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Review

A new front in cell invasion: The invadopodial membrane

Eric L. Hastie, David R. Sherwood *

Department of Biology, Duke University, 124 Science Drive, Box 90388, Durham, NC 27708, USA

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ABSTRACT

Invadopodia are F-actin-rich membrane protrusions that breach basement membrane barriers during cell invasion. Since their discovery more than 30 years ago, invadopodia have been extensively investigated in cancer cells *in vitro*, where great advances in understanding their composition, formation, cytoskeletal regulation, and control of the matrix metalloproteinase MT1-MMP trafficking have been made. In contrast, few studies examining invadopodia have been conducted *in vivo*, leaving their physiological regulation unclear. Recent live-cell imaging and gene perturbation studies in *C. elegans* have revealed that invadopodia are formed with a unique invadopodial membrane, defined by its specialized lipid and associated protein composition, which is rapidly recycled through the endolysosome. Here, we provide evidence that the invadopodial membrane is conserved and discuss its possible functions in traversing basement membrane barriers. Discovery and examination of the invadopodial membrane has important implications in understanding the regulation, assembly, and function of invadopodia in both normal and disease settings.

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1. Introduction

Directed vesicle trafficking to and from the plasma membrane facilitates the generation of membrane extensions, specialized secretion sites, and rapid delivery and removal of specific proteins from cell membranes. Examples of directed membrane trafficking include neurite outgrowth (Hausott and Klimaschewski, 2016), neuronal and immunological synapse function (Gonnord et al., 2012), wound healing (Abreu-Blanco et al., 2011), cell division (Shuster and Burgess, 2002), and cell migration (Maritzen et al., 2015). Targeted vesicle delivery requires a source of internal vesicles/membrane, exocytic trafficking machinery, and when vesicles are dynamically recycled, endocytic recycling components (Grant and Donaldson, 2009). This review will highlight recent studies in *C. elegans* that indicate a role for vesicular trafficking in the regulation of invadopodia, specialized F-actin-rich surface structures that mediate cell invasion through extracellular matrix barriers. Invadopodia in *C. elegans* undergo dynamic addition of a specialized invadopodial membrane. The invadopodial membrane is specifically associated with invadopodia and contains unique lipid and protein components distinct from the surrounding plasma membrane. During invadopodia breakdown, the invadopodial membrane lipid and protein components are rapidly recycled through endolysosomal vesicles then delivered back to the plasma membrane to form new invadopodia. In this review we will provide a brief history of invadopodia, discuss evidence for the conservation of the invadopodial membrane and focus on the regulation of traf-

* Corresponding author.
E-mail address: david.sherwood@duke.edu (D.R. Sherwood).

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ficking and possible functions of the invadopodial membrane. The identification and investigation of this unique membrane is providing a deeper mechanistic understanding of invadopodia formation and cell invasion during normal development and diseases such as cancer.

2. Background: invadopodia are specialized subcellular structures that mediate basement membrane and interstitial matrix invasion

Basement membranes are dense, sheet-like forms of extracellular matrix that underlie all epithelia and endothelia and surround muscle, fat, and Schwann cells (Halfter et al., 2015; Yurchenco, 2011). Independent polymeric laminin and type IV collagen networks as well as a number of associated proteins including perlecan and nidogen contribute to basement membrane composition (Yurchenco, 2011). Functionally, basement membranes create tissue barriers, provide structural support, and facilitate filtration, as well as harbor cues for cell differentiation, polarization, and growth (Breitkreutz et al., 2013; Hay, 1981; Poschl et al., 2004; Rasmussen et al., 2012; Suh and Miner, 2013; Yurchenco, 2011). During development and immune cell surveillance, specialized cells acquire the ability to invade basement membrane barriers to allow cell movement into and out of tissues (Kelley et al., 2014; Madsen and Sahai, 2010; Rowe and Weiss, 2008). Misregulation of invasion through basement membranes underlies the pathology of developmental diseases, immune disorders, and cancer (Barsky et al., 1983; Hagedorn and Sherwood, 2011). Given the importance of basement membrane invasion in development, immune function, and human health, there has been great interest in understanding how cells transmigrate basement membrane barriers.

In 1989 Wen-Tien Chen used the term invadopodia to name highly protrusive, matrix-degrading membrane structures, composed of actin regulators and proteases found in transformed embryonic chicken fibroblasts plated on glass slides with a thin coating of matrix—a surface that mimics the 2D topography of basement membranes (Chen, 1989; Even-Ram and Yamada, 2005; Genot and Gligorijevic, 2014; Murphy and Courtneidge, 2011). Since Chen's initial description, invadopodia have been observed in many metastatic cancer cell lines (Hoshino et al., 2013) and emerged as one of the key subcellular structures that invasive cells use to breach basement membrane barriers (Lohmer et al., 2014; Schoumacher et al., 2013; Schoumacher et al., 2010). Invadopodia also appear to mediate invasion through the more porous type I collagen rich interstitial matrices that reside between cells and tissues. Imaging of invasive cells in *in vitro* 3D type I collagen matrices has revealed that invadopodia (also referred to as "invadopodia equivalents") in these environments take on the morphology of long, thin filopodial structures (Li et al., 2010; Tolde et al., 2010; Wolf et al., 2009). Podosomes are another F-actin based membrane-associated structure similar to invadopodia, but are generally not protrusive and are most often associated with non-transformed cells that mediate matrix remodeling events, such as dendritic cells, osteoclasts, macrophages, and vascular smooth muscle cells (Davies and Stossel, 1977; Gawden-Bone et al., 2010; Hoshino et al., 2013; Linder et al., 2011; Murphy and Courtneidge, 2011; Seano et al., 2014; Zambonin-Zallone et al., 1988). In some culture conditions, however, podosomes extend long protrusions that degrade extracellular matrix, suggesting a possible close relationship between podosomes and invadopodia (Gawden-Bone et al., 2010). To help account for such findings, the term invadosomes has recently been adopted to incorporate both structures (Destaing et al., 2011; Linder, 2009; Linder et al., 2011; Saltel et al., 2011), proposing that invadopodia, podosomes, and possibly other actin-based cellular protrusions that bind and degrade extracellular matrix represent a spectrum of molecularly related structures that may adapt and even interchange in response to the microenvironment (Di Martino et al., 2016; McNiven, 2013). In this review, we will be consistent with the bulk of previously published work that defines invadopodia as highly protrusive invasive structures (Linder et al., 2011; Lohmer et al., 2014). We include within this definition invadopodia observed during developmental and normal physiological invasion events, recognizing that invadopodia are likely a component of a normal invasion program co-opted by tumor cells (Lohmer et al., 2014; Murphy and Courtneidge, 2011).

Through candidate gene approaches, proteomic analysis, and more recent *in vivo* genetic screens, approximately 100 genes have been associated with invadopodia formation, function, and breakdown (see Table 1 and references therein). This includes well-studied actin regulators, matrix metalloproteinases (MMPs), signaling pathways, and integrins, as well as genes involved with glycolysis, metabolism, protein degradation, chaperone activity, and protein synthesis for which an exact role in invadopodia formation has not been determined (Attanasio et al., 2011; Hoshino et al., 2013; Lohmer et al., 2016). The breadth of gene families associated with invadopodia likely reflects the complexity and intricate regulation of invadopodia and suggests that many aspects of their function and control remain unknown.

Although most studies have examined invadopodia in cancer cells *in vitro*, recent imaging advances in *ex vivo* and *in vivo* settings are establishing their existence and physiological importance in basement membrane invasion in both normal and disease settings (Di Martino et al., 2016; Genot and Gligorijevic, 2014; Lohmer et al., 2014). These studies include examination of cancer cell invasion on isolated rat peritoneum basement membranes (Schoumacher et al., 2010), imaging of vascular invasion by cancer cells in mouse and chicken embryos (Gligorijevic et al., 2012; Leong et al., 2014; Roh-Johnson et al., 2014), examination of intestinal epithelial cell invasion in a reactive oxygen species (ROS) disease model in zebrafish (Seiler et al., 2012), and visualizing anchor cell invasion during organogenesis in *C. elegans* (Hagedorn et al., 2013). Studying invadopodia in native contexts is not only confirming the relevance of these structures for cell invasion through basement membrane, but also is revealing new aspects of invadopodia biology. One fascinating example comes from the discovery of the invadopodial membrane in the anchor cell of *C. elegans*.

3. Invadopodia in *C. elegans* are formed from a recycling invadopodial membrane

The *C. elegans* anchor cell is a specialized uterine cell that initiates uterine-vulval attachment following invasion through underlying basement membrane (Sherwood and Sternberg, 2003). Anchor cell invasion is facilitated by dynamic and highly protrusive F-actin-rich invadopodia that localize to the anchor cell-basement membrane interface (the invasive cell membrane). The basement membrane in *C. elegans* is highly conserved and all major basement membrane components and receptors found in vertebrates are also present in *C. elegans* (Kramer, 2005). A suite of unique attributes of *C. elegans* as a model organism—including fluorescently tagged basement membrane components, anchor cell specific expression of fluorescently tagged proteins, the highly stereotyped nature of invasion, and genetic analysis—have allowed detailed experimental dissection of invadopodia *in vivo* (Hagedorn et al., 2014; Hagedorn et al., 2013; Lohmer et al., 2016; Lohmer et al., 2014).

Similar to tumor progression, where cancer cell invasion is promoted by signals from neighboring cells such as tumor associated macrophages (Noy and Pollard, 2014; Roh-Johnson et al., 2014), anchor cell invasion is stimulated by the underlying vulval cells (Sherwood and Sternberg, 2003). The vulval cells direct