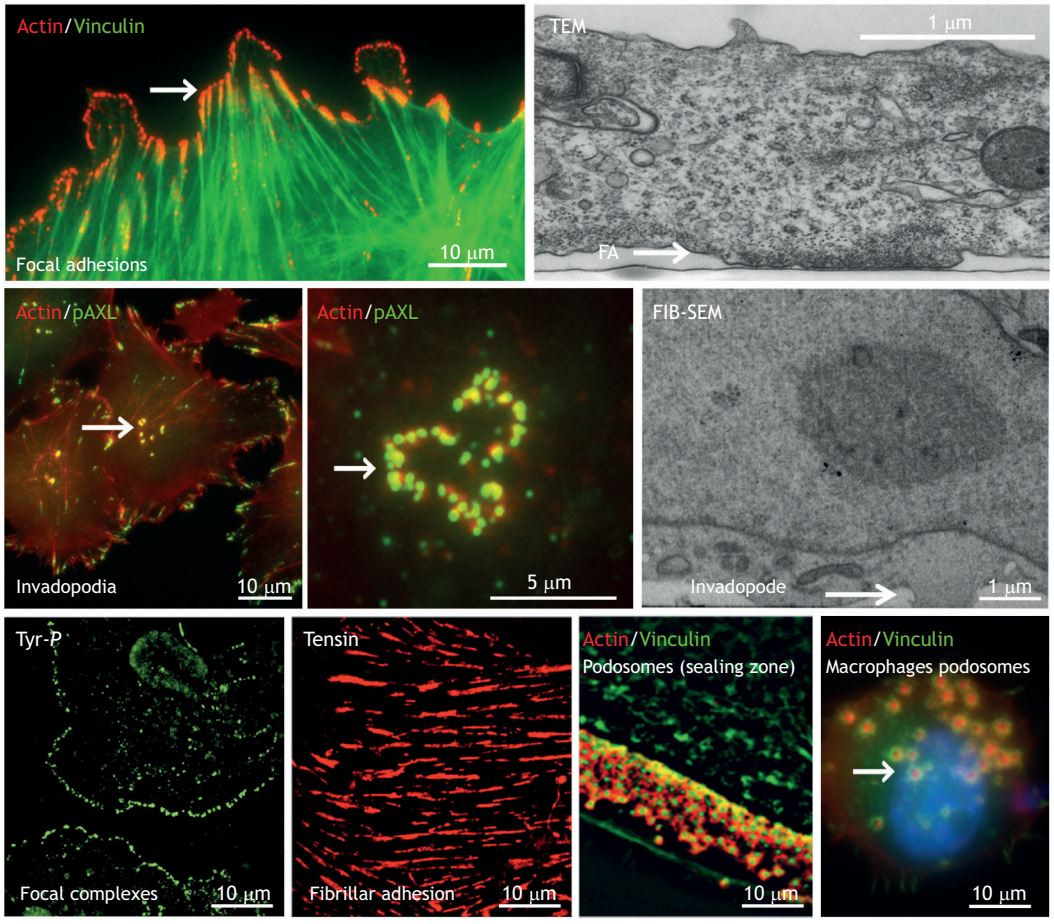

Fig. 1. Examples of the morphological diversity of integrin adhesions formed by different cells in culture. Shown are different types of integrin-mediated adhesion. All of these are potentially assembled by the same core of adhesome components, including integrin receptors, adaptor proteins forming the membrane-associated plaque that anchor the cytoskeleton, the F-actin network as well as associated proteins and signaling molecules, such as cytoplasmic and receptor tyrosine kinases (RTKs). (Top) The left image shows FAs and the associated cytoskeleton in cultured endothelial cells marked for actin (green) and vinculin (red). The right image shows a representative side-view of a FA (indicated by an arrow) in cultured chicken lens cells, obtained using transmission electron microscopy. Both images in have been adapted with permission from Medalia and Geiger, 2010. (Middle) Left and middle panels show invadopodia in cells of the human melanoma A375 cell line at two magnifications, stained for actin (red) and the phosphorylated RTK AXL (pAXL, green). The right image, shows a side-view of a single invadopodium in a cultured A375 cell, obtained by focused ion beam-scanning electron microscopy (FIB-SEM). The invadopod protrusion is indicated by an arrow. (Bottom) Other types of integrin adhesion (panel description from left to right). First: focal complexes in cultured transformed fibroblasts (SV-80 cell line) treated with the contractility inhibitor Y-27623 and stained for phosphorylated tyrosine (Tyr-*P*). Second: fibrillar adhesions in primary human fibroblasts (HFF cells) labeled for tensin. Third and fourth, respectively: podosomes in osteoclasts and macrophages, labeled for F-actin (red) and vinculin (green). The first two of the images in the bottom row have been adapted with permission from Geiger et al., 2001.

different cellular functions. An example for other forms is fibrillar adhesion, long streaks or arrays of integrin-rich clusters that are typically associated with extracellular fibronectin-rich fibers, mediated mainly through integrin α5β1. Unlike FAs, these adhesions are not associated with prominent contractile stress fibers but are most likely to evolve from FAs (De Pascalis and Etienne-Manneville, 2017; Zaidel-Bar et al., 2003). Another type of adhesion is that of podosomes, i.e. small, ring-like adhesions that surround an F-actin core (Block et al., 2008; Linder and Aepfelbacher, 2003; Linder et al., 2011). Podosomes have been shown to be mechanosensitive adhesive cytoskeletal structures (Bouissou et al., 2017; Labernadie et al., 2014; van den Dries et al., 2013, 2019) that are prominent in osteoclasts, i.e. cells that resorb and remodel bone (Jurdic et al., 2006; Luxenburg et al., 2012). However, they are also found in invading immune cells (Labernadie et al., 2014; Linder, 2007; Linder and Aepfelbacher, 2003; Murphy and Courtneidge, 2011) and in other cell types, such as aortic endothelial cells (Moreau et al., 2003). An additional group of integrin adhesions – somewhat similar to podosomes – are invadopodia that drive matrix degradation and invasion; these are often displayed by metastatic cancer cells of different origins, i.e. of breast, lung, colon, squamous epithelia of head and neck, as well as by melanoma, glioblastoma and many more (Gimona et al., 2008; Linder, 2007; Murphy and Courtneidge, 2011). Examples of the diverse forms of integrin adhesions in cultured cells are shown in Fig. 1.

The molecular characterization of the diverse forms of integrin adhesions demonstrated a remarkable similarity, manifested by the presence of a similar set of adaptor molecules that mediate the interaction between integrins and actin (e.g. vinculin, α-actinin, paxilin and talin, see below), actin regulators (e.g. cofilin, profilin, VASP) and signaling enzymes (e.g. Src-family kinases, FAK, specific receptor tyrosine kinases), collectively known as the 'integrin adhesome' (Horton et al., 2016; Revach and Geiger, 2014; Zaidel-Bar et al., 2007; Zaidel-Bar and Geiger, 2010).

An important and characteristic property of integrin adhesions is their mechanosensitivity, namely, the involvement of mechanical forces, applied by the extracellular environment or by the internal cytoskeleton, in their assembly, stability and functionality. The molecular mechanisms underlying this phenomenon has been extensively investigated and partially elucidated in recent years (see, for instance, Bershadsky et al., 2006; Chen et al., 2004; Chorev et al., 2018; Clark et al., 2007; Jansen et al., 2017; Labernadie et al., 2014; Lee et al., 2007; Parekh and Weaver, 2016; Pontes et al., 2017; Riveline et al., 2001; Shemesh et al., 2005; Sun et al., 2016; Young et al., 2009; Zhou et al., 2015; and our discussion below).

In this Hypothesis, we primarily address the formation and functionality of FAs and invadopodia, two integrin adhesions with distinct morphologies and functions, yet comprising a similar molecular 'tool kit'. The former mediate stable and force-dependent matrix adhesions (Wehrle-Haller, 2012; Wu, 2007), whereas the latter are invasive adhesions that eventually destroy the ECM (Murphy and Courtneidge, 2011; Revach and Geiger, 2014). Below, we briefly – step-by-step – present the key processes involved in the assembly of these two forms of integrin adhesions, and argue that the lamellipodium–FA complex bears close molecular and biomechanical resemblance to the invadopodial protrusion and its near-by adhesion domain.

Zoomed-in view of FAs assembly and function

FAs are flat elongated structures comprising several μm^2 and are typically located at the periphery of cultured cells (Burridge et al., 1997; Geiger et al., 2001; Wehrle-Haller, 2012). They mediate close, stable and firm adhesion to the substrate, and anchor – through their cytoplasmic domains – the termini of actomyosin-rich stress fibers that apply considerable contractile forces (~5 $nN/\mu m^2$) to the adhesion site (Balaban et al., 2001; Wehrle-Haller, 2012; Wozniak et al., 2004). These cytoskeletal interactions are mediated by a membrane-associated protein-rich plaque, consisting of many different adhesome proteins (Geiger et al., 2001; Horton et al., 2016; Zaidel-Bar et al., 2007). They include adaptor proteins, such as talin, vinculin and paxillin; actin crosslinkers, such as α-actinin and filamin; as well as others. Collectively, these adhesome proteins, form a physical cytoskeleton-associated scaffold at the adhesion site, and effectively anchor integrin molecules to the actin network. This same plaque also contains a variety of signaling molecules (including kinases, phosphatases) and actin regulators (profilin, cofilin, VASP, Arp2/3) (Geiger et al., 2001; Zamir and Geiger, 2001).

A key mechanistic observation, already made in the 1990s, is that FA assembly and signaling activity are mechanosensitive processes (Geiger et al., 2001; Prager-Khoutorsky et al., 2011), responding both to differences in matrix properties, such as rigidity, ligand density, topography and dynamics, and to the cytoskeletal mechanics (Chen et al., 2004; Geiger et al., 2009; Pelham and Wang, 1997; Riveline et al., 2001; Yeh et al., 2017; Zaidel-Bar et al., 2003). The mechanosensitive process starts at an early stage of FA formation, when nascent focal complexes under the lamellipodium – which are typically formed at the cell periphery – encounter a flow of branched F-actin networks generated by Arp2/3-driven actin polymerization at the leading edge of spreading or migrating cells (Galbraith et al., 2002; Geiger et al., 2001) (Fig. 2). A closer look at these early stages indicated that small focal complexes (0.5–1.0 μm^2) contain a cohort of adhesome molecules, including talin dimers, vinculin and FAK, which interconnect and further activate integrin heterodimers, thereby enhancing both integrin binding to the ECM and recruitment of actin filaments to the newly formed adhesions (Gingras et al., 2008; Smith and McCann, 2007; Tanentzapf et al., 2006). Focal complexes are rather transient structures that – within minutes after their formation – can either disassemble or grow and evolve into FAs, following the application of mechanical traction forces (Chen et al., 2004; Geiger et al., 2001; Shemesh et al., 2005).

The current understanding of the local nanomechanics associated with the transformation of focal complexes to stable FAs is still rather scarce (Craig and Chen, 2003; Nobes and Hall, 1995; Parsons et al., 2010); yet, it is apparent that the 'birthplace' of focal complexes under the lamellipodium has very unique mechanical properties in that it is exposed to shear forces, which are primarily generated by the retrograde flow of the F-actin network (Alexandrova et al., 2008; Cramer, 1997; Gardel et al., 2008; Swaminathan et al., 2017; Vallotton et al., 2005; Yamashiro and Watanabe, 2014). The exact geometry of the molecular interface between the branched actin network and the nascent integrin adhesions is not known. However, the retrograde actin flow, moving centripetally at a rate of 0.5–0.7 μm/min (Alexandrova et al., 2008), indicates that it applies shear stress to them. These forces are believed to be essential for the activation of some of the mechano-responsive adhesome molecules within the focal complexes, including talin and vinculin, which are transformed from an auto-inhibited to an active state (Ciobanasu et al., 2014; Rahikainen et al., 2019; Yao et al., 2015, 2016). Specifically, these mechanical forces can induce conformational changes in talin, facilitating the exposure of its vinculin-binding site (VBS); this enhances the interaction between the two molecules and, apparently, activates vinculin by reducing the intramolecular head-to-tail interaction, thus promoting

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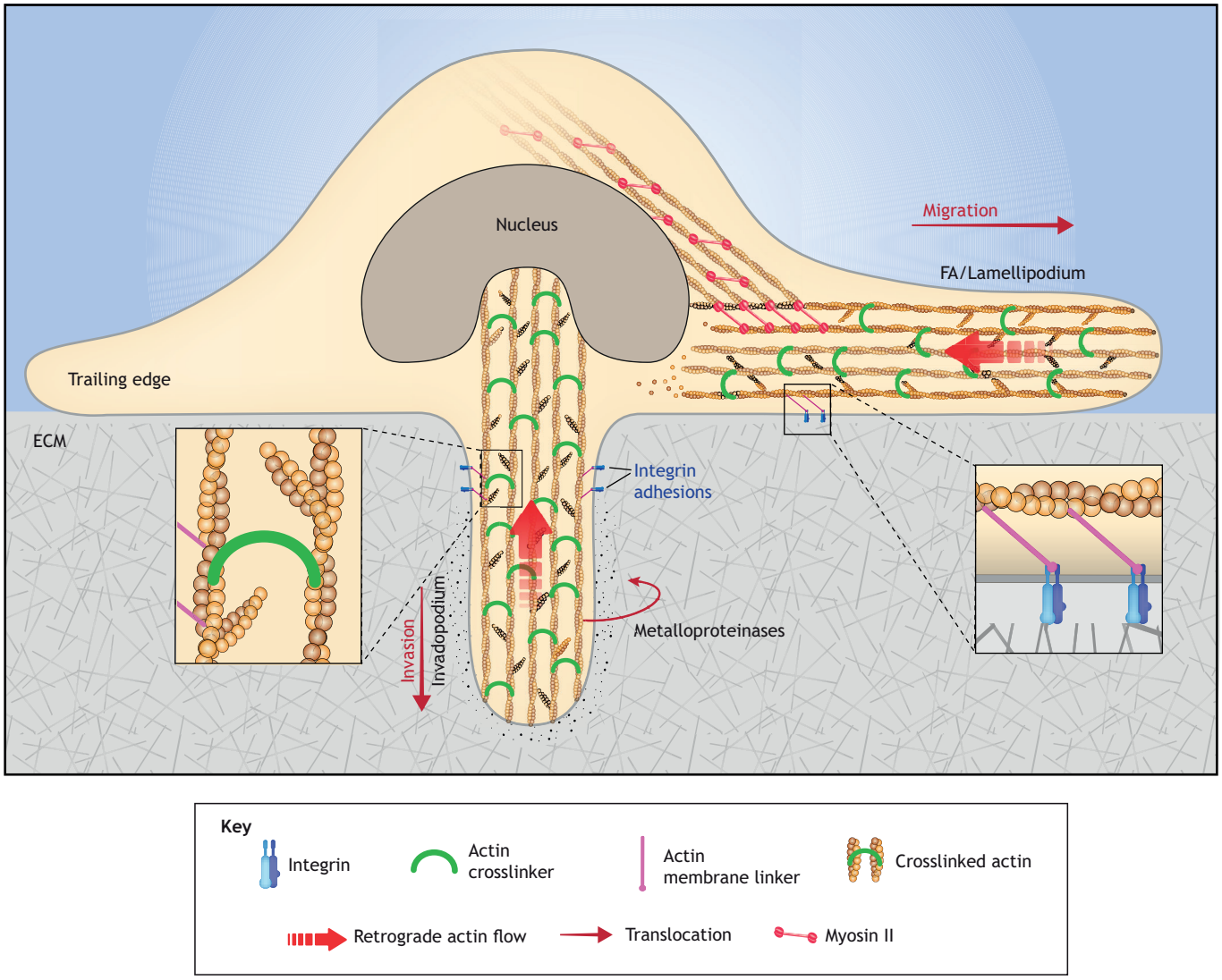

Fig. 2. Proposed model depicting both similar and distinct features of the protrusive machinery of the focal adhesion (FA)–lamellipodial system and invadopodia. Shown is a cell displaying two protrusion-based cellular translocations processes: cell migration (left to right) along the underlying ECM and invasion (top to bottom) into the underlying matrix. These protrusions are not drawn to scale and their dimensions, relative to each other and to the cell body, may vary. We propose that the initial mechanism involved in the formation of these two protrusions is almost identical, i.e. actin polymerization towards the cell center. In this step, shear forces are applied to nascent nearby integrin adhesions to reinforce them. However, the additional stages in the biomechanical processes underlying both protrusion–adhesion systems are quite different. FAs are stabilized by stress fibers; this is essential to support robust lamellipodial extensions, induced by tensile stress applied to FAs. Invadopodia, by contrast, show a physical elongation block of their core actin bundles by the nucleus, which increases the cytoskeletal pressure applied to the protrusion and, so, enhances its penetration into the ECM.

its transformation from an auto-inhibited to an active state (Cohen et al., 2006; Huttenlocher and Horwitz, 2011; Miyamoto et al., 1995; Swaminathan et al., 2017). This process can be followed by the recruitment of additional mechanosensitive proteins, such as FAK and p130Cas (officially known as BCAR1), which produce additional docking sites for multiple signaling molecules and other adhesome components (Janoštiak et al., 2014; Zebda et al., 2012).

The traction force generated by the actin polymerization-driven retrograde flow is crucial for the initiation of FA formation, yet, appears to be insufficient for complete transition of focal complexes into mature, large and stable FAs. This ‘maturation step’ requires an additional force generator, in the form of contractile actomyosin-containing stress fibers that anchor to the adhesion plaque and apply tensile forces of ∼5 nN/μm^2 to it (Balaban et al., 2001; Wehrle-Haller, 2012; Wozniak et al., 2004). Interaction with the contractile machinery, which leads to FA maturation, is manifested in the development of large, robust and stable adhesion sites, and is associated with specific changes in the protein composition of the adhesion plaque (e.g. recruitment of the LIM-domain protein zyxin), increased tyrosine phosphorylation of diverse adhesome components, extension of the adhesion sites, as well as its apparent mechanical reinforcement of the adhesion sites (Galbraith et al., 2002; Geiger et al., 2009). The structure–function relationships that drive FA initiation and maturation are only superficially understood; however, it is clear that the process, initially, is driven by polymerization of actin in the nearby lamellipodium and proceeds with involvement of the contractile actomyosin machinery.

Zoomed-in view of invadopodia assembly and functions

Invadopodia, much like FAs, are actin-based integrin adhesions; yet, they are primarily perceived as protrusive structures that can

mechanically penetrate into the underlying ECM and enzymatically degrade it (Gimona et al., 2008; Linder, 2007; Murphy and Courtneidge, 2011). Invadopodia and the structurally related podosomes were shown to facilitate matrix degradation in various physiological (e.g. tissue remodeling) and pathological contexts (e.g. metastatic invasion) (Linder et al., 2011).

The processes underlying invadopodia formation are still poorly understood. Their formation appears to be initiated by ligand-induced activation of receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR) and AXL (Revach et al., 2019; Yamaguchi et al., 2005, 2011), which trigger diverse signaling pathways that are activated by Src kinases (Bromann et al., 2004; Kelley et al., 2010; Tarone et al., 1985) and protein kinase C (PKC), as well as the cytoskeletal regulators N-WASP and cofilin (Bowden et al., 1999; Destaing et al., 2011; Rodriguez et al., 2009), which nucleate actin polymerization and recruit the adaptor protein SH3PXD2A (also known as and hereafter referred to as TKS5) to the adhesion site (Sharma et al., 2013). Once bound, TKS5 can interact with phosphatidylinositol-3,4-bisphosphate at the plasma membrane, leading to membrane recruitment of the actin regulators NCK1 and cortactin (Murphy and Courtneidge, 2011). These, in turn, activate the local polymerization of actin, i.e. the 'actin core', recruit the membrane type I-matrix metalloproteinase (MMP14, also known as and hereafter referred to as MT1-MMP), which initiates matrix degradation, and direct the local secretion of vesicles containing different metalloproteases (Artym et al., 2006; Murphy and Courtneidge, 2011). It has been shown that these early events of invadopodia formation are independent on forces exerted by actomyosin contractility (Gasparski et al., 2017; Gimona et al., 2008). For a more in-depth description of the mechanisms underlying early invadopodia assembly mechanisms, the reader is referred to previous publications (Murphy and Courtneidge, 2011; Revach and Geiger, 2014).

Shortly after assembly of the initial actin core, integrins and their associated adhesome components are recruited, and form an adhesive ring around the actin core. Continued polymerization of the actin core is believed to push the invadopodia membrane outwards and drive its penetration into the surrounding ECM (Murphy and Courtneidge, 2011; Schoumacher et al., 2010). Notably, besides integrins, the adhesion plaque of invadopodia contains classic scaffolding adhesome components, most of which are also prominent components of FAs, including paxillin, the paxillin-family member Hic-5 (officially known as TFGB1I1), vinculin, zyxin and ILK (Abdel-Rahman et al., 2017; Arnaout et al., 2005; Barczyk et al., 2010; Destaing et al., 2011; Revach et al., 2015). In addition, FAs and invadopodia share, among others, common regulatory molecules, such as Src family kinases and PTK2B (also known as and hereafter referred to as PYK2) (Genna et al., 2018; Kelley et al., 2010; Mader et al., 2011).

The molecular architecture of invadopodia provides basic functional clues on the protrusive activity of the invasive machinery. The ring-shaped adhesion keeps the cell membrane locally attached to the ECM, while the membrane-bound and secreted metalloproteinases 'soften' the nearby matrix, rendering it more susceptible to mechanical perturbation. Furthermore, the protrusive actin polymerization produces the compressive force, which effectively pushes the tip of the invadopodium into the matrix (Revach and Geiger, 2014).

This common view of invadopodia invasiveness requires that additional mechanistic questions are addressed. For example, how can the elongating actin core forcefully push the invadopodium tip outwards, rather than simply elongate towards the cell center? An interesting observation recently made in our laboratory demonstrated that the majority of invadopodia (~80%) in cultured human melanoma (A375) and breast cancer (MDA-MB-231) cells are located 'under' the nucleus, and their core actin bundles push against the nucleus and indent it (Revach et al., 2015). This association with the nucleus apparently interferes with the centripetal elongation of the core actin filaments, thereby increasing the compression force applied by the invading tip to the ECM. Given the known mechanical properties of the nucleus (Krause et al., 2013; Liu et al., 2014), measurements of the nuclear indentation further enabled us to calculate the invasive force applied to the matrix by the invadopodia tip. Based on these considerations, we estimated the compressive force applied by invadopodial protrusions to the ECM to be ~20 nN/μm^2 (Revach et al., 2015), which is a rather strong force relative to the tensile stress applied to FAs (and the underlying ECM), which is ~5.5 nN/μm2 (Balaban et al., 2001; Schwarz et al., 2002). Indeed, the magnitude of this compressive force is of the order or even higher than the rigidity values of mesenchymal connective tissue, which are in the range of 10–40 kPa, and, thus, could be physiologically relevant for invadopodia-mediated cancer invasion (Engler et al., 2006).

Invadopodia and focal adhesions – common assembly mechanisms but different functional properties

Taking into account the different biological properties of FAs and invadopodia, we wonder how it is possible that these two forms of integrin adhesions – which share so many of their 'integrin adhesome' and cytoskeletal constituents – form such different subcellular structures with distinct mechanical and functional properties.

To address this question at a structural/mechanistic level, we here consider two key parameters associated with the development of these structures – temporal and spatial. As discussed above and based on previous studies (Beaty and Condeelis, 2014; Beaty et al., 2013; Branch et al., 2012; Gasparski et al., 2017), and including our own observations (Riveline et al., 2001; Wolfenson et al., 2013; Zaidel-Bar et al., 2003, 2004), there are two clear distinct temporal stages during the development of both FAs and invadopodia, which we will refer to here as 'initiation' and 'maturation'. For FAs, the initiation step starts with the assembly of new nascent focal complexes under the lamellipodium (Alexandrova et al., 2008) and ends when the adhesion becomes dependent on actomyosin and contraction (Riveline et al., 2001; Zaidel-Bar et al., 2004). For invadopodia, the initiation step appears to begin with the formation of the actin core and to end when the core bundle reaches its full length and usually physically interacts, mechanically, with the nucleus (Revach et al., 2015).

Spatially, both adhesions are physically (and mechanically) interacting with neighboring cellular systems that affect the developing structures and can be considered as functional domains of the adhesion sites. For FAs, these are the lamellipodium and the attached stress fiber; for the adhesion ring of invadopodia, these are the invasive protrusion and the nucleus (see Fig. 2). A close look into the crosstalk between these sub-structures reveals a compelling similarity between FAs and invadopodia during the initiation stage and main differences during maturation of the two, which closely corresponds to the distinct functions of these adhesions (see Table 1). During the initial steps, both formation of focal complexes and nucleation of invadopodia are most likely to be triggered by local F-actin flow, owing to polymerization at the leading edge of the cell and in the tip of developing invadopodia, respectively. We hypothesize that shear

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Table 1. Structure and function comparison of FAs and invadopodia, and of forces that regulate their initiation, maturation and functionality (see also Fig. 2)

Function/Structure	Focal adhesions (lamellipodia)	Invadopodia
Basic process	Lamellipodial protrusion	Invadopodial protrusion
Cellular response	Migration	Invasion
Protrusion driver	Actin polymerization	Actin polymerization
Early protrusion–adhesion coupling	Shear stress applied to early adhesions by retrograde actin flow in lamellipodia	Shear stress applied to adhesion rings by retrograde actin polymerization within the core of invadopodia
Maturation and reinforcement of the adhesion–protrusion system	Tensile forces generated by contractile (actomyosin) stress fibers that enhance FA assembly and growth	Increased compressive force at invadopodia tips, due to force orientation by the nucleus, which enhances ECM penetration of invadopodia

forces applied by the 'treadmilling' actin play a similar role in activating the mechanosensitive adhesome proteins and drive initiation of the multi-protein adhesion complexes in the two systems (Table 1 and Fig. 2).

The fundamental difference between the maturation of FAs and invadopodia lies in their differential interactions with distinct cellular regulatory systems, including the actomyosin-based contractile machinery, the microtubular system, the nucleus and different signaling networks – primarily specific Rho-family GTPases, which are key cytoskeletal regulators (Nobes and Hall, 1995). For example, FA maturation depends on actomyosin-generated forces that are stimulated by activated RhoA GTPase (Parekh and Weaver, 2016; Zhou et al., 2015). Nucleation and maturation of the actin machinery in invadopodia, however, appears to be less dependent on actomyosin contractility than FAs and seems to be governed more strictly by the GTPase CDC42, which triggers the formation of finger-shaped (filopodial) protrusions (Murphy and Courtneidge, 2011) and might be responsible for the peculiar cylindrical shape of invasive protrusions. For further information concerning upstream regulatory networks of the Rho-family of GTPases, e.g. phosphoinositide 3-kinases PI3Ks, PKCα, different receptor tyrosine kinases (RTKs) and others, see Hemmings and Restuccia, 2012; Hoshino et al., 2012; Revach et al., 2019; Ségaliny et al., 2015.

The involvement of signaling processes in invadopodia and FA formation is consistent with the fact that both structures are highly tyrosine-phosphorylated (Mader et al., 2011; Maziveyi and Alahari, 2017), and that their formation is differentially affected by specific kinase and phosphatase modulators. For example, constitutively active Src expressed in normal fibroblasts induces a striking transition from a prominence of FAs to the formation of invadopodia-like 'podosomes rosettes' (Tarone et al., 1985). Furthermore, fine-tuning of Src, FAK and PYK2 signaling can differentially regulate FAs and invadopodia, and, consequently, may lead to different outcomes with regard to invasion and migration patterns (Chan et al., 2009; Genna et al., 2018; Kolli-Bouhafs et al., 2014). In part, the invadopodia-promoting effects of specific tyrosine kinases are commonly attributed to their capacity to phosphorylate and activate cortactin, a key promotor of actin polymerization and organization in invadopodia. The complexity of tyrosine-kinase-based signaling and the fine interplay between different kinases goes beyond the scope of this article; yet, we like to add that the balance between different kinases, e.g. ErbB3 and AXL, can have a major effect on invadopodium and FA formation and, consequently, on matrix degradation and cancer cell invasion (Revach et al., 2019).

As indicated, mature FAs and invadopodia differ in their local and global cellular mechanics. Whereas FA maturation strictly depends on contractility, the mechanical regulation of invadopodia can be less dependent on actomyosin-generated tensile forces. Inhibition of non-muscle myosin II, a main generator of tensile force in cells, or its upstream activators myosin light chain kinase and Rho kinase, abrogate not only FAs but also invadopodium-associated ECM degradation, despite the fact that myosin IIA, myosin IIB and phosphorylated myosin light chains do not localize to invadopodia (Alexander et al., 2008). However, the above mentioned experiments with pharmacological contractility inhibitors neither cause complete disassembly of invadopodia, nor fully abolish their ECM-degrading function. ECM rigidity or a high ligand density – which commonly induce higher cellular contractility – also elevate both invadopodium number and ECM-degradation activity (Alexander et al., 2008; Artym et al., 2015; Jerrell and Parekh, 2014). In addition, the length of invadopodia was shown to increase following the transient application of mechanical tugging stress transmitted to matrix fibers by magnetic beads, and increases cofilin activity and secretion of MMP2 (Gasparski et al., 2017).

However, knockdown of myosin light chain kinase was shown to cause a significant increase in the total number of invadopodia and the extent of ECM degradation in squamous cell carcinoma (Jerrell and Parekh, 2019). This is also in line with the finding that the general decrease of actomyosin contractility in response to the ROCK inhibitor Y27632 increases cancer cell invasiveness (Vishnubhotla et al., 2012).

Two molecular mechanisms have been proposed to account for the limited mechanosensitivity of invadopodia. In the first, the phosphorylated forms of the important mechanosensitive regulators p130Cas and FAK, are enriched in invadopodia actively degrading on rigid substrates. Their recruitment was significantly decreased upon treatment with contractility inhibitors and correlated with suppression of their degradation capacity (Alexander et al., 2008). In the second, ROCK1 and ROCK2, two isoforms of one of the key contractility regulator Rho-associated kinase (ROCK), differently affect invadopodia regulation (Jerrel and Parekh, 2016). Interestingly, these ROCK isoforms have opposite effects on invadopodia formation and activity (suppression by ROCK1 and activation by ROCK2), which might be related to the fact that the isoforms use distinct signaling pathways: the contractile, i.e. myosin II-related mechanism is activated by ROCK1, whereas the non-contractile and actin polymerization-dependent, i.e. LIMK–profilin-mediated mechanism is activated by ROCK2 (Jerrell and Parekh, 2016). These results represent an intriguing situation, in which two downstream targets of Rho A exert opposite effects on distinct integrin adhesions, FA and invadopodia, i.e. ROCK1 stimulates FA maturation and ROCK2 supports invadopodia outgrowth. These new results emphasize the importance of the LIMK–profilin pathway in driving actin polymerization in invadopodia.

It is also possible that a precise level of matrix rigidity affects invadopodium functions, as intermediate levels of ECM rigidity

enhance invadopodium-mediated ECM degradation, whereas both stiffer and softer matrices reduce ECM degradation (Jerrell and Parekh, 2016). However, a clear and coherent picture of the relationship between contractile forces and invadopodium dynamics is still missing.

Our reference to similar and distinct properties of FAs and invadopodia has, so far, been directly related to the involvement of the actin-based cytoskeleton and its associated signaling systems. However, another cytoskeletal regulatory activity is provided by the microtubule system (Revach et al., 2015; Schoumacher et al., 2010). For example, in breast cancer cells, intact microtubules are not essential for invadopodia initiation; yet, destruction of microtubule reduces invadopodia elongation and maturation (Schoumacher et al., 2010). This observation might be related to our findings that microtubules in A375 melanoma cells form a 'cage' around the actin cores of invadopodia, thus, potentially supporting their mechanical stability (Revach et al., 2015). By contrast, in FAs, microtubule depolymerization, induced by different drugs, reinforces FA formation due to the increased tension on stress fibers and Rho A activation (Liu et al., 1998). A main mechanism, which is currently thought to be responsible for this effect, is the release of the upstream Rho-activator GEF-H1 from depolymerizing microtubules, thereby triggering the Rho A pathway, a key activator of acto-myosin contraction (Chang et al., 2008; Krendel et al., 2002). In addition, extension of microtubules to the vicinity of FAs plays a key role in FA dissociation (Kaverina et al., 1999); this can be achieved by multiple mechanisms, such as integrin turnover and delivery of regulatory factors, among others (Stehbens and Wittmann, 2012).

In summary, based on the findings presented above, we propose that the initial assembly of both FAs and invadopodia is mediated by actin polymerization and treadmilling, occurring either at the edge of the lamellipodium or at the tip of the invading protrusion (Table 1 and Fig. 2). Application of shear stress by the treadmilling of actin to nearby adhesion molecules – mostly integrins and the associated components, i.e. talin, vinculin, and possibly FAK and/or PYK2 – then possibly triggers the assembly of functional adhesion structures. Binding to integrin, combined with the local shear force, is essential and sufficient to activate these mechanosensitive adhesome components, resulting in the development of focal complexes or adhesion rings in FAs and invadopodia. The subsequent assembly and growth of an 'adhesion plaque' enriched with actin-binding adhesome components (including talin and vinculin) in both systems, also augments outward pressure, either by acting on the leading edge of the lamellipodium (FAs) or the invasive protrusion (invadopodium), thus driving either cell migration or invasion by the respective systems.

Furthermore, the initial shear stress that is generated by the retrograde actin flow is estimated to be 30 pN/μm^2 in lamellipodia (Gardel et al., 2008) and is likely to be of the same order in invadopodia. However, this might be insufficient to support a deep penetration of invadopodia into the ECM or to develop robust and stable FAs. To amplify the level of force, the two types of adhesion have developed quite distinct solutions. The maturing FAs associate with contractile stress fibers that can apply relatively high tensile forces (Balaban et al., 2001), which are needed for FA maturation, stability and growth. By contrast, invadopodia appear to utilize quite a different mechanism to enhance the invasive-protrusive force; we propose that this is based on physically blocking elongation of the polymerizing actin bundle with the nucleus, thereby increasing the compressive force that is applied locally to the ECM at ∼20nN/μm^2 (Revach et al., 2015) (Fig. 2). In line with this view, Ferrari and colleagues recently claimed that forces generated by actin polymerization per se are insufficient to effectively penetrate the matrix and suggested that frictional forces, generated in the course of invadopodia bending, play a role in reinforcing the ECM-penetration phase (Ferrari et al., 2019a). Our proposed model is also highly relevant to invadopodium dynamics and mechanics during 3D matrix invasion *in vivo*. Although specific information regarding the 3D organization of invadopodia *in vivo* is scarce, we suggest that core actin bundles that are elongating towards the cell center have a high likelihood of encountering the centrally located nucleus, thereby enhancing the radial protrusion of invadopodia. Further, a nuclear involvement is relevant for cancer cell invasiveness in 3D matrices, as interaction between invadopodia and the nucleus might have additional effects besides the direct mechanical support of radial invadopodia extension, e.g. modulation of transcription.

A recently published study described an additional mechanosensitive process that can contribute to invadopodia-mediated matrix degradation (Infante et al., 2018). It was shown that the MT1-MMP-secretory compartment localizes anteriorly to the nucleus in the direction of cell invasive migration, thereby increasing the efficient delivery of proteolytic enzymes to invadopodia. Such polarization was observed in cells migrating through 3D collagen matrix of high rigidity but not through soft gels. The authors attribute this localization of the secretory compartment to the mechanical stress experienced by the nucleus during invasion through spatially-confined conditions. In this situation, the adaptor proteins linker of nucleoskeleton and cytoskeleton (LINC) complex and Lis1 (officially known as PAFAH1B1) that connect the nucleus to the cytoskeleton direct microtubules, which serve to deliver MT1-MMP-containing vesicles in a polarized manner towards invadopodia (Infante et al., 2018; reviewed by Ferrari et al., 2019b).

After combining these recent observations with our data (Revach et al., 2015), we propose that the nuclear indentation – beyond directly reinforcing penetration of invadopodia – also stimulate and direct MT1-MMP secretion, thereby facilitating the invasive functionality of invadopodia.

Conclusions and perspectives

In this Hypothesis, we discuss common mechanisms that drive early stages during the development of focal adhesions and invadopodia, and review distinct processes that lead to the maturation of the two integrin-associated adhesions. We hypothesize that adhesions to the ECM in both structures are initiated by shear forces that are generated by nearby polymerizing actin filaments and further reinforced by mechanical stress – in the case of FAs through actomyosin based traction, and through compression by the nucleus and the actin bundle of invadopodia. These distinct maturation mechanisms contribute to formation of adhesion with different functionalities – namely robust and stable adhesions to ECM via FAs, and ECM degradation and invasion by invadopodia. Further structural and biophysical studies are needed to obtain the still-missing information regarding the proposed mechanisms as well as their differential regulation in adhesive versus invasive cellular contexts.

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Competing interests

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