© 2018. Published by The Company of Biologists Ltd | Journal of Cell Science (2018) 131, jcs203661. doi:10.1242/jcs.203661

CELL SCIENCE AT A GLANCE

Tks adaptor proteins at a glance

Priyanka Saini[1] and Sara A. Courtneidge[1,2,3,*]

ABSTRACT

Tyrosine kinase substrate (Tks) adaptor proteins are considered important regulators of various physiological and/or pathological processes, particularly cell migration and invasion, and cancer progression. These proteins contain PX and SH3 domains, and act as scaffolds, bringing membrane and cellular components in close proximity in structures known as invadopodia or podosomes. Tks proteins, analogous to the related proteins p47^{phox}, p40^{phox} and NoxO1, also facilitate local generation of reactive oxygen species (ROS), which aid in signaling at invadopodia and/or podosomes to promote their activity. As their name suggests, Tks adaptor proteins are substrates for tyrosine kinases, especially Src. In this Cell Science at a Glance article and accompanying poster, we discuss the known structural and functional aspects of Tks adaptor proteins. As the science of Tks proteins is evolving, this article will point out where we stand and what still needs to be explored. We also underscore pathological conditions involving these proteins, providing a basis for future research to develop therapies for treatment of these diseases.

KEY WORDS: Invadopodia, Podosomes, Tks adaptors

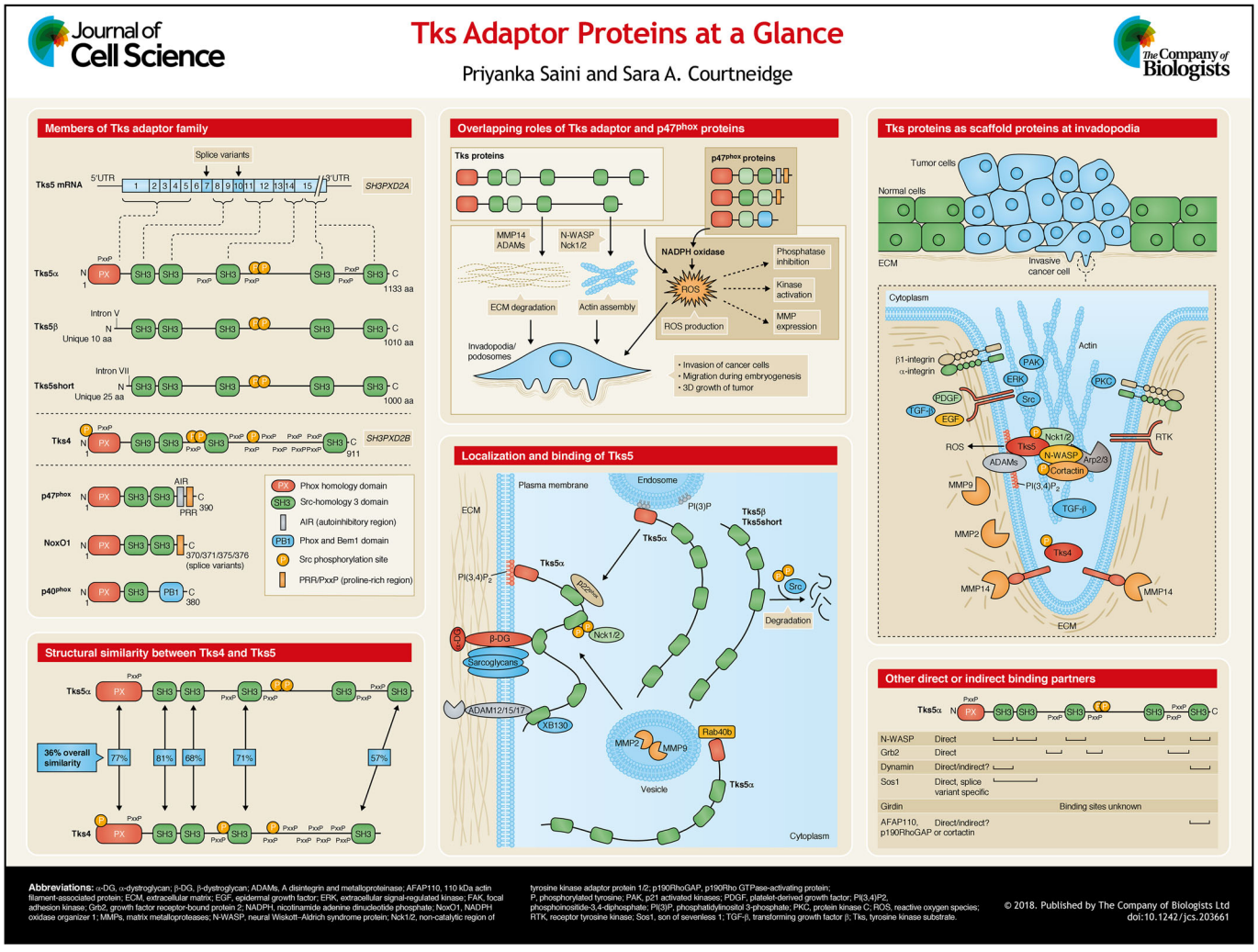
[1]Department of Cell, Developmental & Cancer Biology, Oregon Health and Science University, Portland, OR, USA. [2]Department of Biomedical Engineering, Oregon Health and Science University, Portland, OR, USA. [3]Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA.

*Author for correspondence (courtneidge@ohsu.edu)

S.A.C., 0000-0001-7889-265X

Introduction

The Tks adaptor proteins consist of an N-terminal phox homology (PX) domain, multiple Src homology 3 (SH3) domains, proline-rich



Journal of Cell Science (2018) 131, jcs203661. doi:10.1242/jcs.203661

regions (PRR) and Src phosphorylation sites (Lock et al., 1998; Courtneidge et al., 2005; Buschman et al., 2009) (see poster). The PX domain belongs to the phosphoinositide-binding module superfamily (Ponting, 1996) and is involved in binding of Tks proteins to anionic phospholipids (Abram et al., 2003; Buschman et al., 2009). It is this distinct function of the PX domain that determines localization of proteins to different membranous compartments as a consequence of interactions with specific phosphatidylinositol lipids (Ellson et al., 2002; Sato et al., 2001). SH3 domains, tyrosine phosphorylation sites and PRR motifs each facilitate binding to other proteins (Pawson and Gish, 1992). These four interactions together spatially coordinate signal transduction events. Tks5 (also known as SH3PXD2A) and the related Tks4 (SH3PXD2B), both have N-terminal PX domains but differ in the number of SH3 domains (five and four, respectively), their Src phosphorylation sites, PRR motifs and linker sequences (see poster). In humans, Tks4 and Tks5 proteins have an overall structural similarity of 36% (Buschman et al., 2009). Both Tks4 and Tks5 proteins are found in various species of vertebrates, ranging from fish to human. Vertebrates also have three related genes with similar architecture which function as organizers of reactive oxygen species (ROS) production, known as $p47^{phox}$ (also known as NCF1 or SH3PXD1A), $p40^{phox}$ (NCF4 or SH3PXD4) and NoxO1. The simplest animals known to have Tks- or p47-related genes are the urochordate *Ciona intestinalis* and the echinoderm *Strongylocentrotus purpuratus*, which each has a single gene encoding a protein with a PX domain that is followed by three or four SH3 domains, respectively (Kawahara and Lambeth, 2007). In addition, the unicellular choanoflagellate *Monosiga brevicollis*, a close relative of multicellular animals, has a single gene encoding a PX domain that is followed by two SH3 domains (Kawahara and Lambeth, 2007), and thus appears to represent a precursor to the entire Tks/p47 gene family.

Tks isoforms

Three isoforms of Tks5 have been reported, namely, Tks5α (also referred to as Tks5long), Tks5β and Tks5short (Lock et al., 1998; Li et al., 2013; Cejudo-Martin et al., 2014) each arising from a distinct promoter. Tks5α is the only isoform containing the PX domain, whereas Tks5β and Tks5short both initiate prior to the first SH3 domain (see Box 1 for information on distinguishing between isoforms). In addition, there are two known splice variants of Tks5, flanking the first SH3 domain (Lock et al., 1998). Quantitative PCR analysis reveals that forms with none, one or both splices exist (our unpublished results), increasing the potential complexity. In contrast, neither alternative promoters nor splice isoforms have been described for Tks4. Both *Tks4* and *Tks5* mRNAs are widely expressed; however, the amount of each protein expressed in different tissues is still relatively unknown. Like Tks5α, Tks5β can be detected in almost all tissues, but, depending on the tissue, its mRNA level can be substantially lower than that for Tks5α (Cejudo-Martin et al., 2014). The expression pattern of Tks5short in various tissues still needs to be determined. Tks5β and Tks5short proteins are highly expressed in embryonic fibroblasts, whereas cancer cells widely express the Tks5α form (Cejudo-Martin et al., 2014; Seals et al., 2005; Iizuka et al., 2016). The biological functions of the Tks5β and Tks5short isoforms remain to be elucidated.

Tks proteins in invadopodia and podosomes

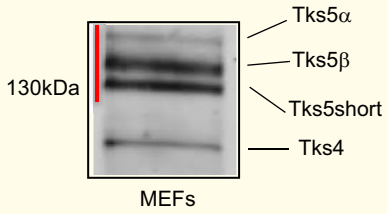
Podosomes and invadopodia (together sometimes called invadosomes) are similar structures found in normal cells and cancer cells, respectively. They are defined as actin-rich protrusions that are observed at the ventral surface of invasive cells in 2D culture and mediate focal degradation of the extracellular matrix (ECM) (Tarone et al., 1985; Chen, 1990; Murphy and Courtneidge, 2011). Tks proteins are found localized to invadopodia in many invasive cancer cells (Seals et al., 2005; Iizuka et al., 2016). Moreover, Tks5α and Src are considered to be defining elements of invadopodia. These structures were first defined in Src-transformed fibroblasts (Tarone et al., 1985), and the exogenous expression of Tks5α along with active Src induces formation of invadopodia in non-invasive cancer cell lines, which usually lack them (Seals et al., 2005). Furthermore, both integrin and receptor tyrosine kinase signaling, which both result in Src activation (Mitra and Schlaepfer, 2006; Bromann et al., 2004), can stimulate invadopodia formation. However, whether Tks4 and Tks5 are phosphorylated exclusively by Src family kinases in cancer cells has not been addressed.

In keeping with its role as an essential invadopodia scaffold protein, Tks5 is required for invadopodia formation and degradation of ECM- and protease-dependent invasion through Matrigel in Src-transformed NIH3T3 cells (a mouse model cancer cell line with full invasive and metastatic properties), as well as in numerous human cancer cells (Iizuka et al., 2016; Seals et al., 2005). Tks4 knockdown has similar effects in Src3T3 cells and melanoma cell lines (Buschman et al., 2009; Iizuka et al., 2016). What about *in vivo*? Recently, using high-resolution intravital imaging in model systems, invadopodia have been visualized in tumor cells as they escape the primary tumor to enter the bloodstream (intravasation) (Gligorijevic et al., 2014) and exiting the bloodstream to enter secondary sites (extravasation) (Leong et al., 2014). Importantly, knockdown of Tks adaptors profoundly inhibited both processes.

Interestingly, both Tks4 and Tks5 also promote the growth of cancer cells in three-dimensional cultures of the ECM protein collagen-I, as well as both primary tumor growth and metastatic outgrowth of cancer cells *in vivo* (Blouw et al., 2008; Blouw et al., 2015; Iizuka et al., 2016). Analysis of *in vivo* growth of both Src3T3 fibrosarcoma cells and MDA-MB-231 breast cancer cells with and without Tks5 knockdown revealed less tumor growth, increased apoptosis and diminished and/or abnormal tumor vasculature (Blouw et al., 2008, 2015). Knockdown of Tks-interacting proteins such as membrane type 1-matrix metalloproteinase 1 (MT1-MMP) (Buschman et al., 2009), or invadopodia regulators

Box 1. Distinguishing Tks5 isoforms and Tks4 by immunoblotting

Different isoforms of Tks5 can be identified on an immunoblot based on their size. Tks5α runs at higher molecular mass compared with Tks5β and Tks5short because of the presence of the PX domain in Tks5α, which Tks5β and Tks5short lack.



Tks5β and Tks5short are transcribed from in-frame ATGs upstream of exon 6 and exon 8, respectively. It is believed that owing to its higher molecular mass, Tks5β migrates slower than Tks5short, although this has not been directly confirmed. Thus, all three isoforms of Tks5 can be visualized on the same blot (provided the antibody recognizes a common region). There are specific antibodies available commercially that recognize either Tks5 or Tks4.

Journal of Cell Science (2018) 131, jcs203661. doi:10.1242/jcs.203661

such as cortactin (Weaver, 2008) and Cdk5 (Quintavalle et al., 2011) can also impair growth of implanted tumors (Clark et al., 2009; Feldmann et al., 2010; Hotary et al., 2003). This suggests that invadopodia might not only facilitate passage across basement membranes, but also interface with, and modulate, the tumor microenvironment, perhaps by increasing synthesis, release or processing of growth factors (such as vascular endothelial growth factor, VEGF), to promote tumor growth.

What evidence is there that Tks adaptors influence outcome in cancer? Expression of the Tks5α protein was first correlated with worse outcome in patients with glial-derived brain tumors (Stylli et al., 2012). Analysis of gene expression databases also suggested the involvement of Tks5 in several cancers (Stylli et al., 2014), although in this case, inability to distinguish among Tks5 isoforms, as well as the under-representation of the very long (>11 kb) *Tks5* mRNA (Lock et al., 1998), likely complicated data interpretation. More recently, the availability of RNA sequencing (RNA-seq) databases has allowed the specific analysis of Tks5α expression by focusing on the first 5 exons of *SH3PXD2A*, which encode the PX domain. In lung cancer, both high levels of Tks5α, and a high ratio of Tks5α to Tks5β and Tks5short, predicted poor survival of patients diagnosed in the early stages of disease (Li et al., 2013). In breast cancer, high expression of the Tks5α isoform also correlated with reduced survival, particularly in patients diagnosed with stage I/II disease. In this case, neither the levels of Tks5β or Tks5short, nor the relative expression of short to long isoforms, influenced outcome (Blouw et al., 2015). Both Tks5 and Tks4 proteins were highly expressed in melanoma tissues compared with nevi, and Tks5, but not Tks4, levels increased with disease progression (Iizuka et al., 2016). Finally, increased Tks5 levels (isoform unknown) were observed during progression of prostate cancer (Burger et al., 2014).

Podosomes are frequently found in hematopoietic cells, such as macrophages and dendritic cells, and upon stimulation in various other adult cell types, including fibroblasts, smooth muscle, epithelial and endothelial cells (Hoshino et al., 2013; Linder et al., 2011). However, a role for Tks adaptors in podosome formation and/or function has been interrogated in only a few studies. Tks5-dependent podosome formation of circumferential podosomes mediated osteoclast cell–cell fusion (Oikawa et al., 2012). In the same study, Tks5- and podosome-dependent fusion of osteoclasts with melanoma cells was also observed, although the physiological or pathological relevance of this finding awaits further exploration (Oikawa et al., 2012). Vascular smooth muscle cells use podosomes to migrate and invade tissues (Lener et al., 2006) in a Tks5-dependent manner (Crimaldi et al., 2009). Finally, Tks5 was more recently identified in podosomes in primary macrophages and was found to be a major contributor to their invasive behavior (Burger et al., 2011).

Tks proteins in cell migration and embryogenesis

Tks adaptor proteins have been linked to cell migration processes during embryonic development. Loss of Tks5 hinders dorsal to ventral migration and formation of podosome-like protrusions in migrating neural crest cells in zebrafish *in vivo*, ultimately causing developmental defects (Murphy et al., 2011). Analysis of mouse neural crest stem cells and their derivatives *in vitro* revealed the presence of podosomes. Tks5 knockdown resulted in reduced podosome formation and migration under 2D conditions, as well as fewer protrusive structures in 3D (Murphy et al., 2011), providing the first evidence for podosomes in a developmental cell type. Gene-trap targeting of the *Sh3pxd2a* (*Tks5*) gene in mice resulted in loss of Tks5α and resulted in neonatal lethality, with obvious defects in palate formation, further supporting a role for Tks5 in neural crest-derived cell types (Cejudo-Martin et al., 2014). It has also been shown that during neural development, axon guidance involved the elaboration of Tks5-expressing podosomes in growth cones (Santiago-Medina et al., 2015). In humans, mutations in *SH3PXD2B* (Tks4) are found frequently in individuals with Frank–ter Haar syndrome (FTHS), a developmental disorder characterized by skeletal, cardiovascular and eye abnormalities (Iqbal et al., 2010; Cejudo-Martin and Courtneidge, 2011; Zrhidri et al., 2017; Bendon et al., 2012), as well as in one case of Borrone dermato-cardio-skeletal syndrome (Wilson et al., 2014). Mouse knockout or mutation of *Sh3pxd2b* phenocopied all of the essential features of FTHS (Iqbal et al., 2010; Mao et al., 2011). Several different mutations in Tks4 have been reported, with many predicted to truncate the protein, although one intriguing missense mutation (R43W) has been reported in a structurally conserved arginine in the lipid-binding PX domain. While the phenotypes of the Tks5α and Tks4 knockout mice are somewhat distinct, the fact that double knockout embryos died soon after implantation suggests that Tks4 and Tks5 also share some overlapping functions during embryogenesis (Cejudo-Martin et al., 2014).

The interactome of Tks adaptor proteins

Tks adaptors lack catalytic activity, and are therefore likely to manifest their functions through their interactions with proteins and lipids. Several interacting proteins have been identified, including metalloproteases of the ADAM family, actin regulators, such as Nck and N-WASP, and components of the NADPH oxidase complex. In many of these cases, it remains to be established whether the associations are direct, and exist within the cell. We will next describe the binding properties of the domains of the Tks adaptors and how they might act in concert to promote function.

The PX domain of Tks5 adaptor proteins has affinity for inositol phospholipids, in particular phosphatidylinositol 3-phosphate (PtdIns3P) and PtdIns(3,4)P_2 (Abram et al., 2003). Early expression studies with the GFP-tagged PX domain of Tks5 showed its presence in endosomal structures (which are rich in PtdIns3P), whereas full-length Tks5 showed a generalized cytoplasmic localization (Abram et al., 2003). At the time, it was proposed that the full-length molecule adopted a 'closed' conformation, in which the lipid-binding cleft of the PX domain was occluded in some way (Abram et al., 2003). In a different study, live cell imaging analysis revealed that PtdIns(3,4)P_2 accumulated near focal adhesions and recruited Tks5 in a Src-dependent manner, which, in turn, suggested that phosphorylation by Src caused Tks5 to adopt the 'open' conformation and allowed the PX domain access to phosphoinositides and perhaps other proteins (Oikawa et al., 2012). However, we now understand that the most abundant Tks5 isoforms in normal fibroblasts are Tks5β and Tks5short (which lack the PX domain), and that Src transformation results in increased expression of Tks5α and the destabilization of Tks5β and/or Tks5short (Cejudo-Martin et al., 2014). Therefore, the cytoplasmic localization of Tks5 in normal fibroblasts is perhaps more likely to be the result of absence of the PX domain, rather than a conformation that prevents the PX domain from binding lipids. Ultrastructural studies will be required to fully elucidate the domain interactions of Tks5. Fewer studies have evaluated the PX domain of Tks4, although it has been reported to bind to PtdIns3P and PtdIns(3,4)P_2 (Buschman et al., 2009), as well as other phosphoinositides, including PtdIns(3,4,5)P_3 (Lányi et al., 2011; Fekete et al., 2013). An affinity of the PX domain of Tks4 for PtdIns(3,4,5)P_3 might allow its accumulation at lamellipodia and membrane ruffles (Lányi et al., 2011).

Tks proteins are known substrates of the tyrosine kinase Src, which is activated by growth factors such as platelet-derived growth factor

Journal of Cell Science (2018) 131, jcs203661. doi:10.1242/jcs.203661

and epidermal growth factor, and integrins in normal and cancer cells (Bromann et al., 2004; Lock et al., 1998; Abram et al., 2003; Buschman et al., 2009; Parsons and Parsons, 1997), as well as by changes in actin polymerization (Lock et al., 1998). Src phosphorylates Tks5 at residues Y558 and/or Y620 in humans (Y557/Y619 in mice), which is essential for Tks5α-mediated invadopodia formation and cancer cell invasion (Oikawa et al., 2012; Stylli et al., 2009; Burger et al., 2014), as well as neural crest cell migration and podosome formation (Murphy et al., 2011). However, in Src-3T3 cells, Src-mediated phosphorylation of the Tks5β isoform marks it for proteasomal degradation (Cejudo-Martin et al., 2014) (see poster). Three residues, tyrosines 25, 373, and 508, in Tks4 protein are sites of Src phosphorylation, and are required for functional invadopodia formation (Buschman et al., 2009). The effects of mutation of individual SH3 domains, PRR sequences or the many putative serine/threonine phosphorylation sites have not yet been comprehensively evaluated for either Tks4 or Tks5.

Three main functions have been ascribed to the Tks adaptor proteins in invadopodia; firstly, they act as scaffold proteins for the actin polymerization machinery, secondly, they promote the localization and/or activation of proteases at invadopodia and thirdly, they facilitate the localized generation of ROS (see poster).

Early studies using Src-3T3 cells suggested that invadopodia formation was initiated with the recruitment of Tks5 to sites near focal adhesions by binding of the PX domain of Tks5 to PtdIns(3,4)P_2 and an SH3 domain of adaptor protein Grb2 to one of the PRR sequences in Tks5 (Oikawa et al., 2008). It was further suggested that all the SH3 domains of Tks5 separately associated with N-WASP to promote actin polymerization. Other studies have revealed an interaction between the tyrosine phosphorylation sites of Tks5 and Nck1/2, which could also act as a focal point for actin polymerization by activating the actin nucleation complex Arp2/3 (Stylli et al., 2009). An alternative model has been provided by studies in a rat mammary carcinoma cell line (Sharma et al., 2013). In this system, cortactin, N-WASP, cofilin and actin first formed invadopodia precursor structures. Tks5 recruitment then occurred through interaction of its PX domain with PtdIns(3,4)P_2, which acted to stabilize invadopodia formation (Sharma et al., 2013). Neither the kinetics of Tks4 arrival at invadopodia, nor its possible interactions with actin regulators/nucleators have been investigated. However, in one study that used Src-3T3 cells, the reduced actin content of invadopodia caused by Tks4 knockdown was compensated over time by an increase in Tks5 expression (although matrix degradation was not rescued) (Buschman et al., 2009). SH3 domain capture experiments have suggested associations between Tks5 SH3 domains and dynamin (Oikawa et al., 2008; Rufer et al., 2009), a GTPase essential for podosome and invadopodia formation (McNiven et al., 2004), although in this case co-association could not be confirmed in cells. An engineered form of Tks5 that contains a mitochondrial targeting sequence caused the mitochondrial relocalization of the podosome and/or invadopodia regulators AFAP-110, p190RhoGAP (ARHGAP35) and cortactin, and inhibition of podosome formation in vascular smooth muscle cells (Crimaldi et al., 2009). However, mutational and association studies have not confirmed these interactions, or whether they were direct or indirect. In myoblasts, the cell adhesion receptor dystroglycan has been shown to interact with Tks5 (perhaps via the third SH3 domain) to regulate podosome formation in these cells (Thompson et al., 2008). More recently, an interaction between the fifth SH3 domain of Tks5 and the actin-binding protein XB130 was reported. Interference with this interaction affected cell survival and proliferation of bronchial epithelial cells (Moodley et al., 2015), as well as cell migration (Moodley et al., 2016). Whether this interaction impacts invadosome formation remains to be tested. Finally, an interaction between Tks5 and the actin-binding protein Girdin has recently been reported (Ke et al., 2017). To summarize, Tks5 brings together proteins, either by direct or indirect association, which regulate the actin machinery at membranes to form podosomes and invadopodia (see poster).

There are also several ways in which Tks adaptors facilitate the proteolytic activity associated with invadopodia. For example, the fifth SH3 domain of Tks5 (which has very high homology to the fourth SH3 domain of Tks4) interacts with members of the ADAM family of disintegrin and metalloproteinases (Abram et al., 2003), which was confirmed by co-immunoprecipitation in cell lysates. However, the functional relevance of this interaction in cancer cells is currently unknown (see Box 2 for more information about the interaction in neuronal cells). Knockdown of either Tks4 or Tks5 had no effect on the total secretion of the matrix metalloproteases MMP2 and MMP9 (Seals et al., 2005, Buschman et al., 2009). Nevertheless, Tks adaptors could directly integrate proteases with the actin assembly machinery. In this context, any overlap between the functions of Tks4 and Tks5 may be dependent on the cell type. For example, in Src-3T3 cells, Tks5 is absolutely required for both formation and function of invadopodia, whereas Tks4 plays a more prominent role in ECM degradation through a direct or indirect interaction with MT1-MMP (Buschman et al., 2009). In melanoma cells, knockdown of either Tks4 or Tks5 inhibited invadopodia formation and decreased surface expression of MT1-MMP, even though here too Tks4 co-localized with MT1-MMP to a greater extent than Tks5 (Iizuka et al., 2016). Perhaps the relative expression of Tks5 and Tks4 in different cell types has an effect on compensatory behavior, although given their structural differences, it is also likely that there are intrinsic differences in their interactomes. Recently, an association between Tks5 and the small GTPase Rab40b was reported, which acted to tether transport vesicles containing MMP2 and MMP9 to invadopodia sites (Jacob et al., 2016) (see poster). Surprisingly, the PX domain of Tks5 mediated this interaction.

Box 2. Tks5 and its interaction partners in tumor growth and other diseases

Knockdown of Tks-interacting proteins such as membrane type 1-matrix metalloproteinase 1 (MT1-MMP) (Buschman, et al., 2009) or invadopodia regulators such as cortactin (Weaver, 2008) and Cdk5 (Quintavalle, et al., 2011) can also impair growth of implanted tumors (Hotary et al., 2003; Clark et al., 2009; Feldmann et al., 2010). This suggests that invadopodia might not only facilitate passage across basement membranes, but may also interface with, and modulate, the tumor microenvironment, perhaps by increasing synthesis, release or processing of growth factors (such as vascular endothelial growth factor, VEGF), to promote tumor growth. As discussed in the main text, Tks5 interacts with members of the ADAMs family of matrix metalloproteases. Binding of Tks5 to ADAMs is not only observed in the context of invadopodia and/or podosomes, but there are also studies showing that association of Tks5 and ADAM12 upon exposure of neurons to amyloid-β peptide causes neurotoxicity in Alzheimer's disease (Malinin et al., 2005). Interference with this interaction reduces neuronal death. Furthermore, recent reports describe the importance of ADAM12 in the formation of invadopodia, as well as subsequent to their formation (Diaz et al., 2013; Eckert et al., 2017). Thus, this interaction might be explored therapeutically to treat Alzheimer's disease and perhaps also invasive cancer.

As discussed earlier, the Tks adaptor proteins have structural similarity to the organizer proteins of the NADPH oxidase complex (Kawahara and Lambeth, 2007; Lock et al., 1998; Courtneidge, 2012), which prompted us to evaluate the role of ROS in invadosome formation and function. We found that inhibition of ROS, and/or knockdown of Nox subunits, markedly reduced podosome and invadopodia formation (Diaz et al., 2009). Mapping experiments *in vitro* suggested an association of the first two SH3 domains of Tks5 with the proline-rich region of the $p22^{phox}$ subunit of the NADPH oxidase. ROS are short-lived, yet important, molecules in signal transduction, which, for example, inhibit phosphatases, activate kinases and modify actin (Bedard and Krause, 2007). One of the substrates for the ROS that are generated at invadopodia is tyrosine protein phosphatase non-receptor type 12 (PTP-PEST), which can be oxidized and subsequently transiently inactivated by ROS (Tonks, 2005), thereby augmenting tyrosine phosphorylation of Tks5 and Tks4 (Diaz et al., 2009). Intriguingly, PTP-PEST is found at invadopodia (Diaz et al., 2009) and podosomes (Chellaiah et al., 2001), where it perhaps facilitates the turnover of these structures by balancing the active and inactive states of Tks adaptors (Diaz et al., 2009). ROS may also increase MMP expression and/or activation to increase the degradation of extracellular matrix at invadopodial sites (Bedard and Krause, 2007) (see poster). More studies are warranted to determine mechanisms by which ROS mediate function at invadopodia.

Classically, the Tks-related organizer protein $p47^{phox}$ interacts with $p40^{phox}$, $p67^{phox}$ and Rac1 and/or Rac2 to form a complex that activates NADPH oxidase or its isoform Nox2 (Groemping and Rittinger, 2005). NoxO1, a homologue of $p47^{phox}$ found in non-phagocyte cells, has a similar role in the activation of Nox1 and Nox3 (Bánfi et al., 2003). Tks4 and Tks5 can also act as organizers to activate Nox1 as well Nox3, in a manner that is analogous to NoxO1 (Gianni et al., 2009). Indeed, when NoxO1 was overexpressed in DLD1 colon cancer cells, it reduced formation and function of invadopodia, perhaps because it competes with Tks4 for binding to the NADPH oxidase, but cannot recruit key invadopodia proteins because it lacks important SH3 domains and/or Src phosphorylation sites (Gianni et al., 2009). More recently, it was also shown that Tks4 and Tks5, in a Src-directed manner, can directly bind to NoxA1 (homologue of $p67^{phox}$) via its N-terminal PRR domain (Gianni et al., 2011; Gianni et al., 2010). These findings suggest that there are overlapping functions between Tks adaptor proteins and the closely related members of the $p47^{phox}$ superfamily, which have established roles in various cellular functions through the production of ROS (see poster).

Concluding remarks

Despite some remarkable progress in the molecular characterization of Tks adaptor proteins, there is still much to be done, particularly with regards to Tks4. We also lack a complete understanding of the Tks interactomes, as well as a comprehensive mutational analysis of domains, motifs and phosphorylation sites, and structural insights into their regulation. It is important to use the genetic tools now available to determine the interplay between invadopodia and the tumor microenvironment, as well as the *in vivo* roles for podosomes in many cell types.

Competing interests

The authors declare no competing or financial interests.

Funding

Work in the Courtneidge lab in recent years has been supported by grants R01 CA129686, R01 CA154002 and R21 CA177382 from the National Cancer Institute, and by funds from the Knight Cancer Institute. Deposited in PMC for release after 12 months.

Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.203661.supplemental.

References

Abram, C. L., Seals, D. F., Pass, I., Salinsky, D., Maurer, L., Roth, T. M. and Courtneidge, S. A. (2003). The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. *J. Biol. Chem.* **278**, 16844-16851.

Bánfi, B., Clark, R. A., Steger, K. and Krause, K.-H. (2003). Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. *J. Biol. Chem.* **278**, 3510-3513.

Bedard, K. and Krause, K. H. (2007). The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* **87**, 245-313.

Bendon, C. L., Fenwick, A. L., Hurst, J. A., Nürnberg, G., Nürnberg, P., Wall, S. A., Wilkie, A. O. and Johnson, D. (2012). Frank-ter Haar syndrome associated with sagittal craniosynostosis and raised intracranial pressure. *BMC Med. Genet.* **13**, 104.

Blouw, B., Seals, D. F., Pass, I., Diaz, B. and Courtneidge, S. A. (2008). A role for the podosome/invadopodia scaffold protein Tks5 in tumor growth in vivo. *Eur. J. Cell Biol.* **87**, 555-567.

Blouw, B., Patel, M., Iizuka, S., Abdullah, C., You, W. K., Huang, X., Li, J.-L., Diaz, B., Stallcup, W. B. and Courtneidge, S. A. (2015). The invadopodia scaffold protein Tks5 is required for the growth of human breast cancer cells in vitro and in vivo. *PLoS ONE*, **10**, e0121003.

Bromann, P. A., Korkaya, H. and Courtneidge, S. A. (2004). The interplay between Src family kinases and receptor tyrosine kinases. *Oncogene* **23**, 7957-7968.

Burger, K. L., Davis, A. L., Isom, S., Mishra, N. and Seals, D. F. (2011). The podosome marker protein Tks5 regulates macrophage invasive behavior. *Cytoskeleton (Hoboken, N.J.)* **68**, 694-711.

Burger, K. L., Learman, B. S., Boucherle, A. K., Sirintrapun, S. J., Isom, S., Diaz, B., Courtneidge, S. A. and Seals, D. F. (2014). Src-dependent Tks5 phosphorylation regulates invadopodia-associated invasion in prostate cancer cells. *Prostate* **74**, 134-148.

Buschman, M. D., Bromann, P. A., Cejudo-Martin, P., Wen, F., Pass, I. and Courtneidge, S. A. (2009). The novel adaptor protein Tks4 (SH3PXD2B) is required for functional podosome formation. *Mol. Biol. Cell* **20**, 1302-1311.

Cejudo-Martin, P. and Courtneidge, S. A. (2011). Podosomal proteins as causes of human syndromes: a role in craniofacial development? *Genesis* **49**, 209-221.

Cejudo-Martin, P., Yuen, A., Vlahovich, N., Lock, P., Courtneidge, S. A. and Díaz, B. (2014). Genetic disruption of the Sh3pxd2a gene reveals an essential role in mouse development and the existence of a novel isoform of Tks5. *PLoS ONE* **9**, e107674.

Chellaiah, M. A., Biswas, R. S., Yuen, D., Alvarez, U. M. and Hruska, K. A. (2001). Phosphatidylinositol 3,4,5-trisphosphate directs association of Src homology 2-containing signaling proteins with gelsolin. *J. Biol. Chem.* **276**, 47434-47444.

Chen, W. T. (1990). Transmembrane interactions at cell adhesion and invasion sites. *Cell Differ. Dev.* **32**, 329-335.

Clark, E. S., Brown, B., Whigham, A. S., Kochaishvili, A., Yarbrough, W. G. and Weaver, A. M. (2009). Aggressiveness of HNSCC tumors depends on expression levels of cortactin, a gene in the 11q13 amplicon. *Oncogene* **28**, 431-444.

Courtneidge, S. A. (2012). Cell migration and invasion in human disease: the Tks adaptor proteins. *Biochem. Soc. Trans.* **40**, 129-132.

Courtneidge, S. A., Azucena, E. F., Pass, I., Seals, D. F. and Tesfay, L. (2005). The SRC substrate Tks5, podosomes (invadopodia), and cancer cell invasion. *Cold Spring Harb. Symp. Quant. Biol.* **70**, 167-171.

Crimaldi, L., Courtneidge, S. A. and Gimona, M. (2009). Tks5 recruits AFAP-110, p190RhoGAP, and cortactin for podosome formation. *Exp. Cell Res.* **315**, 2581-2592.

Diaz, B., Shani, G., Pass, I., Anderson, D., Quintavalle, M. and Courtneidge, S. A. (2009). Tks5-dependent, nox-mediated generation of reactive oxygen species is necessary for invadopodia formation. *Sci. Signal.* **2**, ra53.

Diaz, B., Yuen, A., Iizuka, S., Higashiyama, S. and Courtneidge, S. A. (2013). Notch increases the shedding of HB-EGF by ADAM12 to potentiate invadopodia formation in hypoxia. *J Cell Biol.* **201**, 279-292.

Eckert, M. A., Santiago-Medina, M., Lwin, T. M., Kim, J., Courtneidge, S. A. and Yang, J. (2017). ADAM12 induction by Twist1 promotes tumor invasion and metastasis via regulation of invadopodia and focal adhesions. *J. Cell Sci.* **130**, 2036-2048.

Ellson, C. D., Andrews, S., Stephens, L. R. and Hawkins, P. T. (2002). The PX domain: a new phosphoinositide-binding module. *J. Cell Sci.* **115**, 1099-1105.

Fekete, A., Bőgel, G., Pesti, S., Péterfi, Z., Geiszt, M. and Buday, L. (2013). EGF regulates tyrosine phosphorylation and membrane-translocation of the scaffold protein Tks5. *J. Mol. Signal.* **8**, 8-8.

Feldmann, G., Mishra, A., Hong, S. M., Bisht, S., Strock, C. J., Ball, D. W., Goggins, M., Maitra, A. and Nelkin, B. D. (2010). Inhibiting the cyclin-dependent

kinase CDK5 blocks pancreatic cancer formation and progression through the suppression of Ras-Ral signaling. *Cancer Res.* **70**, 4460-4469.
Gianni, D., Diaz, B., Taulet, N., Fowler, B., Courtneidge, S. A. and Bokoch, G. M. (2009). Novel p47(phox)-related organizers regulate NADPH oxidase 1 (Nox1) activity and localization. *Sci. Signal.* **2**, ra54-ra54.
Gianni, D., Taulet, N., Dermardirossian, C. and Bokoch, G. M. (2010). c-Src-mediated phosphorylation of NoxA1 and Tks4 induces the reactive oxygen species (ROS)-dependent formation of functional invadopodia in human colon cancer cells. *Mol. Biol. Cell* **21**, 4287-4298.
Gianni, D., Dermardirossian, C. and Bokoch, G. M. (2011). Direct interaction between Tks proteins and the N-terminal proline-rich region (PRR) of NoxA1 mediates Nox1-dependent ROS generation. *Eur. J. Cell Biol.* **90**, 164-171.
Gligorijevic, B., Bergman, A. and Condeelis, J. (2014). Multiparametric Classification Links Tumor Microenvironments with Tumor Cell Phenotype. *PLOS Biol.* **12**, e1001995.
Groemping, Y. and Rittinger, K. (2005). Activation and assembly of the NADPH oxidase: a structural perspective. *Biochem. J.* **386**, 401-416.
Hoshino, D., Branch, K. M. and Weaver, A. M. (2013). Signaling inputs to invadopodia and podosomes. *J. Cell Sci.* **126**, 2979-2989.
Hotary, K. B., Allen, E. D., Brooks, P. C., Datta, N. S., Long, M. W. and Weiss, S. J. (2003). Membrane type I matrix metalloproteinase usurps tumor growth control imposed by the three-dimensional extracellular matrix. *Cell* **114**, 33-45.
Iizuka, S., Abdullah, C., Buschman, M. D., Diaz, B. and Courtneidge, S. A. (2016). The role of Tks adaptor proteins in invadopodia formation, growth and metastasis of melanoma. *Oncotarget* **7**, 78473-78486.
Iqbal, Z., Cejudo-Martin, P., De Brouwer, A., Van Der Zwaag, B., Ruiz-Lozano, P., Scimia, M. C., Lindsey, J. D., Weinreb, R., Albrecht, B., Megarbane, A. et al. (2010). Disruption of the Podosome Adaptor Protein TKS4 (SH3PXD2B) Causes the Skeletal Dysplasia, Eye, and Cardiac Abnormalities of Frank-Ter Haar Syndrome. *Am. J. Hum. Genet.* **86**, 254-261.
Jacob, A., Linklater, E., Bayless, B. A., Lyons, T. and Prekeris, R. (2016). The role and regulation of Rab40b-Tks5 complex during invadopodia formation and cancer cell invasion. *J. Cell Sci.* **129**, 4341-4353.
Kawahara, T. and Lambeth, J. D. (2007). Molecular evolution of Phox-related regulatory subunits for NADPH oxidase enzymes. *BMC Evol. Biol.* **7**, 178.
Ke, Y., Bao, T., Zhou, Q., Wang, Y., Ge, J., Fu, B., Wu, X., Tang, H., Shi, Z., Lei, X. et al. (2017). Discs large homolog 5 decreases formation and function of invadopodia in human hepatocellular carcinoma via Girdin and Tks5: Dlg5 regulates HCC invadopodia. *Int. J. Cancer.* **141**, 364-376.
Lányi, Á., Baráth, M., Péterfi, Z., Bőgel, G., Orient, A., Simon, T., Petrovszki, E., Kis-Tóth, K., Sirokmány, G., Rajnavölgyi, É. et al. (2011). The Homolog of the Five SH3-Domain Protein (HOFI/SH3PXD2B) Regulates Lamellipodia Formation and Cell Spreading. *PLoS ONE* **6**, e23653.
Lener, T., Burgstaller, G., Crimaldi, L., Lach, S. and Gimona, M. (2006). Matrix-degrading podosomes in smooth muscle cells. *Eur. J. Cell Biol.* **85**, 183-189.
Leong, H. S., Robertson, A. E., Stoletov, K., Leith, S. J., Chin, C. A., Chien, A. E., Hague, M. N., Ablack, A., Carmine-Simmen, K., Mcpherson, V. A. et al. (2014). Invadopodia are required for cancer cell extravasation and are a therapeutic target for metastasis. *Cell Rep.* **8**, 1558-1570.
Li, C. M.-C., Chen, G., Dayton, T. L., Kim-Kiselak, C., Hoersch, S., Whittaker, C. A., Bronson, R. T., Beer, D. G., Winslow, M. M. and Jacks, T. (2013). Differential Tks5 isoform expression contributes to metastatic invasion of lung adenocarcinoma. *Genes Dev.* **27**, 1557-1567.
Linder, S., Wiesner, C. and Himmel, M. (2011). Degrading devices: invadosomes in proteolytic cell invasion. *Annu. Rev. Cell Dev. Biol.* **27**, 185-211.
Lock, P., Abram, C. L., Gibson, T. and Courtneidge, S. A. (1998). A new method for isolating tyrosine kinase substrates used to identify fish, an SH3 and PX domain-containing protein, and Src substrate. *EMBO J.* **17**, 4346-4357.
Malinin, N. L., Wright, S., Seubert, P., Schenk, D. and Griswold-Prenner, I. (2005). Amyloid-beta neurotoxicity is mediated by FISH adaptor protein and ADAM12 metalloprotease activity. *PNAS* **102**, 3058-3063.
Mao, M., Hedberg-Buenz, A., Koehn, D., John, S. W. and Anderson, M. G. (2011). Anterior segment dysgenesis and early-onset glaucoma in nee mice with mutation of Sh3pxd2b. *Invest. Ophthalmol. Vis. Sci.* **52**, 2679-2688.
Mcniven, M. A., Baldassarre, M. and Buccione, R. (2004). The role of dynamin in the assembly and function of podosomes and invadopodia. *Front. Biosci.* **9**, 1944-1953.
Mitra, S. K. and Schlaepfer, D. D. (2006). Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr. Opin. Cell Biol.* **18**, 516-523.
Moodley, S., Hui Bai, X., Kapus, A., Yang, B. and Liu, M. (2015). XB130/Tks5 scaffold protein interaction regulates Src-mediated cell proliferation and survival. *Mol. Biol. Cell* **26**, 4492-4502.
Moodley, S., Derouet, M., Bai, X. H., Xu, F., Kapus, A., Yang, B. B. and Liu, M. (2016). Stimulus-dependent dissociation between XB130 and Tks5 scaffold proteins promotes airway epithelial cell migration. *Oncotarget* **7**, 76437-76452.
Murphy, D. A. and Courtneidge, S. A. (2011). The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. *Nat. Rev. Mol. Cell Biol.* **12**, 413-426.
Murphy, D. A., Diaz, B., Bromann, P. A., Tsai, J. H., Kawakami, Y., Maurer, J., Stewart, R. A., Izpisua-Belmonte, J. C. and Courtneidge, S. A. (2011). A Src-Tks5 pathway is required for neural crest cell migration during embryonic development. *PLoS ONE* **6**, e22499.
Oikawa, T., Itoh, T. and Takenawa, T. (2008). Sequential signals toward podosome formation in NIH-src cells. *J. Cell Biol.* **182**, 157.
Oikawa, T., Oyama, M., Kozuka-Hata, H., Uehara, S., Udagawa, N., Saya, H. and Matsuo, K. (2012). Tks5-dependent formation of circumferential podosomes/invadopodia mediates cell–cell fusion. *J. Cell Biol.* **197**, 553-568.
Parsons, J. T. and Parsons, S. J. (1997). Src family protein tyrosine kinases: cooperating with growth factor and adhesion signaling pathways. *Curr. Opin. Cell Biol.* **9**, 187-192.
Pawson, T. and Gish, G. D. (1992). SH2 and SH3 domains: from structure to function. *Cell* **71**, 359-362.
Ponting, C. P. (1996). Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: binding partners of SH3 domains? *Protein Sci.* **5**, 2353-2357.
Quintavalle, M., Elia, L., Price, J. H., Heynen-Genel, S. and Courtneidge, S. A. (2011). A cell-based high-content screening assay reveals activators and inhibitors of cancer cell invasion. *Sci. Signal.* **4**, ra49.
Rufer, A. C., Rumpf, J., Von Holleben, M., Beer, S., Rittinger, K. and Groemping, Y. (2009). Isoform-selective interaction of the adaptor protein Tks5/FISH with Sos1 and dynamins. *J. Mol. Biol.* **390**, 939-950.
Santiago-Medina, M., Gregus, K. A., Nichol, R. H., O'toole, S. M. and Gomez, T. M. (2015). Regulation of ECM degradation and axon guidance by growth cone invadosomes. *Development* **142**, 486-496.
Sato, T. K., Overduin, M. and Emr, S. D. (2001). Location, location, location: membrane targeting directed by PX domains. *Science* **294**, 1881-1885.
Seals, D. F., Azucena, E. F., Jr, Pass, I., Tesfay, L., Gordon, R., Woodrow, M., Resau, J. H. and Courtneidge, S. A. (2005). The adaptor protein Tks5/Fish is required for podosome formation and function, and for the protease-driven invasion of cancer cells. *Cancer Cell* **7**, 155-165.
Sharma, V. P., Eddy, R., Entenberg, D., Kai, M., Gertler, F. B. and Condeelis, J. (2013). Tks5 and SHIP2 regulate invadopodium maturation, but not initiation, in breast carcinoma cells. *Curr. Biol.* **23**, 2079-2089.
Stylli, S. S., Stacey, T. T., Verhagen, A. M., Xu, S. S., Pass, I., Courtneidge, S. A. and Lock, P. (2009). Nck adaptor proteins link Tks5 to invadopodia actin regulation and ECM degradation. *J. Cell Sci.* **122**, 2727-2740.
Stylli, S. S., I, S. T., Kaye, A. H. and Lock, P. (2012). Prognostic significance of Tks5 expression in gliomas. *J. Clin. Neurosci.* **19**, 436-442.
Stylli, S. S., Luwor, R. B., Kaye, A. H., I, S. T., Hovens, C. M. and Lock, P. (2014). Expression of the adaptor protein Tks5 in human cancer: prognostic potential. *Oncol. Rep.* **32**, 989-1002.
Tarone, G., Cirillo, D., Giancotti, F. G., Comoglio, P. M. and Marchisio, P. C. (1985). Rous sarcoma virus-transformed fibroblasts adhere primarily at discrete protrusions of the ventral membrane called podosomes. *Exp. Cell Res.* **159**, 141-157.
Thompson, O., Kleino, I., Crimaldi, L., Gimona, M., Saksela, K. and Winder, S. J. (2008). Dystroglycan, Tks5 and Src mediated assembly of podosomes in myoblasts. *PLoS ONE* **3**, e3638.
Tonks, N. K. (2005). Redox redux: revisiting PTPs and the control of cell signaling. *Cell* **121**, 667-670.
Weaver, A. M. (2008). Cortactin in tumor invasiveness. *Cancer Lett.* **265**, 157-166.
Wilson, G. R., Sunley, J., Smith, K. R., Pope, K., Bromhead, C. J., Fitzpatrick, E., Di Rocco, M., Van Steensel, M., Coman, D. J., Leventer, R. J. et al. (2014). Mutations in SH3PXD2B cause Borrone dermato-cardio-skeletal syndrome. *Eur. J. Hum. Genet.* **22**, 741-747.
Zrhidri, A., Jaouad, I. C., Lyahyai, J., Raymond, L., Egea, G., Taoudi, M., Mouatassim, S. E. L. and Sefiani, A. (2017). Identification of two novel SH3PXD2B gene mutations in Frank-Ter Haar syndrome by exome sequencing: case report and review of the literature. *Gene* **628**, 190-193.