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Research paper

Significance of kinase activity in the dynamic invadosome

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ABSTRACT

Invadosomes are actin rich protrusive structures that facilitate invasive migration in multiple cell types. Comprised of invadopodia and podosomes, these highly dynamic structures adhere to and degrade the extracellular matrix, and are also thought to play a role in mechanosensing. Many extracellular signals have been implicated in invadosome stimulation, activating complex signalling cascades to drive the formation, activity and turnover of invadosomes. While the structural components of invadosomes have been well studied, the regulation of invadosome dynamics is still poorly understood. Protein kinases are essential to this regulation, affecting all stages of invadosome dynamics and allowing tight spatiotemporal control of their activity. Invadosome organisation and function have been linked to pathophysiological states such as cancer invasion and metastasis; therapeutic targeting of invadosome regulatory components is thus warranted. In this review, we discuss the involvement of kinase signalling in every stage of the invadosome life cycle and evaluate its significance.

1. Introduction

Cell migration is an essential physiological process in development, wound healing and immune system-mediated protection from pathogens. Many different cell types must migrate through tissues and basement membranes as part of their normal function or in response to environmental cues. Methods of cell migration vary depending on the cell type and extracellular environment; cells can move collectively or as single entities, using amoeboid or mesenchymal modes of migration (Friedl and Wolf, 2010). Cancer cells make use of various migration methods to pathologically invade tissues and disseminate from primary tumours, forming new tumours around the body in a process called metastasis (Bravo-Cordero et al., 2012).

In order to facilitate migration, tissue cells can adhere to the extracellular matrix (ECM), produce force via the modulation of the actomyosin cytoskeleton, and degrade the surrounding matrix and basement membranes blocking the path of migration (Parsons et al., 2010). A key feature of migrating cells is cell-matrix adhesions, of which there are different classes with varying functions. This review will focus on two closely related cell-matrix adhesions: podosomes and invadopodia, collectively termed invadosomes (Murphy and Courtneidge, 2011). These are small multi-molecular complexes formed at the plasma membrane, linking the actin cytoskeleton to the extracellular membrane via integrin receptors. While some types of adhesion such as focal adhesions predominantly function to anchor the cell to the ECM, invadosomes are utilised for cell invasion.

Invadosomes consist of a filamentous actin (f-actin) core which contains proteins regulating actin nucleation, elongation and branching, and a ring structure of integrins and integrin-associated proteins (Murphy and Courtneidge, 2011; Schachtner et al., 2013b). Integrins provide a transmembrane link between the actin cytoskeleton and the ECM; in invadosomes, proteins within the ring structure such as paxillin, talin and vinculin link integrins to the f-actin core. As well as cell-matrix adhesion, invadosomes are sites of matrix degradation. At invadosomes, cells secrete various matrix metalloproteases (MMPs) to degrade ECM proteins and basement membranes and consequently facilitate cell migration through tissues (Saltel et al., 2011). Invadosomes also exhibit rigidity sensing capabilities (Alexander et al., 2008; Collin et al., 2008; Labernadie et al., 2014). Atomic force microscopy revealed that podosomes exert traction on the substrate as well as being protrusive (Labernadie et al., 2014) and thus are sensitive to matrix rigidity, suggesting that they play a role in both outside-in and inside-out mechanosensing (Collin et al., 2008).

Invadosomes are formed by many different cell types in response to different signalling events. Monocyte derived cells

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including macrophages, immature dendritic cells, osteoclasts and megakaryocytes constitutively form podosomes in order to carry out their inherent immune or cell specific functions (Dovas and Cox, 2011; Schachtner et al., 2013b). Macrophages and dendritic cells produce podosomes in order to adhere to the ECM and migrate through tissues towards sites of infection or tissue damage; osteoclast podosomes enable bone remodeling via the formation of podosome superstructures called sealing zones, which act to confine the area of bone resorption; megakaryocyte podosomes penetrate basement membranes to allow proplatelet arm extension into blood vessels for platelet release (Schachtner et al., 2013a). In all these cell types, podosome assembly occurs upon adhesion to ECM proteins and engagement of integrin receptors. Matrix stiffness therefore plays an important role in podosome assembly, and can also affect podosome lifetime and invasiveness (Collin et al., 2008). Clinically, the importance of podosomes is highlighted in Wiskott-Aldrich syndrome, a well-described immunodeficiency disorder (Kirchhausen, 1998; Thrasher and Burns, 2010). Additionally, osteopetrosis is characterised by increased bone mass due to impaired osteoclast-mediated bone resorption (Tolar et al., 2004).

Invadopodia can be described as a cancer cell's mimic of a podosome. They are formed as adhesive and invasive structures to enable migrating cancer cells to penetrate tissues during metastasis and contain many of the same molecular components as podosomes (Revach and Geiger, 2014). Despite their similarities however, invadopodia are generally observed to be longer lived, sometimes lasting several hours (Sharma et al., 2013) compared to the highly dynamic podosome which typically lasts less than ten minutes (Calle et al., 2004; Evans et al., 2003; Yamaguchi et al., 2005). Also, invadopodia protrude further from the cell body (Enderling et al., 2008) and are more degradative than podosomes (Artym et al., 2011); this is in keeping with the notion that cancer cells adapt normal cellular processes for their own pathological function (Murphy and Courtneidge, 2011). The clinical relevance of invadopodia in cancer progression has been studied in animal models (Eckert et al., 2011; Lohmer et al., 2014). The recent development of multiphoton microscopy in intravital imaging has transformed our ability to visualise cancer cell movement *in vivo* and confirmed the importance of invadopodia in tumour cell intravasation during metastasis (Condeelis and Segall, 2003; Gligorijevic et al., 2012).

The exact mechanisms that regulate invadosome dynamics are not fully understood, however many proteins are known to be involved and regulation is thought to be cell-type specific (Murphy and Courtneidge, 2011). Invadosome formation is initiated in response to signalling events promoting a migratory and invasive phenotype. This includes a variety of stimuli, including matrix adhesion, growth factors and cytokines (Destaing et al., 2011; Hoshino et al., 2013). At the plasma membrane, integrins and receptor kinases relay extracellular signals to downstream signalling molecules such as kinases, GTPases and adaptor proteins. These signalling pathways initiate invadosome formation via the nucleation of branched actin filaments by Wiskott-Aldrich Syndrome Protein (WASP) family members and the actin-related protein 2/3 (Arp2/3) complex; this will form the invadosome actin core (Beaty and Condeelis, 2014; Calle et al., 2008; Hurst et al., 2004; Kaverina et al., 2003; Machesky and Insall, 1998; Thrasher and Burns, 2010).

The invadosome is subsequently stabilised by elongation and cross-linking of actin filaments, and recruitment of various molecules to the region surrounding the core to make up the invadosome ring. The ring structure has been proposed to form a link between integrin receptors contacting the ECM and the intracellular actin core (Murphy and Courtneidge, 2011; Schachtner et al., 2013b). Mature, invadosomes can then recruit and secrete MMPs (Marchesin et al., 2015). Less is known about invadosome disassembly; in dendritic cells, myosin IIA has been suggested to induce contractions of the actin cytoskeleton, which disrupt the structural integrity of podosomes and lead to their dissolution (van Helden et al., 2008). Calpain, a cysteine protease, has also been linked to podosome disassembly, through its cleaving of podosome-related proteins such as talin and WASP (Calle et al., 2006; Macpherson et al., 2012).

While the invadosome life cycle can be described in various stages including formation, proteolytic activity and dissolution (Artym et al., 2006; Branch et al., 2012; Hoshino et al., 2013), the identification of regulatory factors acting at a precise stage has proved experimentally challenging due to the dynamic nature of these structures. This is further complicated by the observations that podosome f-actin cores can undergo oscillations in stiffness in response to localised actomyosin contractility (Labernadie et al., 2010) and can also undergo fission and fusion events (Evans et al., 2003). Following perturbation of a regulatory factor, the presence or absence of invadosomes can be used to determine whether this factor is necessary for the initiation and formation of the invadosome. The number of adhesions per cell allows further conclusions to be drawn: an increased number of invadosomes per cell would suggest either a promotion of invadosome formation or a defect in invadosome dissolution (Hoshino et al., 2013). Live cell imaging is crucial in determining between these stages, allowing invadosome lifetime measurements, as well as comparisons between the timing of regulatory factor localisation and recruitment of ring proteins or MMPs (Artym et al., 2006; Branch et al., 2012). Additionally, studies have assessed the size of invadosomes or tertiary structures, as well as their ability to degrade matrices such as gelatin (Beaty and Condeelis, 2014; Schachtner et al., 2013b).

Using a combination of such analytical techniques, the key proteins that make up the structure of the invadosome have been well described (Beaty and Condeelis, 2014; Schachtner et al., 2013b), however less is known about the regulatory proteins involved. Kinases are a large group of protein enzymes which function to add phosphate groups to their substrates, resulting in extremely diverse cellular actions. Kinases often act as signal transducers within intracellular signalling cascades in order to affect cell physiology or phenotype; in relation to invadosomes, many different kinases have been implicated in the dynamics of these structures (Hoshino et al., 2013; Saykali and El-Sibai, 2014; Winograd-Katz et al., 2011). Each stage of the invadosome is tightly regulated by signalling factors influencing where and when invadosomes are formed. Kinases are integral components of these signalling pathways, and while certain phosphorylation events have been identified as important, there is much still to be explored. In this review, we aim to describe known kinase involvement in invadosome stimulation, formation, stabilisation, activity and dissolution (Fig. 1), and highlight where current knowledge is lacking.

2. Kinases in invadosome stimulation

The stimuli inducing invadosome formation are varied; they include growth factors, cytokines, ECM proteins, micro RNAs, calcium and reactive oxygen species (ROS) (Murphy and Courtneidge, 2011). The extracellular signals inducing invadosome formation are well studied (Hoshino et al., 2013), as well as the downstream involvement of Rho GTPases (Spuul et al., 2014) and the molecules responsible for the formation of branched f-actin, such as WASP/N-WASP and the Arp2/3 complex (Saykali and El-Sibai, 2014; Schachtner et al., 2013b); however, little is known about the factors regulating these processes. Kinases play a critical role as signal transducers to regulate invadosome initiation; many kinases and phosphorylation events have been shown to be involved, yet it is still unclear how these signals are integrated.