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Review

Cytoskeleton networks in basement membrane transmigration

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ABSTRACT

The basement membrane (BM) is a dense, tightly cross-linked matrix that acts as physiological barrier to maintain tissue homeostasis. Studies on *Caenorhabditis elegans*, leucocytes diapedesis and cancer cell invasion have shown that BM transmigration is a conserved three-stage process. Firstly, invadopodia-like protrusions form at the ventral surface of invasive cells; later, one protrusion elongates that lastly drives the infiltration of cells into the underlying compartment. This review illustrates the mechanism used by invasive cancer cells to cross the BM barrier by focusing on the role of key cytoskeleton components. We also describe currently available in vitro assays to study each step of the BM transmigration program.

Introduction

Acquisition of an invasive behavior is a prerequisite for cancer metastasis. The majority of cancers arise from epithelial tissues that are separated from the stromal compartment by the basement membrane (BM) (Thiery, 2002). The BM is a thin, dense and highly cross-linked matrix that regulates tissue architecture and normal cell function (Kalluri, 2003). To escape from the primary tumor and infiltrate the surrounding tissues, invasive cancer cells must first traverse this BM barrier. While there is great interest to understand cancer cell invasion, the mechanism of BM transmigration by tumor cells remains partially understood.

In vitro, when invasive cancer cells are cultured on the top of an extracellular matrix (gelatin, fibronectin, laminin or matrigel), they develop finger-like protrusions on their ventral side, called invadopodia, that degrade the underlying matrix (Chen, 1989). Since the discovery of these "invasive feet", it has been assumed that they perforate the BM and thus initiate the invasion process. Nevertheless, it is still unknown whether invadopodia exist in vivo and how invasive cells use invadopodia to infiltrate into the subjacent stromal compartment.

New insights into the mechanism of how invasive cells cross the BM come from in vivo studies of the larval development of the nematode *Caenorhabditis elegans* (*C. elegans*) (Sherwood, 2006), work on diapedesis of immune cells (Carman, 2009) and ex vivo studies using a native BM isolated from the rat peritoneum (Hotary et al., 2006; Schoumacher et al., 2010).

During larval development in *C. elegans*, one specialized gonadal cell, the anchor cell, traverses two BM layers in order to invade the vulval epithelium. This invasion process forms the uterine–vulval connection, essential for mating and moving embryos in the outside environment. The invading cell develops small protrusions reminiscent to invadopodia that break BMs and penetrate between vulval cells to promote attachment of the anchor cell into the vulval epithelium (Fig. 1A) (Sherwood, 2006).

Immune cells often cross endothelial barriers as they enter (intravasation) or exit (extravasation) the blood circulation. Leucocytes can cross endothelia by either infiltrating between two endothelial cells (paracellular diapedesis), or invading directly through one endothelial cell (transcellular diapedesis). Interestingly, transcellular diapedesis resembles the invasion of the anchor cell in *C. elegans*. After arrest on endothelial wall, leucocytes migrate laterally and form invadopodia-like invaginations that probe the endothelium for less resistant zones. These invaginations can then protrude nearly the entire endothelial cell length. Such protrusive behavior seems to precede and to be required for an efficient diapedesis/transmigration of leucocytes (Fig. 1B) (Carman, 2009).

We have recently showed that invasive tumor cells cultured on a BM isolated from the rat peritoneum initially perforate the

Abbreviations: BM, basement membrane; ECM, extracellular matrix; MMPs, metalloproteinases; EM, electron microscopy.

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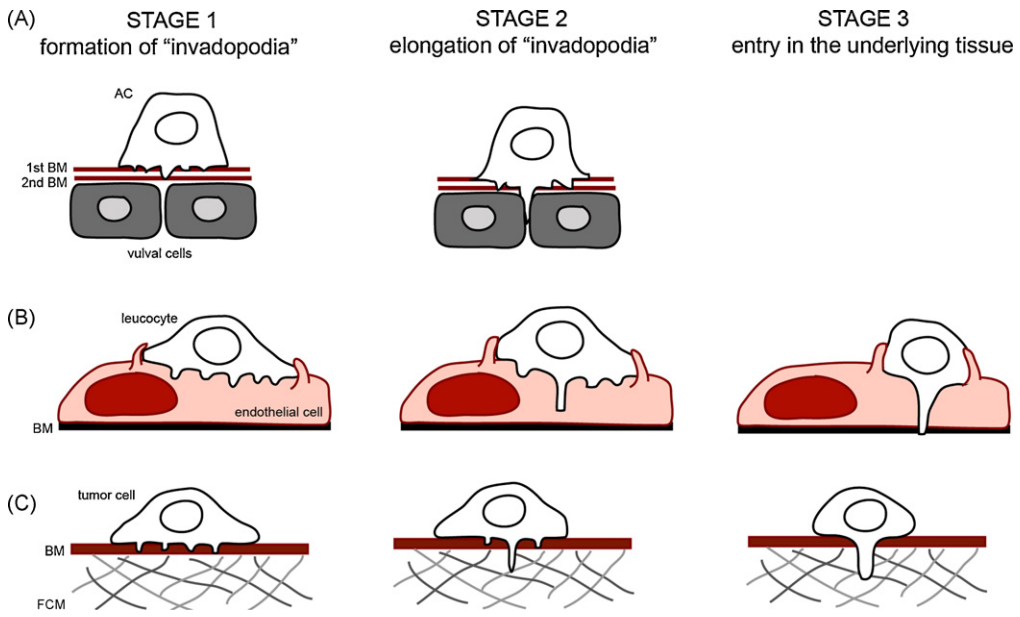

Fig. 1. The basement membrane transmigration program is a conserved mechanism. The crossing of basement membranes (BMs) by invasive cells is a conserved three-stage process: invadopodia-like protrusions form at the ventral cell surface (stage 1), then these protrusions elongate (stage 2) to later infiltrate to the underlying compartment (stage 3). (A) Invasion of the anchor cell (AC) in *C. elegans*. The AC breaks two BM layers before infiltrating between two vulval cells. Adapted from Sherwood (2006). (B) Transcellular diapedesis of leucocytes. Leucocytes probe endothelial cell wall for less resistant zones by invadopodia-like structures before traversing the whole cell length. Adapted from Carman et al. (2007). (C) Crossing of native BM by invasive cancer cells. Invasive tumor cells perforate the epithelial BM before penetrating the underlying extracellular matrix (ECM) compartment. Adapted from Schoumacher et al. (2010).

membrane by developing actin-rich protrusions (Schoumacher et al., 2010). These protrusions were characterized as invadopodia by the presence of the main invadopodia markers cortactin, MT1-MMP and c-src (Buccione et al., 2004; Linder, 2007; Weaver, 2006). Invadopodia further elongate and lastly develop into larger protrusions that guide the cell into the subjacent 3D compartment (Fig. 1C) (Hotary et al., 2006; Rowe and Weiss, 2008).

Hence, invasive cancer cells use a similar mechanism to leucocytes and the anchor cell to cross physiological barriers (BM or endothelium). In these three illustrated examples, the BM transmigration program is executed in three steps: (1) invasive protrusions (invadopodia) form and perforate the BM; (2) invadopodia elongate and mature in the degraded zone; (3) cell penetrate into the stromal compartment (Figs. 1 and 3A) (Schoumacher et al., 2010). However, it is yet undefined whether the first stage of leucocytes diapedesis or anchor cell invasion depends on MMP activity as the invasion of cancer cells.

Genes that are only expressed during embryonic development and/or wound healing are often again switched on when cancer cells become invasive (Thiery and Sleeman, 2006). The cytoskeleton plays an essential role in cell motility and many cytoskeletal proteins are often misregulated during cancer invasion (Condeelis et al., 2005; Vignjevic and Montagnac, 2008). How cancer cells reprogram the cytoskeletal machinery to fulfill their new ability to penetrate through physiological barriers such as BM is not fully understood.

Currently, it is difficult to assess the molecular mechanism responsible for BM transmigration by cancer cells in vivo. The relative tissue inaccessibility and the limitations of the whole body imaging techniques of living mice as well as the time consumption that genetic manipulations of the animals require make the in vivo approach challenging. It is therefore essential to design robust and physiologically relevant in vitro models.

In this review, we will describe in vitro experimental approaches used to study each step of BM transmigration and focus on the roles of key cytoskeleton components during this process.

Methods to study basement membrane transmigration by cancer cells

Stage 1: invadopodia formation

The classical invadopodia assay consists of plating cells on a thin layer of extracellular matrix (ECM) with a maximum of 2 μm thickness, made of either fluorescently labeled gelatin or unlabeled gelatin overlaid with fluorescent fibronectin. Cancer cells degrade the matrix by producing dark spots in the fluorescent background. Invadopodia are then defined as actin puncta that localize to sites of matrix degradation (Fig. 2A). Invadopodia could also be studied by plating invasive cells on a thick layer of matrigel, about tens to hundreds of microns thick (Lizarraga et al., 2009). Matrigel is a gelatinous mixture of collagen IV, laminin and proteoglycans secreted by Engelbreth–Holm–Swarm (EHS) mouse sarcoma cells, whose molecular composition is similar to the one of native BMs (Kalluri, 2003). Matrigel can be visualized by labeling one of its main components, laminin or collagen IV, with a desired fluorophore (Schoumacher et al., 2010) (Fig. 2A). However, neither cross-linked gelatin (or fibronectin) nor matrigel faithfully mimic native BM (Table 1). Therefore, they should be used as complementary approaches to study invadopodia formation.

How do we identify invadopodia? In transmission electron micrographs, invadopodia appear as long and thin projections at the ventral surface of the cells that enter the ECM (Buccione et al., 2004; Chen, 1989; Lizarraga et al., 2009; Weaver, 2008). At the light microscopy level, active invadopodia are defined as actin-rich structures, associated with matrix degradation capacity. In addition to the metalloproteinase MT1-MMP which is crucial for matrix dissolution (Artym et al., 2006; Hotary et al., 2006; Poincloux et al., 2009), the presence of the active c-src kinase and/or a high level of phosphotyrosinated proteins in actin puncta are often used as invadopodia markers (Bowden et al., 2006; Buccione et al., 2004; Linder, 2007).

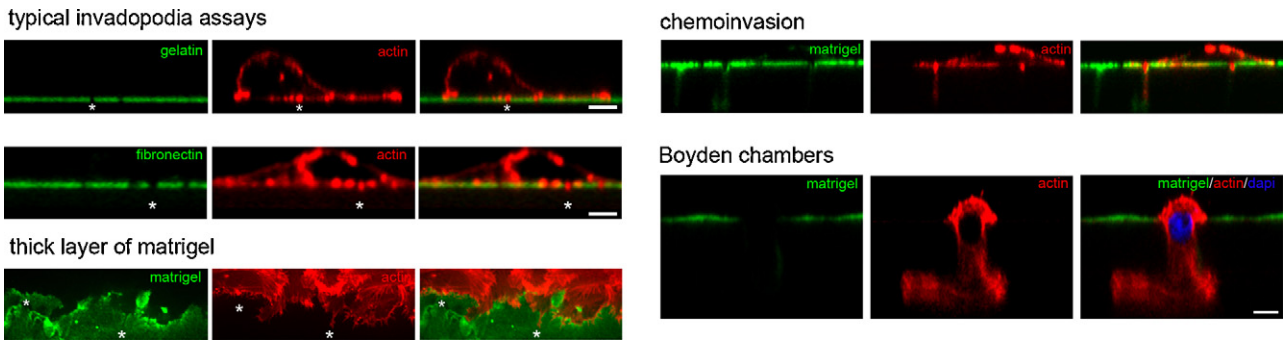

Fig. 2. In vitro assays to study basement membrane transmigration. Invasion of the breast cancer cells MDA-MB-231 was assessed in the different models. Cells were immunostained with phalloidin-Cy3 to visualize actin cytoskeleton. Scale bar: 5 μm. Left panels: models to study stage 1 of BM crossing. Typical invadopodia assays. Top: thin layer of gelatin (green) coated on glass. Bottom: thin fibronectin layer (green) atop of gelatin coated on glass. Thick layer of matrigel: matrigel (green) coated on glass. Right panels: models to study stages 2 and 3 of BM crossing. Top: chemoinvasion assay (stage 2 of invasion). Fluorescently labeled matrigel (green) coated on a filter containing 1 μm-diameter pores. Bottom: Boyden chamber assay. Matrigel coated on a filter containing 8 μm-diameter pores and visualized by immunostaining for laminin and collagen IV (green).

Stage 2: invadopodia elongation

In the second stage of invasion, invadopodia elongate before the invasive cell penetrates the underlying tissue (Schoumacher et al., 2010; Sherwood, 2006). All methods described above have limitations that preclude analysis of invadopodia elongation. For example, in the gelatin degradation assay, cells are plated on thin layers of ECM coated directly onto glass, thus the extension of invadopodia beyond the thin matrix layer is physically blocked. In the assays using thick layers of matrigel or fibronectin on the top of gelatin, cells are not chemoattracted and thus they mostly migrate in the *x*–*y* plane. As a consequence, invadopodia penetrate into the matrix only superficially and rarely elongate more than 2 μm (our unpublished data).

It has been recently developed a new robust in vitro chemoinvasion assay, which allows elongation of invadopodia (Schoumacher et al., 2010). This assay resembles the modified Boyden chamber in which cells are plated on a filter containing 8 μm-diameter pores and are chemoattracted using growth factors enriched medium in the lower chamber. In the chemoinvasion assay, the filter contains 1 μm-diameter pores and is coated with a thin layer of fluorescently labeled matrigel to mimic the BM. Besides covering the entry of the pores, the matrix also penetrates and fills the first few microns. The size of the pores corresponds to the average diameter of invadopodia, but it is smaller than the cell nucleus. As a consequence, cells form invadopodia without passing through the filter. In addition, the thickness of the filter (about 10 μm) allows the elongation of invadopodia inside the pores (Fig. 2B; Table 1).

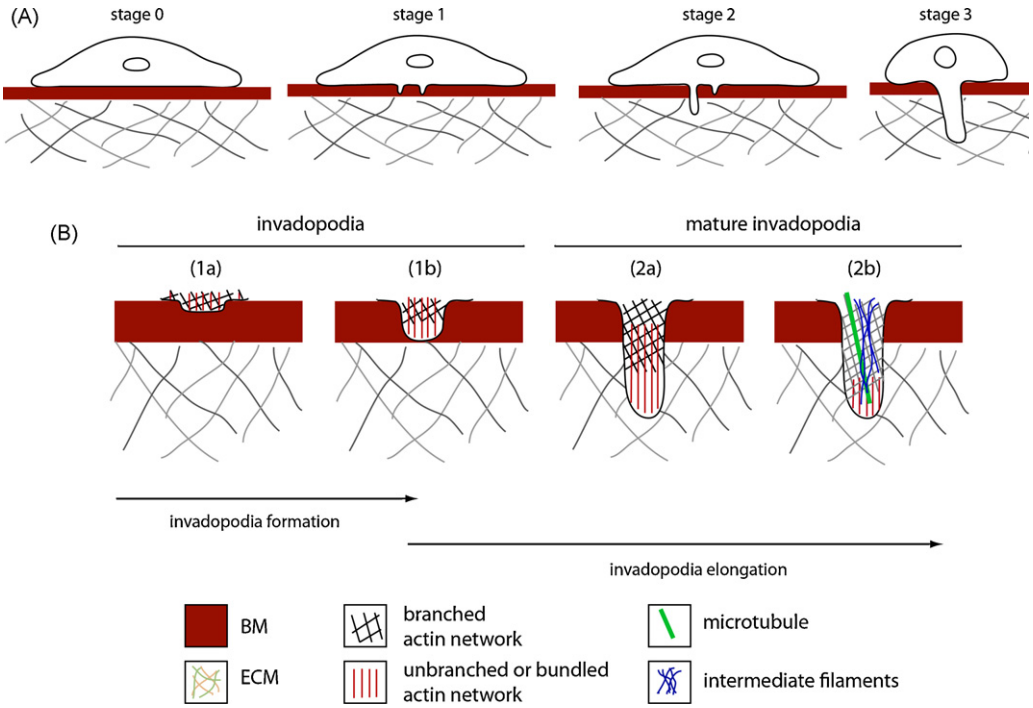

Fig. 3. Remodeling of the cytoskeleton during transmigration of basement membrane. (A) Stages of basement membrane (BM) breaching by invasive cancer cells. Non-invasive tumor cells do not degrade the BM (stage 0). Once cancer cells become invasive, invadopodia form and degrade the BM (stage 1). Further, invadopodia elongate (stage 2), and lead the cell to infiltrate into the stromal compartment (stage 3). (B) Cytoskeleton organization in invasive protrusions. Formation of invadopodia requires the assembly of dendritic/diagonal and bundled actin networks (1a). Elongation of invadopodia is achieved by growth of actin bundles, sustained by the dendritic/diagonal actin network (1b and 2a). Microtubules and intermediate filaments penetrate mature invadopodia, while actin bundles are replaced by the expansion of the dendritic/diagonal actin network (2b).

Table 1
In vitro assays to study basement membrane transmigration.

	Gelatin/fibronectin	Thick layer of matrigel	Chemoinvasion	Boyden chambers	Native BM (rat peritoneum)	3D-culture system
Scheme						
Stage	Stage 1	Stage 1	Stage 2	Stage 3	Stages 1/2/3	Stages 1/2/3
Advantages	Rapid and robust evaluation of invadopodia molecular composition Quantifiable measurement of invadopodia activity/matrix degradation Thin and cross-linked matrix as native BM	Chemical composition similar to BM	Rapid and robust spatial distribution of invadopodia components Quantifiable measurement of invadopodia elongation	Rapid and robust evaluation of cell "diapedesis" Quantifiable measurement of cell invasion efficiency	3D organization of matrices similar to in vivo Native BM	3D organization of matrices similar to in vivo Quantifiable measurement of the efficiency of cell invasion
Disadvantages	Physical obstruction of invadopodia elongation Chemical composition different from native BM	Less stringent barrier (absence of cross-links) Absence of chemoattraction: migration in 2D without invadopodia elongation	Less stringent barrier (absence of cross-links) Physical constraints imposed by the pores	Less stringent barrier (absence of cross-links) Absence of matrix in the pores: migration rather than invasion	Ethical constraints: use of animals Time consuming	Less stringent barrier (absence of cross-links)
Level of difficulty	Easy	Easy	Moderate	Easy	Difficult	Moderate
References	Artym et al. (2009), Artym et al. (2006), Monsky et al. (1994)	Hooper et al. (2006), Lizarraga et al. (2009)	Schoumacher et al. (2010)	Albini and Benelli (2007), Allavena et al. (1988)	Hotary et al. (2006), Schoumacher et al. (2010)	Kikuchi and Takahashi (2008)

Stage 3: cell entrance into the stromal compartment

Boyden chambers are commonly used to assess the ability of cells to traverse a BM barrier. In this assay, cells must degrade a thin matrigel layer before passing through the empty pores of the filter (Fig. 2C; Table 1). This contrasts with the in vivo situation where cancer cells, after breaching the BM, penetrate the underlying stroma mostly composed of a collagen I network, which molecular composition and stiffness differ from the BM. Even though the use of matrigel and the absence of matrix in the pores do not fully recapitulate the physiological condition (Rowe and Weiss, 2008), this assay is widely used for screenings of molecules that are potentially involved in cancer cell invasion. However, since the mechanism of cell invasion is regulated by the rigidity and molecular composition of matrices (Even-Ram and Yamada, 2005; Friedl and Wolf, 2010; Kumar and Weaver, 2009), it is important to validate findings obtained with the Boyden chamber assay, using for example the native BM model.

In this model developed by Weiss' group, the rat peritoneal BM is tightened on a plastic transwell and collagen I is added on the lower surface, recapitulating the organization of epithelial tissues (Hotary et al., 2006). This allows testing the ability of cancer cells to pass through the BM and to enter the collagen matrix. Although this assay represents a physiologically relevant method to study cancer cell invasion in vitro, the preparation of the BM requires a high number of animals, thus precluding high-throughput studies.

More recently, Kikuchi and Takahashi (2008) developed an assay in which a thin layer of fluorescently labeled matrigel is coated on the top of a thick collagen I matrix, recapitulating in vivo conditions (Fig. 2C; Table 1). Highly invasive cells have been shown to degrade the matrigel layer and penetrate the subjacent collagen matrix.

Role of the cytoskeleton in basement membrane transmigration

The actin cytoskeleton is essential for invadopodia formation

Microfilaments are the only cytoskeletal component described to be in invadopodia (Linder, 2007). Actin filaments can be organized in a dendritic/diagonal actin network as in lamellipodia, or in actin bundles as in filopodia. While the dendritic/diagonal actin array pushes the plasma membrane allowing cells to crawl on a two-dimensional substrate, actin bundles produce the required stiffness for the formation of finger-like extensions, filopodia, that are used to explore the environment.

The first actin-binding proteins discovered in invadopodia were lamellipodial proteins: the Arp2/3 complex, cortactin and cofilin (Bowden et al., 1999, 2006; Yamaguchi et al., 2005). The Arp2/3 complex and its regulator cortactin are necessary for invadopodia formation while cofilin is essential for invadopodia stability and their degradation activity. A functional relationship between these proteins was recently shown, providing a molecular mechanism for the initiation of invadopodia. In "latent" conditions, cortactin binds to cofilin and inhibits its severing activity. Phosphorylation of cortactin by c-src or other invadopodia-associated kinases releases cofilin allowing Arp2/3-dependent actin polymerization and thereby initiating the formation of an invadopodium (Oser et al., 2009; Webb et al., 2007). Hence, invadopodia formation relies on the dendritic/diagonal actin network, similarly to lamellipodia.

Recently, several studies demonstrated the requirement of filopodial proteins for invadopodia biogenesis. Mena, one isoform of the VASP family of proteins, localizes in invadopodia and is necessary for their stability (Philippar et al., 2008). Diaphaneous-related formins (DRF/mDia) are also present in invadopodia and they are required for invadopodia formation, gelatin degradation

and protrusion into matrigel (Lizarraga et al., 2009). In addition, fascin, the major actin-bundling protein of filopodia, and myosinX, the filopodia-specific molecular motor, are also enriched in invadopodia and are essential for their formation (Li et al., 2010; Schoumacher et al., 2010). Further, besides similar morphology, both invadopodia and filopodia depend on the activity of the same GTPase Cdc42 (Sakurai-Yageta et al., 2008; Yamaguchi et al., 2005). These observations demonstrate that both dendritic/diagonal and bundled actin arrays cooperate to give rise to functional invadopodia.

Depletion of the Arp2/3 complex or fascin reduces the degradation capacity of invadopodia as well as their lifetime (Schoumacher et al., 2010), suggesting that both dendritic/diagonal and bundled actin networks are required to stabilize the protruding invadopodium. Interestingly, depletion of myosinX decreases matrix degradation without significantly affecting invadopodia lifetime (Schoumacher et al., 2010), arguing that myosinX plays a role in the formation of invadopodia rather than in the stabilization of the actin core. One could imagine that myosinX transports proteins, like MMPs, along the invadopodia shaft as the protrusion grows, which could explain its requirement for matrix degradation. Live imaging analysis of MMPs delivery in invadopodia could help to test this hypothesis.

The key feature of invadopodia is their ability to degrade matrices, which is the prerequisite for BM crossing. It is believed that the rod-like shape of invadopodia allows focal delivery of MMPs to restrictive areas of the BM. A potential mechanism for MMPs delivery to invadopodia has emerged with the discovery of the role of the exocyst complex and SNARE proteins in MT1-MMP trafficking and invadopodia formation (Poincloux et al., 2009; Sakurai-Yageta et al., 2008; Steffen et al., 2008). However, the coupling of MMPs delivery to invadopodia with the growth of the actin core is yet only partially understood. Only one study addressed this point by analyzing the time sequence of the recruitment of cortactin and MT1-MMP to invadopodia. Cortactin is recruited to invadopodia before MT1-MMP showing that actin assembly precedes clustering of MMPs (Artym et al., 2006). Moreover, cortactin has been shown to regulate secretion of major MMPs (MT1-MMP, MMP-2 and MMP-9) at invadopodia (Clark et al., 2007), suggesting that cortactin may coordinate actin assembly and focal delivery of MMPs at invadopodia sites (Clark and Weaver, 2008).

Based on these observations, it is plausible that actin cytoskeleton reorganization might be required to concentrate MMPs and for focused matrix degradation at specific sites of the BM. Interestingly, depletion of the MT1-MMP or inhibition of MMPs activity impairs accumulation of actin and cortactin at the ventral surface of invasive cells, suggesting a positive feedback loop in which MT1-MMP itself or ECM degradation products contribute to the initiation or maturation of invadopodia (Ayala et al., 2008; Sakurai-Yageta et al., 2008; Steffen et al., 2008).

Actin cytoskeleton cooperate with microtubules and vimentin intermediate filaments to elongate invadopodia

Invasive cells sitting on the BM usually form several invadopodia at their ventral surface, but only elongate one protrusion (our unpublished data). It remains unclear whether several invadopodia are merging or whether a single invadopodium elongates to give rise to longer invasive protrusion.

All three cytoskeletal networks are required for invadopodia elongation, the second step of BM crossing (Schoumacher et al., 2010).

Invadopodia formation is dependent on both dendritic/diagonal and bundled actin networks, but the internal architecture of the invadopodial actin core is still under debate (Baldassarre et al., 2006; Linder, 2007; Lorenz et al., 2004). While the activator of Arp2/3 complex, N-WASP, is enriched at the base of invadopodia (Lorenz et al., 2004; Schoumacher et al., 2010), the distribution of the Arp2/3 complex is variable depending on the cell type, being restricted only to the base or present all along invadopodia (Baldassarre et al., 2006; Linder, 2007; Lorenz et al., 2004). The actin-bundling protein fascin is mostly present along invadopodial shafts, while myosinX and an active form of the formin mDia2 are often found at the tip of elongated invadopodia. These data together with electron microscopy analyses show that elongated invadopodia have a structure reminiscent to the roots of filopodia with actin bundles embedded in a dendritic/diagonal actin array. Moreover, depletion of the filopodial or lamellipodial machinery decreases the length of the protrusions, showing that both dendritic/diagonal and bundled actin networks are required for the second step of BM transmigration. While the dendritic/diagonal network could be necessary to generate actin filaments and stabilize the roots of actin bundles, the filopodial machinery could be required for the elongation of actin bundles allowing invadopodia to protrude beyond the perforated basement membrane. Dynamic analysis of the recruitment of key actin-binding proteins would help to support this model.

Different types of cells exploit the force produced by actin bundling to penetrate other cells or tissues. In invasive cancer cells, actin bundles could provide the necessary stiffness to penetrate the rigid tumor tissue. During tumor progression, the ECM is indeed progressively stiffened (Paszek et al., 2005), which enhances invadopodia formation (Alexander et al., 2008; Enderling et al., 2008). Similarly, during the spreading of the bacteria Listeria from one host cell into another, the actin network in the comet tails is reorganized from a dendritic/diagonal to tight actin bundles (Vignjevic and Montagnac, 2008). In the acrosome reaction, spermatozoids also employ actin bundles to penetrate into oocytes (Tilney, 1975). Hence, actin bundling seems to be a general mechanism for “penetration” (Vignjevic and Montagnac, 2008).

An intact microtubule network is required for the function of invadopodia-related structures, podosomes, formed in monocytes and osteoclasts (Kopp et al., 2006; Linder, 2007). However, stabilization or disruption of the microtubule network does not impair invadopodia formation (Kikuchi and Takahashi, 2008; Schoumacher et al., 2010). Even though microtubules are not present in invadopodia, they are required for invadopodia elongation (Kikuchi and Takahashi, 2008; Schoumacher et al., 2010). Thus, they play a role in the transition between the second and third step of BM transmigration. Given their function in intracellular trafficking, it is possible that microtubules provide tracks for delivery of specific proteins (such as MMPs) necessary for invadopodia elongation (Linder, 2007; Poincloux et al., 2009; Sakurai-Yageta et al., 2008). In agreement with this hypothesis, the exocytosis of MMP-2 and MMP-9 in melanoma cells is microtubules-dependent (Schnaeker et al., 2004).

Furthermore, the key cell-polarity regulator IQGAP1 is present in invadopodia (Sakurai-Yageta et al., 2008). IQGAP1 can simultaneously bind to actin filaments and the microtubule +TIP protein CLIP170 (Brown and Sacks, 2006), suggesting that cooperation between actin and microtubule networks may take place in invadopodia. Indeed, both cytoskeletons as well as numerous vesicles are present in the mature invadopodia as observed by electron microscopy (Schoumacher et al., 2010).

Cytoplasmic intermediate filaments (IFs) play a role in cell cortex resistance and stability of cell–cell adhesions. As a result, they are considered as a stress-buffering cytoskeleton that maintains cell and tissue integrity (Eriksson et al., 2009; Herrmann et al., 2007). The two main cytoplasmic IFs are cytokeratins and vimentin. Cytokeratins represent a large family of proteins specific to epithelial cells, while vimentin is expressed by mesenchymal cells. During cancer invasion, carcinoma cells often undergo a complete or par-

tial epithelial-to-mesenchymal transition (EMT): they completely or partially lose their epithelial markers and gain mesenchymal ones (Thiery, 2002; Yilmaz and Christofori, 2009). For instance, invasive cancer cells often change their repertoire of cytokeratins and start expressing vimentin. Vimentin is widely used as an EMT marker, but its precise function in cancer cell invasion is just emerging. Vimentin is normally expressed in highly migratory or invasive cells: mesodermal and neural crest cells during embryonic development and cells at the edge of the lesion during wound healing (Thiery et al., 2009). Vimentin filaments also control changes in cell shape, adhesion and motility observed during EMT (Mendez et al., 2010). Even though vimentin localizes with the actin-bundling protein, fimbrin, in podosomes of macrophages (Correia et al., 1999), neither vimentin nor cytokeratins are present and involved in invadopodia formation (Schoumacher et al., 2010). However, an intact vimentin filament network is required for invadopodia elongation. This is the first direct evidence that IFs have a role in cancer cell invasion, suggesting that vimentin filaments could confer cancer cells the physical and mechanical resistance necessary to infiltrate the stromal compartment.

Role of the cytoskeleton during cell translocation into the 3D compartment

The last step of BM crossing most likely depends on the same molecular components as invadopodia elongation.

Requirement of fascin (Hwang et al., 2008; Jawhari et al., 2003; Vignjevic et al., 2007), cortactin (Hill et al., 2006; Patel et al., 1998; Rothschild et al., 2006) and formins (Lizarraga et al., 2009) for the invasion of cancer cells in Boyden chambers suggests that both intact dendritic/diagonal and bundled actin networks are required for the infiltration of cancer cells into the underlying tissues.

Similarly, in the Boyden chamber assay, migration and invasion of cancer cells is blocked when microtubules are stabilized by paclitaxel (Taxol) (Stearns and Wang, 1992; Westerlund et al., 1997). While delivering essential proteins for cell invasion (see above), microtubules could also provide the force required to move the nucleus during cell translocation as it has been shown during neuronal migration (Schaar and McConnell, 2005; Umeshima et al., 2007). Interactions between dynamic microtubules and actin filaments are essential in a wide variety of biological events (Geraldo and Gordon-Weeks, 2009; Rodriguez et al., 2003). However, it remains to be demonstrated whether the interaction of these two cytoskeletal networks has a role in the last step of cancer cell translocation.

Depletion of vimentin in prostate cancer cell decreases their capacity to invade, using the Boyden chamber assay (Zhao et al., 2008). After BM crossing, tumor cells are invading the surrounding parenchyma. As they move inside this three-dimensional (3D) matrix, they push against collagen fibers thereby exchanging mechanical forces with their environment (Kumar and Weaver, 2009). The switch from cytokeratins to vimentin could be fundamental for cell resistance to these mechanical forces. Invasive carcinoma cells can invade either as individual cells or as a collective group of cells that maintain cell–cell adhesions (Friedl and Wolf, 2010). Cells migrating individually have a mesenchymal phenotype and express vimentin. In collective migration, the tip cell of the cell cluster has an analogous morphology. However, whether tip cells undergo at least a partial EMT and whether they express vimentin remains to be analyzed (Friedl and Wolf, 2010). Individual cells invading a 3D collagen matrix encounter a resistance force while pushing against collagen fibers. On contrary, this force is shared between all cells in the cluster while migrating collectively. Since intermediate filaments regulate cell shape and cell resistance, one could imagine that the expression of vimentin in isolated migratory cells is required to resist constraints and maintain cell shape.

Conclusion

The dissolution of the BM by invadopodia represents the first step of the metastasis cascade. Invasive cancer cells use all three cytoskeleton networks to execute their infiltration into the stromal compartment. They turn on cytoskeleton-related genes that are expressed by mesodermal or neural crest cells during embryonic development (Thiery, 2002; Thiery and Sleeman, 2006), such as fascin and/or vimentin. Cancer cells seem to simply reactivate latent morphogenic programs already encoded in their genome to counteract physiological barriers and promote cancer progression. Several open but fascinating points remain to be challenged. What are the signals that trigger invadopodia formation, activating the whole invasion cascade? How are MMPs delivered to invadopodia? What controls the interaction between microfilaments, microtubules and intermediate filaments? Are invadopodia maintained after crossing of the BM and do they have a role in invasive migration in the ECM? A better understanding of the initial step of the invasion cascade will bring new ideas for the design of efficient drugs to stop or at least to limit the metastatic spread.

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