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Review

Spatiotemporal regulation of Src and its substrates at invadosomes

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ABSTRACT

In the past decade, substantial progress has been made in understanding how Src family kinases regulate the formation and function of invadosomes. Invadosomes are organized actin-rich structures that contain an F-actin core surrounded by an adhesive ring and mediate invasive migration. Src kinases orchestrate, either directly or indirectly, each phase of the invadosome life cycle including invadosome assembly, maturation and matrix degradation and disassembly. Complex arrays of Src effector proteins are involved at different stages of invadosome maturation and their spatiotemporal activity must be tightly regulated to achieve effective invasive migration. In this review, we highlight some recent progress and the challenges of understanding how Src is regulated temporally and spatially to orchestrate the dynamics of invadosomes and mediate cell invasion.

Introduction

Invadosomes are highly dynamic, actin-rich, protrusive structures that promote adhesion to and degradation of the extracellular matrix (ECM), facilitating invasive cell migration. The collective term invadosomes includes podosomes that form in macrophages, dendritic cells, osteoclasts and endothelial cells, and invadopodia that are associated with cancer cells (Saltel et al., 2011). Invadosomes are generally composed of an actin-rich core with actin-nucleating components including cortactin, N-WASP and Arp2/3, surrounded by a ring of adhesion and adaptor proteins such as vinculin, paxillin, and integrins. These protrusive structures promote localized secretion of degradative enzymes including matrix metalloproteinases (MMPs) and can exist independently as dot-like structures or they can be arranged into complex metastructures such as clusters and rosettes (Fig. 1). In osteoclasts, podosomes can mature further into a sealing belt that forms a cavity to mediate bone degradation and resorption (Jurdic et al., 2006). The dynamic formation, disassembly and degradation activity of both podosomes and invadopodia have been implicated in invasive cell migration (Linder et al., 2011; Pan et al., 2011; Badowski et al., 2008; Calle et al., 2006; Varon et al., 2006; Seals et al., 2005).

Cell migration and invasion are necessary for a variety of physiological functions including leukocyte trafficking, development, and wound repair. Defective podosome formation can be seen in inherited disorders including Wiskott Aldrich syndrome (Linder et al., 1999; Nusblat et al., 2011), PAPA syndrome (Cortesio et al., 2010), and potentially Frank-Ter-Haar syndrome (Iqbal et al., 2010; Buschman et al., 2009), while defects in osteoclast podosomes are associated with osteopetrosis (Gil-Henn et al., 2007). Moreover, cancer invasion and metastasis have been associated with the formation of dynamic, actin rich invadopodia with the capacity for matrix degradation both in vitro and in vivo (Eckert et al., 2011; Gertler and Condeelis, 2010; Philippar et al., 2008; Packard et al., 2009). Although podosomes and invadopodia are important during invasive migration, it has been suggested that podosome rosettes of smooth muscle cells, vascular endothelial cells, aortic endothelial cells, or fibroblasts may also function in ECM remodeling (Daubon et al., 2011; Rottiers et al., 2009), mechanosensing (Collin et al., 2008) and adhesion to the ECM (Boateng et al., 2012; Kocher et al., 2009; Collin et al., 2006).

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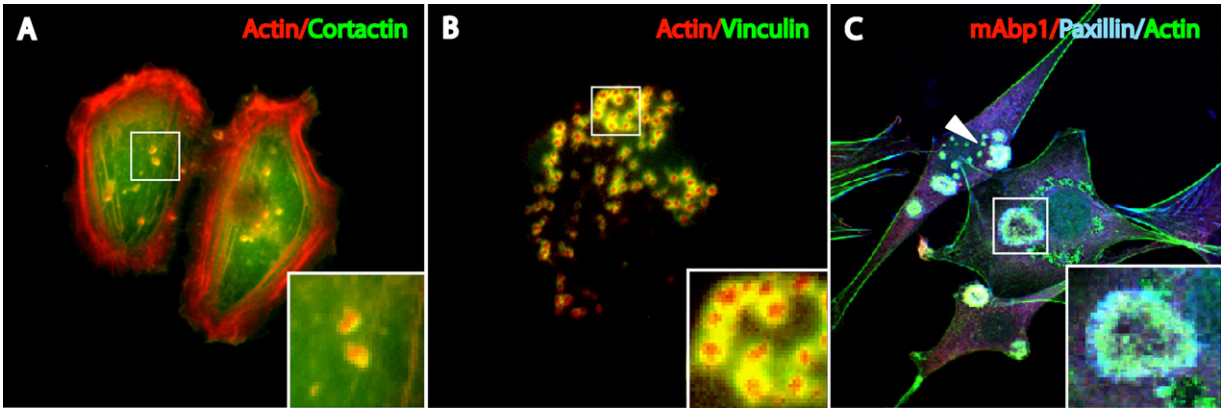

Fig. 1. Podosomes and invadopodia from different cell types. (A) Actin and cortactin co-localize at invadopodia in human MDA-231-MD breast cancer cells. (B) Vinculin forms a ring around the actin cores of podosomes in primary human macrophages. (C) NIH 3T3 cells transformed with constitutively active c-Src527F form both dot (arrowhead) and rosette podosomes (inset). Magnified views of podosomes or invadopodia are shown within insets.

Podosomes and invadopodia are highly dynamic and require tight regulation to control their rapid formation and turnover. In contrast to other adhesion structures like focal adhesions, podosomes and invadopodia are primary sites of rapid actin polymerization and are not associated with stabilized actin filament bundles (Destaing et al., 2003; Ochoa et al., 2000). Invadosome cores contain signaling molecules such as Rho GTPases (Bravo-Cordero et al., 2011) and Src family kinases (Gavazzi et al., 1989), as well as actin regulatory proteins including cortactin (Bowden et al., 1999), WASP (Linder et al., 1999), and the actin nucleating Arp2/3 complex (Yamaguchi et al., 2005). Other components generally concentrate in the surrounding ring structure including integrins and integrin-associated proteins like vinculin, talin and paxillin (Gavazzi et al., 1989; Marchisio et al., 1988; Linder and Aepfelbacher, 2003). As highly dynamic structures, invadosomes can assemble and disassemble within minutes, but in some cases can stabilize and exist for hours.

The invadosome lifetime is divided into stages including assembly, maturation and disassembly (reviewed by Murphy and Courtneidge, 2011). During invadosome precursor formation, signaling proteins such as transmembrane growth factor receptors (Rottiers et al., 2009; Varon et al., 2006) and/or cytoplasmic kinases, Src and protein kinase C (PKC) (Tatin et al., 2006; Gatesman et al., 2004), organize with structural and adaptor proteins including Tks5, Nck1, and cortactin (Gatesman et al., 2004; Stylii et al., 2009; Oser et al., 2009, 2010; Crimaldi et al., 2009) to recruit the Arp2/3 complex and mediate actin polymerization (Yamaguchi et al., 2005). Under some conditions, actin may be organized into metastructures such as clusters and rosettes. Next, the maturation stage includes protrusion mediated by actin bundling or cross-linking proteins (Li et al., 2010; Guiet et al., 2012) and microtubules (Schoumacher et al., 2010), stabilization of actin filaments through cortactin (Oser et al., 2009) and secretion or localization of proteases for ECM degradation (Clark et al., 2007; Chen and Wang, 1999; Nakahara et al., 2007). Finally, during disassembly, the actin core is dismantled and invadosome components disassociate (Badowski et al., 2008; Cortesio et al., 2008). Understanding the signaling mechanisms and functional components of invadosome formation and turnover has been a key focus for invadosome research and has implications to developing drug targets that control cell invasion.

A major candidate therapeutic target is the non-receptor tyrosine kinase, Src (Wadhawan et al., 2011). Src kinase, often referred to as "the oldest oncogene", has received considerable attention due to its role in cell transformation and cancer cell invasion. v-Src was initially discovered as the transforming agent of the Rous sarcoma virus (David-Pfeuty and Singer, 1980; Tarone et al., 1985), and its cellular counterpart, c-Src, have been the focus of intensive investigation in cancer research. Src is over-expressed or constitutively active in many cancers including breast (Biscardi et al., 1998; Ottenhoff-Kalff et al., 1992), prostate (Nam et al., 2005), and colon cancer (Cartwright et al., 1989; Talamonti et al., 1993), and plays an integral role in regulating each stage of the formation and turnover of invadosomes by targeting distinct substrates. The Src family kinases (SFKs) are composed of nine members: Src, Yes, Fyn, Fgr, Yrk, Hck, Lck, Lyn and Blk (Martin, 2001), with Src, Fyn, and Yes being ubiquitously expressed in non-hematopoietic cells. Src is a non-receptor tyrosine kinase and its mechanism of activation has been well studied over the past several decades (Martin, 2001; Sicheri et al., 1997; Xu et al., 1997; Yeatman, 2004). At the amino terminus, Src has an SH3 and SH2 domain that mediate protein–protein interactions, followed by a linker region and a kinase domain at the C-terminus. During its inactive state, Src is phosphorylated at Y527, which maintains inhibitory intramolecular interactions. When active, the SH2 and SH3 domains are released to initiate intermolecular interactions, and the kinase domain autophosphorylates tyrosine 416 in the activation loop of the catalytic domain for full activity (Kmiecik et al., 1988).

Src can be regulated by kinases and phosphatases, or protein–protein interactions with its SH2 and SH3 domains. Negative regulators of Src kinase activity include the non-receptor C-terminal Src kinase, Csk (Ia et al., 2010; Okada and Nakagawa, 1989) and the Csk homologous kinase, Chk (Zrihan-Licht et al., 1997). Csk and Chk phosphorylate Src at Y527 to induce Src folding and autoinhibition. Conversely, phosphatases that activate Src by dephosphorylation of the C-terminal phosphotyrosine, including PTPα (Zheng et al., 1992), SHP-1 (Somani et al., 1997), SHP-2 (Hakak et al., 2000), PTP1B (Bjorge et al., 2000; Cortesio et al., 2008), and PTP-PEST (Chellaiah and Schaller, 2009), release the autoinhibitory configuration of Src, thereby leading to its activation. Both PTP1B and PTP-PEST regulate Src activity at invadopodia and podosomes, respectively (Cortesio et al., 2008; Chellaiah and Schaller, 2009). PTP1B regulates Src phosphorylation at the C-terminal tyrosine during invadopodia formation and proteolysis and activation of PTP1B by calpain-2 can amplify Src activity during invadopodia assembly (Cortesio et al., 2008). PTP-PEST localizes to osteoclast podosomes (Chellaiah et al., 2001) and is important for the control of rosette formation in Src-transformed fibroblasts (Diaz et al., 2009); however, how PTP-PEST regulates Src activity at podosomes is not clear.

In this review, we focus on the challenges of understanding how Src participates in different steps during the invadosome