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Short communication

Nck1 and Grb2 localization patterns can distinguish invadopodia from podosomes

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ABSTRACT

Invadopodia are matrix-degrading ventral cell surface structures formed in invasive carcinoma cells. Podosomes are matrix-degrading structures formed in normal cell types including macrophages, endothelial cells, and smooth muscle cells that are believed to be related to invadopodia in function. Both invadopodia and podosomes are enriched in proteins that regulate actin polymerization including proteins involved in N-WASp/WASp-dependent Arp2/3-complex activation. However, it is unclear whether invadopodia and podosomes use distinct mediators for N-WASp/WASp-dependent Arp2/3-complex activation. We investigated the localization patterns of the upstream N-WASp/WASp activators Nck1 and Grb2 in invadopodia of metastatic mammary carcinoma cells, podosomes formed in macrophages, and degradative structures formed in Src-transformed fibroblasts and PMA-stimulated endothelial cells. We provide evidence that Nck1 specifically localizes to invadopodia, but not to podosomes formed in macrophages or degradative structures formed in Src-transformed fibroblasts and PMA-stimulated endothelial cells. In contrast, Grb2 specifically localizes to degradative structures formed in Src-transformed fibroblasts and PMA-stimulated endothelial cells, but not invadopodia or podosomes formed in macrophages. These findings suggest that distinct upstream activators are responsible for N-WASp/WASp activation in invadopodia and podosomes, and that all these ventral cell surface degradative structures have distinguishing molecular as well as structural characteristics. These patterns of Nck1 and Grb2 localization, identified in our study, can be used to sub-classify ventral cell surface degradative structures.

Introduction

Podosomes and invadopodia were both initially identified in Rous Sarcoma Virus-Transformed (described hereafter as Src-transformed) fibroblasts (Chen, 1989). Podosomes were first described as adhesive structures in close contact with the substratum containing actin and vinculin (Tarone et al., 1985). Similar to podosomes, invadopodia were also identified as ventral cell surface structures enriched with actin and vinculin, but the added feature of invadopodia was their ability to focally degrade the underlying extracellular matrix (ECM) using matrix metalloprotease (MMP) activity (Chen, 1989). Several studies have now demonstrated that podosomes formed in many cell types also have the ability to degrade the underlying ECM using MMP activity (Linder, 2007; Yamaguchi et al., 2006) suggesting that the ventral cell surface structures formed in Src-transformed fibroblasts, originally termed invadopodia and podosomes (Chen, 1989; Tarone et al., 1985), were essentially the same structures.

Since podosomes were identified in Src-transformed fibroblasts, researchers began to discover that many non-transformed normal cell types form podosomes in vitro including: myeloid-derived cells such as primary monocytes (Worthylake et al., 2001), macrophages, dendritic cells, osteoclasts and neutrophils (Szczur et al., 2006), smooth muscle cells, endothelial cells and lymphocytes (Carman et al., 2007; Redondo-Munoz et al., 2006) (for reviews see Linder, 2007, 2009). In these cell types, podosomes could form in the absence of Src transformation suggesting that they play physiological, rather than pathological, roles in adhesion, protrusion and matrix remodeling during normal physiological processes. Currently, podosomes in non-transformed cells are structurally defined as ventral cell surface structures that contain an F-actin core accompanied by other actin regulatory proteins including Wiskott Aldrich Syndrome protein (WASp), neural (N)-WASp, cortactin, the Arp2/3-complex, surrounded by a ring of adhesion

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plaque proteins including vinculin and paxillin, that are linked to the ECM via integrins (Gimona et al., 2008; Linder et al., 1999).

Since invadopodia were identified in Src-transformed fibroblasts (Chen, 1989), it was discovered that several invasive cancer cell types form invadopodia including: metastatic mammary carcinoma cells, melanoma cells, and aggressive head and neck squamous carcinoma cells (Baldassarre et al., 2003; Clark et al., 2007; Yamaguchi et al., 2005). Invadopodia that form in invasive cancer cells share many similar structural and functional features to the invadopodia first identified in Src-transformed fibroblasts, although their morphological features are distinct. Invadopodia formed in invasive cancer cells are relatively small (~1–2 μm diameter) circular punctate structures (Yamaguchi et al., 2005), while the invadopodia formed in Src-transformed fibroblasts appear as large (~5 μm diameter) rosettes (Webb et al., 2007). Today, invadopodia are defined as F-actin enriched membrane protrusions with matrix degradation activity formed on the ventral cell surface of invasive tumor cells (Caldieri et al., 2009; Lizarraga et al., 2009; Oser and Condeelis, 2009; Weaver, 2008b; Yamaguchi and Condeelis, 2007). Invadopodia are believed to play an important role in tumor cell invasion by degrading the ECM via MMP activity (Poincloux et al., 2009; Yamaguchi and Condeelis, 2007).

Both invadopodia and podosomes are enriched in F-actin and proteins involved in regulating the actin cytoskeleton including: cortactin, N-WASp/WASp, cofilin, and the Arp2/3-complex (Albiges-Rizo et al., 2009; Linder, 2007; Oser et al., 2009). In addition, both invadopodium and podosome formation requires the coordination of many cell biological processes including integrin and growth factor receptor signaling (Buccione et al., 2009; Calle et al., 2006; Yamaguchi et al., 2005), membrane trafficking (Liu et al., 2009; Sakurai-Yageta et al., 2008), and MMP localization, activation, and secretion (Artym et al., 2006; Clark et al., 2007; Sakurai-Yageta et al., 2008). In fact, podosomes and invadopodia share so many features that the umbrella term “invadosome” has been used to group these ventral cell surface degradative structures (Linder, 2009).

Although invadopodia and podosomes share many proteins in common and functional characteristics, there are also several unique features of each organelle that suggest that they may have some distinct functions. For example, invadopodia do not contain an adhesion plaque ring (containing vinculin and paxillin) that surrounds the F-actin core, which is a defining feature of podosomes, indicating that invadopodia may not mediate adhesion to the ECM. In fact, the absence of vinculin in invadopodia may be a useful structural marker to distinguish invadopodia from podosomes (Chan et al., 2009; Gimona, 2008; Linder, 2009). Apart from some structural differences, invadopodia have longer lifetimes, a greater protrusive capacity, and increased matrix degradation activity compared to podosomes (Linder, 2007). These characteristics support the hypothesis that invadopodia are critical for tumor cell invasion during metastasis (Condeelis and Segall, 2003; Philippar et al., 2008). Podosomes are smaller in size, but each podosome-forming cell generates a much greater number of podosomes compared to invadopodia (Linder, 2007). Lastly, invadopodia form in invasive cancer cells, while podosomes form in normal, non-pathological cell types.

Ironically, although the terms invadopodia and podosomes were first coined in studies of Src-transformed fibroblasts (Chen, 1989; Tarone et al., 1985), the ventral cell surface degradative structures formed in Src-transformed fibroblasts (referred to hereafter as degradative structures formed in Src-transformed fibroblasts) have several features that suggest they are unique from what is currently described as invadopodia and podosomes. Unlike podosomes, degradative structures formed in Src-transformed fibroblasts do not contain an adhesion ring with vinculin and paxillin surrounding a punctate F-actin core, but rather appear as much larger rosette-like structures with matrix degradation activity containing adhesion proteins (e.g. vinculin and paxillin) that colocalize with the F-actin core (Buschman et al., 2009; Oikawa et al., 2008). In that respect, both podosomes and degradative structures formed in Src-transformed fibroblasts appear to be *bona fide* adhesion structures. Unlike both podosomes and degradative structures formed in Src-transformed fibroblasts, invadopodia are not enriched with vinculin (Chan et al., 2009).

Recent evidence suggests that invadopodia, podosomes, and ventral cell surface degradative structures formed in Src-transformed fibroblasts may contain a distinct set of proteins used to regulate the actin cytoskeleton (Oikawa et al., 2008; Yamaguchi et al., 2005). Although N-WASp/WASp localizes to and is necessary for the formation of invadopodia, degradative structures formed in Src-transformed fibroblasts, and podosomes formed in macrophages (Linder et al., 1999; Mizutani et al., 2002; Oikawa et al., 2008; Yamaguchi et al., 2005), distinct activators of N-WASp are responsible for invadopodium formation in metastatic mammary carcinoma cells compared to degradative structure formed in Src-transformed fibroblasts (Oikawa et al., 2008; Oser et al., 2009; Yamaguchi et al., 2005). Specifically, Nck1, an upstream activator of N-WASp, localizes to invadopodia and is important for invadopodium formation and matrix degradation activity of invadopodia in both metastatic mammary carcinoma cells (Yamaguchi et al., 2005) and melanoma cells (Stylli et al., 2009). In contrast, Grb2, another upstream activator of N-WASp, does not localize to nor is important for invadopodium formation in the same mammary carcinoma cell type (Yamaguchi et al., 2005). However, Grb2 localizes to degradative structures formed in Src-transformed fibroblasts early during their assembly and is critical for their formation, while Nck1 knockdown has no effect (Oikawa et al., 2008). These findings suggest that the specific localization patterns of Nck1 and Grb2 can be used to distinguish invadopodia from degradative structures formed in Src-transformed fibroblasts. It is not known whether Nck1 or Grb2 localizes to podosomes formed in non-transformed cell types, such as macrophages. Based on these results, we hypothesized that Nck1 and Grb2 localization patterns could distinguish invadopodia from podosomes formed in macrophages.

In this short communication, we investigated whether the endogenous localization patterns of Nck1 and Grb2 could be used to distinguish between the degradative structures formed in metastatic mammary carcinoma cells, macrophages, Src-transformed fibroblasts, and PMA-stimulated endothelial cells. We hypothesized that Nck1 and Grb2 localization patterns could be used as markers to distinguish among these structures.

Materials and methods

Antibodies

For immunofluorescence (IF), cortactin (ab-33333) and Nck1 (ab-14588) were from Abcam, and Grb2 (sc-255(C-23)) was from Santa Cruz. 4G10 anti-phosphotyrosine monoclonal antibody was from Millipore. Monoclonal antibody against vinculin (hVin1) was from Sigma. Secondary antibodies Alexa488 donkey anti-rabbit, and Alexa568 donkey anti-mouse, and Alexa647-phalloidin were from Invitrogen, and Cy5-conjugated goat anti-mouse was from Jackson Laboratories. For immunoblot analysis, Nck1 (ab-14588) was from Abcam, Grb2 (sc-255(C-23)) was from Santa Cruz, β-actin (AC-15) monoclonal antibody was from Sigma and GAPDH mouse antibody was from Biodesign. Horseradish peroxidase (HRP)-conjugated secondary antibodies against rabbit and mouse IgG were from Jackson Immuno research.