TKS4 and TKS5 Scaffold Proteins

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Scaffold proteins are typically thought of as multi-domain “bridging molecules.” They serve as crucial regulators of key signaling events by simultaneously binding multiple participants involved in specific signaling pathways. In the case of epidermal growth factor (EGF)-epidermal growth factor receptor (EGFR) binding, the activated EGFR contacts cytosolic SRC tyrosine-kinase, which then becomes activated. This process leads to the phosphorylation of SRC-substrates, including the tyrosine kinase substrates (TKS) scaffold proteins. The TKS proteins serve as a platform for the recruitment of key players in EGFR signal transduction, promoting cell spreading and migration. The TKS4 and the TKS5 scaffold proteins are **t**yrosine **k**inase **s**ubstrates with **f**our or **f**ive SH3 domains, respectively.

Their structural features allow them to recruit and bind a variety of signaling proteins and to anchor them to the cytoplasmic surface of the cell membrane.

TKS4 and TKS5 had been recognized for their involvement in cellular motility, reactive oxygen species-dependent processes, and embryonic development. Furthermore, TKS4 has also been implicated in the regulation of homeostasis of mature adipose and bone tissue.

Keywords: scaffold protein ; tyrosine kinase substrates ; TKS4 ; TKS5 ; Beige adipose tissue ; osteoporosis ; EMT ; EGFR ; Src ; SH3 domai ; adaptor protein ; mesenchymal stem cells ; MSC ; FTHS ; podosome ; invadopodia ; rare hereditary disease ; Frank-ter Haar syndrome ; OMIM:249420 ; Tks4-KO mice ; SH

1. Introduction

Scaffold proteins modulate intracellular signaling by bringing regulatory proteins, enzymes, or cytoskeletal structures in close proximity [1]. TKS molecules are large scaffold proteins earning their name from the early observation that they serve as tyrosine kinase substrates of SRC kinase [2][3][4]. TKS4 and TKS5 contain one Phox Homology (PX) domain, conserved linear motifs, e.g., several proline-rich motifs (PRMs), and four or five SRC Homology 3 (SH3) domains, respectively. Other names for TKS5 are SH3 and PX domain-containing protein 2A (SH3PXD2A) and Five SH3 domains (FISH), while TKS4 is also known as SH3 and PX domain-containing protein 2B (SH3PXD2B), Homolog of FISH (HOFI), and a factor of adipocyte differentiation 49 (Fad49), reflecting some of their known characteristics [3][5]. The main function of the PX domain is to link the TKS scaffold proteins to the cell membrane via phosphoinositide binding [2][6]. The SH3 domains serve as docking sites for signaling molecules and mediate protein-protein interactions [7]. It is likely that the PRMs of the TKS proteins represent contact sites for SH3 domain-containing molecules (Figure 1). The TKS proteins are phylogenetically related and are expressed in vertebrates, and TKS-like genes are widely present in invertebrates [8]. TKS scaffold proteins are broadly expressed in tissues except for the testis for TKS4, and the spleen and testis for TKS5 [2][3]. They are also expressed in several transformed cell lines [2][9].

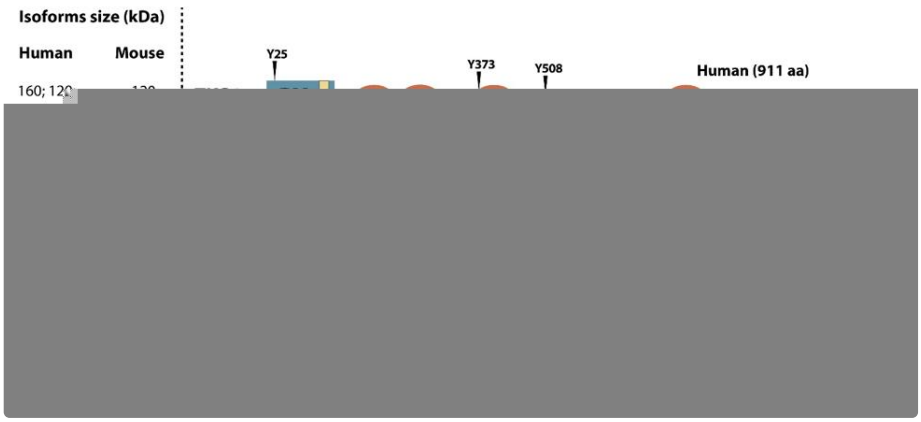

Figure 1. Members of the p47 organizer protein family. The p47 organizer family consists of five structurally similar adaptor/scaffold proteins containing an N-terminal PX domain followed by several SH3 domains, namely $p40^{phox}$, NOXO1, $p47^{phox}$, TKS4, and TKS5. Experimentally confirmed SRC kinase tyrosine phosphorylation sites ("Y") in the human and mouse TKS proteins are shown above and below the depicted domain architecture, respectively.

2. TKS4 and TKS5 Affect Multiple Biological Processes from Growth Factor Receptor Signaling to Metastasis to Homeostasis of Adipose and Bone Tissue

EGFR Signaling via TKS4 and TKS5

Receptor tyrosine kinases (RTK) are transmembrane proteins that control several cellular processes, ranging from proliferation to differentiation and cell migration. Following the binding of their extracellular ligands, RTKs dimerize, undergo auto-phosphorylation on multiple tyrosine residues in their cytoplasmic region, and associate with intracellular signaling molecules. Diverse molecular cascades transmit the signal from RTKs to their final effector molecules, ultimately leading to the modulation of distinct biological processes within the cell [20].

Epidermal growth factor receptor (EGFR) is one of the most well-studied RTKS. Upon activation, it initiates several signal transduction cascades, including the RAS-RAF-MEK, phosphatidylinositol 3 (PI3)- kinase-AKT, PLCγ, and JAK-STAT pathways [21]. Moreover, active EGFR binds cytosolic SRC tyrosine-kinase, which then becomes activated [22][23][24][25]. This process leads to the phosphorylation of SRC-substrates, including the TKS scaffold proteins, which are known to be involved in EGFR signaling [11][26][27]. The TKS proteins serve as a platform for the recruitment of key players in EGFR signal transduction (Figure 2, Table 1), promoting cell spreading and migration [9][11][28][29][30]. In response to EGFR activation, PI3 kinases are activated, and lipids are phosphorylated in the plasma membrane. For example, phosphatidylinositol (4,5)-bisphosphate ($PI(4,5)P_2$) is converted to phosphatidylinositol (3,4,5)-trisphosphate ($PI(3,4,5)P_3$) [31]. According to a model proposed by Bögel et al., the phosphorylated lipid residues anchor the PX domain of TKS4 and translocate the scaffold protein from the cytoplasm to the plasma membrane [11]. On the other arm of the signaling pathway, SRC kinase is also activated by binding to the intracellular tail of EGFR [22][23][24][25] subsequently phosphorylating tyrosine residues on TKS4 (i.e., Tyr25, Tyr373, Tyr508) (Figure 1) [2]. Phosphorylated TKS4 can bind activated SRC by interacting with both its SH2 and SH3 domains. In this complex, SRC remains active for a prolonged period of time and may phosphorylate multiple downstream molecules/partners [32]. This direct interaction between TKS4 with SRC was shown to involve the proline-rich region PSRPLPDAP (residues 466–474) and the tyrosine-phosphorylated pYEEI motif (residues 508–511) of TKS4 (both located between the third and fourth SH3 domains) and the SH3 and SH2 domains of SRC, respectively [32]. Upon PI3 kinase activation, TKS5 also translocates to the plasma membrane in epidermal growth factor (EGF)-stimulated cells [26]. The PX domain of both TKS4 and 5 was found to be essential for the participation of the molecules in EGFR signaling and for the phosphorylation of TKS4 and 5 by activated SRC [11][26]. TKS4 forms a complex with EGFR in which either SRC or a yet unidentified protein may serve as a bridge between the two molecules [11][32]. For example, growth factor receptor binding protein 2 (GRB2) has been identified as a binding partner of both EGFR and TKS4 [28]. No strong interaction between TKS5 and EGFR or SRC has been detected so far, suggesting that, despite their structural similarities, there is only a partial overlap between the regulation of TKS4 and TKS5 in EGF signaling [11][26].

Figure 2. The role of TKS proteins in the recruitment of signaling molecules. ADAM12/15/19 – a disintegrin and metalloprotease 12/15/19, ARP2/3 – actin-related protein 2/3, GRB2 – growth factor receptor binding protein 2, MT1MMP – membrane type 1 matrix metalloprotease, NCK – non-catalytic region of tyrosine kinase adaptor protein, N-WASP –

neural Wiskott-Aldrich syndrome protein, PtdIns – phosphatidylinositol, RUK/CIN85 - regulator of ubiquitous kinase/Cbl-interacting protein of 85 kDa, SRC - proto-oncogene tyrosine-protein kinase Src.

Table 1. Known protein binding partners of TKS4 and TKS5. The known binding partners of (**a**) TKS4 and (**b**) TKS5 are shown with the methods of detection and the binding sites within the TKS molecules. Some of the well-described functions of the binding partners are also listed. ECM – extracellular matrix, EMT – epithelial-mesenchymal transition, ITC – isothermal titration calorimetry, NOX1 – NADPH oxidase 1, PRR – proline-rich region, ROS – reactive oxygen species, RTK – receptor tyrosine kinase. * The first and second SH3 domains cooperate to form a common “super SH3 platform” and allow the binding of the proline-rich region of the partner protein [33].

Table 1a.

TKS4

Partner	Method	TKS4-Interacting Site	Function
ADAM15 [34]	GST pull-down assay	4th SH3 domain	Ectodomain shedding, cell adhesion, and signaling [35]
Cortactin [9]	Co-immunoprecipitation, GST pull-down assay, immunofluorescence co-localization	Unknown	Regulation of actin cytoskeleton [36]
CR16 [37]	GST pull-down assay	Weak interaction with the 2nd, 3rd, and 4th SH3 domains	Reorganization of actin cytoskeleton [38]
DNM2 [37]	GST pull-down assay	3rd SH3 domain	Endo-/exocytosis [37]
FasL (CD178) [39]	Phage display screening	3rd and 4th SH3 domains	Apoptosis induction [40]
GRB2 [28]	Affinity purification–selected reaction monitoring mass spectrometry	Unknown	Adaptor protein involved in the regulation of RTK signaling, cycle progression, actin-based cell motility, podosome formation [41]
NOXA1 [42] [43]	Co-immunoprecipitation, GST pull-down assay	Unknown	ROS generation through NOX1 activation [44]
N-WASP [37]	GST pull-down assay	2nd SH3 domain	A scaffold protein regulating actin cytoskeleton reorganization, and actin polymerization during cell motility and invasion [45]
OPHN1 [37]	GST pull-down assay	3rd SH3 domain	Endo-/exocytosis [37]
RUK/CIN85 [46]	GST pull-down assay	Unknown	Adaptor protein that recruits endocytotic regulatory proteins, and regulates RTK internalization, trafficking, and degradation [47]

SRC [9][32]	Co-immunoprecipitation; GST pull-down and fluorescence-polaziation assays, Duolink proximity ligation assay	PRR (aa: 466–474); P-Tyr motif (aa: 508–511)	Regulation of cell growth, differentiation, proliferation, survival, adhesion, migration, and motility [9][32]
SYNJ1 [37]	GST pull-down assay	3rd SH3 domain and weak interaction with the 4th SH3 domain	Endo-/exocytosis [37]

Table 1b.

TKS5

Partner	**Method**	**TKS5-Interacting Site**	**Function**
ADAM12 [6]	Co-immunoprecipitation, immunofluorescence co-localization	5th SH3 domain	Cell adhesion and fusion, extracellular matrix restructuring, reorganization of actin cytoskeleton, regulation of ectodomain shedding [48]
ADAM15 [6]	Co-immunoprecipitation	5th SH3 domain	Cell adhesion, degradation of ECM components, ectodomain shedding of membrane-bound growth factors [35]
ADAM19 [6]	Phage display screen, co-immunoprecipitation	5th SH3 domain	Extracellular matrix breakdown and reconstruction, ectodomain shedding, role in embryogenesis, cardiovascular system development, obesity, and insulin resistance [49]
b-dystroglycan [50]	Phage display screen, GST pull-down assay, co-immunoprecipitation, immunofluorescence co-localization	3rd SH3 domain	Links the extracellular matrix to the intracellular actin cytoskeleton [50]
CircSKA3 [51]	Co-immunoprecipitation, pull-down assay	Not specified	Circular RNA, an inducer of invadopodium formation [51]
Drebrin [52]	Co-immunoprecipitation	Unknown	An actin-binding protein involved in the regulation of actin filament organization, role in cell migration, cell process formation, intercellular communication, metastasis, and brain development [53]

Dynamin [29][33]	Peptide spot membrane assay, GST pull-down assay, ITC, immunofluorescence co-localization, GST pull-down assay, mass spectrometry/Western blotting	1st and 2nd SH3 domains; 1st and 5th SH3 domains	Regulation of actin cytoskeleton, podosome/invadopodium formation, role in endocytosis [54]
F-actin [29]	GST pull-down assay, and mass spectrometry	5th SH3 domain	Component of cytoskeleton [55]
FasL (CD178) [39]	Phage display screening	5th SH3 domain	Apoptosis induction [40]
FGD1 [56]	Co-immunoprecipitation and mass spectrometry, GST pull-down assay, immunofluorescence co-localization	4th and 5th SH3 domains	A guanine nucleotide exchange factor for the Rho-GTPase CDC42, assembly of podosomes and invadopodia, control of secretory membrane-trafficking, and cell cycle [56][57]
Girdin [58]	Co-immunoprecipitation, immunofluorescence co-localization	Unknown	actin-binding protein regulating actin remodeling and cell polarity, collective migration of neuroblasts, epithelial and cancer cells [59]
GRB2 [28][29]	Co-immunoprecipitation	Polyproline sequences	An adaptor protein involved in cell cycle progression and actin-based cell motility, podosome formation [41]
IRTKS [60]	GST pull-down assay	First binding site located in the segment comprising the 1st and 2nd SH3 domains, second binding site located in the segment comprising the 3rd and 4th SH3 domains	Regulation of plasma membrane dynamics, actin cytoskeleton remodeling, cell migration and polarization, insulin signaling [61]
MT4-MMP [62]	Co-immunoprecipitation	Unknown	Induction of invadopodia and amoeboid movement, degradation of ECM components, role in hypoxia-mediated metastasis [62]
NCK [52]	Co-immunoprecipitation, fluorescence co-localization	Linker region between the 3rd and 4th SH3 domains containing pY557	Adaptor protein involved in cytoskeletal remodeling, invadopodium formation, cell proliferation [63]

Nogo-B [29]	GST pull-down assay and mass spectrometry	5th SH3 domain	Roles in vascular remodeling, cell migration and proliferation, and EMT [64]
NOXA1 [42][43]	Co-immunoprecipitation, GST pull-down assay	One or more of the five SH3 domains	ROS generation through NOX1 activation [44]
N-WASP [29]	GST pull-down assay and mass spectrometry/Western blotting, co-immunoprecipitation	All five SH3 domains	A scaffold protein regulating actin cytoskeleton reorganization, and actin polymerization during cell motility and invasion [45]
p22^{phox} [65]	Co-immunoprecipitation	1st and 2nd SH3 domains	Subunit of NADPH oxidases involved in ROS generation through NOX activity [66]
Rab40b [67]	GST pull-down assay, co-immunoprecipitation,	PX-domain: sites 14-KRR-19 and Y24 in 23-YVYI-28	A GTPase required for the sorting and secretion of MMP2 and MMP9, promotion of migration, invasion, and metastasis of cancer cells [67][68]
RET [69]	Co-immunoprecipitation, immunofluorescence co-localization	Unknown	A receptor tyrosine kinase mediating stress fiber formation, cell polarization, directional migration and invasion, enhancement of proteolytic activity [69]
SOS1 [33]	Immunofluorescence co-localization, peptide spot membrane assay, GST pull-down assay, isothermal titration calorimetry	1st and 2nd SH3 domains *	A guanine nucleotide exchange factor promoting Ras and Rac activation downstream of a variety of receptors such as RTKs [70]
Tubulin [29]	GST pull-down assay and mass spectrometry	3rd SH3 domain	Component of microtubules, affects cell division, differentiation, intracellular transport, motility [71]
WIP [29]	GST pull-down assay and mass spectrometry	3rd and 5th SH3 domains	Regulation of actin cytoskeleton assembly and remodeling [72]
XB130 [73]	Yeast two-hybrid screening, co-immunoprecipitation, GST pull-down assay, immunofluorescence co-localization	5th SH3 domain	A scaffold protein influencing cell growth, survival, and migration [73]
Zyxin [29]	GST pull-down assay and mass spectrometry	3rd and 5th SH3 domains	A focal adhesion protein involved in actin cytoskeleton assembly [74]

In recent years, more possible interaction partners of TKS4 have been identified (Table 1(A)). One possible partner is cortactin [9], a well-known substrate of SRC localized to cortical actin structures within cells. Cortactin can bind the actin-related protein-2/3 (ARP2/3) and neural Wiskott-Aldrich syndrome protein (N-WASP) proteins, and it mediates actin polymerization [75][76][77]. Therefore, TKS4 was expected to be involved in EGFR signaling-mediated actin cytoskeleton

assembly and rearrangement. This proposed mechanism was confirmed by Lányi et al. [9]. They found that, in response to EGF stimulation, TKS4 associates with cellular motility-associated membrane ruffles. They also showed that, when constitutively active SRC is present, TKS4 accumulates in podosomes (actin-rich membrane protrusions involved in cell motility, see below) while forming a complex with SRC and cortactin [9]. TKS5 has also been reported to bind cortactin and other proteins important in the regulation of actin cytoskeleton assembly, including N-WASP, non-catalytic region of tyrosine kinase adaptor protein (NCK), and GRB2 (for a full list, see Table 1b) [10][29][52][78][79].

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