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Molecules in focus

PLEKHA7: Cytoskeletal adaptor protein at center stage in junctional organization and signaling

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ABSTRACT

PLEKHA7 is a recently characterized component of the cytoplasmic region of epithelial adherens junctions (AJ). It comprises two WW domains, a pleckstrin-homology domain, and proline-rich and coiled-coil domains. PLEKHA7 interacts with cytoplasmic components of the AJ (p120-catenin, paracingulin, afadin), stabilizes the E-cadherin complex by linking it to the minus ends of noncentrosomal microtubules, and stabilizes junctional nectins through the newly identified interactor PDZD11. Similarly to afadin, and unlike E-cadherin and p120-catenin, the localization of PLEKHA7 at AJ is strictly zonular (in the *zonula adhaerens* subdomain of AJ), and does not extend along the basolateral contacts. Genome-wide association studies and experiments on animal and cellular models show that although PLEKHA7 is not required for organism viability, it is implicated in cardiovascular physiology, hypertension, primary angle closure glaucoma, susceptibility to staphylococcal α-toxin, and epithelial morphogenesis and growth. Thus, PLEKHA7 is a cytoskeletal adaptor protein important for AJ organization, and at the center of junction-associated signaling pathways which fine-tune important pathophysiological processes.

1. Introduction

PLEKHA7 stands for Pleckstrin Homology Domain Containing, Family A Member 7, and was discovered as a new AJ protein independently by the Takeichi laboratory through its interaction with the N-terminal region of p120-catenin (Meng et al., 2008), and by the Citi laboratory as an interactor of the N-terminal region of paracingulin (CGNL1) (Pulimeno et al., 2010; Pulimeno et al., 2011). Genomic and genetic studies also identified PLEKHA7 as a protein associated with human hypertension (Levy et al., 2009), and implicated in cardiac development and contractility (Wythe et al., 2011). PLEKHA7 is involved not only in stabilizing the two main transmembrane components of AJ, e.g. E-cadherin (Meng et al., 2008) and nectins (Guerrera et al., 2016), but also in a wider array of signaling functions, which are relevant to its role in tissue physiology and pathology.

2. Gene and protein structure

PLEKHA7 is coded by the *plekha7* gene, which is found only in vertebrate organisms, although other members of Pleckstrin Homology Domain Containing Family A of genes are expressed in lower metazoa. In the human genome the *plekha7* gene maps to chromosome 11 and spans a 237 kB region. In the mouse the *plekha7* gene maps to chromosome 7 and spans a 185 kB region.

The amino acid sequence of the PLEKHA7 protein is well conserved throughout vertebrate species: the sequence identity between the human protein sequence and those of dog, mouse, chicken, *Xenopus laevis* and *Danio rerio* are 94%, 89%, 73%, 61% and 64%, respectively.

Human PLEKHA7 is predicted to exist in two isoforms, comprising either 1121 or 1271 amino acids, and PLEKHA7 is detected as polypeptides with apparent sizes of 135–145 kDa in lysates of mammalian cells and tissues (Pulimeno et al., 2010). The functional differences between the isoforms are not known. The N-terminal half of PLEKHA7 contains a pleckstrin-homology (PH) domain (residues 166–281) (Fig. 1A), which allows PLEKHA7 to interact in vitro with phosphorylated phosphatidyinositol lipids (Wythe et al., 2011) (PI in Fig. 2B). A region of PLEKHA7 comprising the PH domain, but lacking the WW domain (residues 120–374) is

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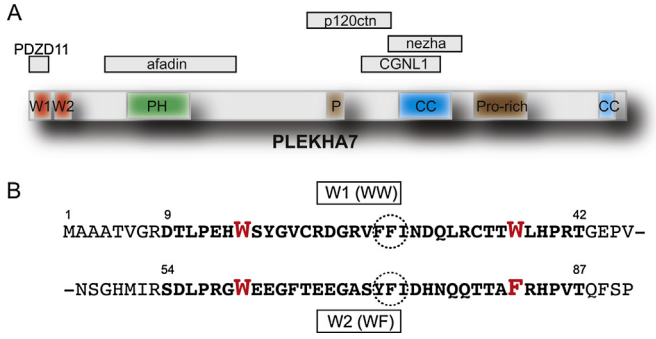

Fig. 1. The structure and molecular interactors of PLEKHA7. (A) Scheme of human PLEKHA7, with domains highlighted in: red (the two WW domains); green (PH); brown (proline-rich); blue (coiled-coil). The regions of interaction with PDZD11, afadin, p120-catenin, paracingulin (CGNL1) and nezha, based on in vitro binding studies, are indicated. (B) Alignement of the two WW domains of PLEKHA7 (highlighted in bold type): the first (W1) containing two Trp residues, and the second (W2) containin a Trp and a Phe residue. Signature Trp and Phe are highlighted in bold, red and larger size. Dotted circles outline the hydophobic residues at the core of the structure.

involved in PLEKHA7 interaction with the scaffold protein afadin (Kurita et al., 2013) (Fig. 1A). Although in some studies only one WW domain was annotated (Meng et al., 2008; Kurita et al., 2013), PLEKHA7 comprises two WW domains in its N-terminal region (Pulimeno et al., 2011; Wythe et al., 2011; Endres et al., 2014; Popov et al., 2015): one canonical class 1 WW domain (with the two signature Trp residues) and a second WW domain, comprising a Trp and a Phe residue, a feature detected in other class-I WW domains (Kasanov et al., 2001), separated by a spacer region of 11 residues (Fig. 1B). Both domains are 33-residue long, and contain a cluster of aromatic residues in the second β-sheet strand (dotted circle in Fig. 1B), which are implicated in the stabilization of the hydrophobic core of the structure (Martinez-Rodriguez et al., 2015). We showed that the region of PLEKHA7 comprising the WW domains specifically interacts with the small PDZ protein PDZD11, and is required for the recruitment of PDZD11 to AJ (Guerrera et al., 2016) (Fig. 1A). The C-terminal half of PLEKHA7 comprises coiled-coil (CC) and proline-rich (Pro) domains (Fig. 1A). p120-catenin binds to a central region of PLEKHA7, that includes the first proline-rich region but lacks the coiled-coil region. Paracingulin and nezha (calmodulin-regulated spectrin-associated protein 3, CAMSAP3) interact with overlapping C-terminal regions of PLEKHA7, comprising the larger coiled-coil domain (Fig. 1A). The recruitment of PLEKHA7 to AJ probably depends on its redundant interactions with phospholipids, p120-catenin and afadin (Meng et al., 2008; Wythe et al., 2011; Kurita et al., 2013; Pulimeno et al., 2010). Additional interactors of PLEKHA7, for which no direct in vitro binding validation is yet available, have been identified through mass spectrometry analysis of PLEKHA7 immunoprecipitates (Kourtidis et al., 2015; Kourtidis and Anastasiadis, 2016).

3. Expression and localization

Immunofluorescence (Meng et al., 2008; Pulimeno et al., 2010) and immuno-electron microscopy (Pulimeno et al., 2010) analyses show that PLEKHA7 is localized at adherens junctions (AJ) of epithelial cells, and specifically in its zonular subdomain (*zonula adhaerens*) (Fig. 2A), which has important signaling functions (Citi et al., 2014). The distance of PLEKHA7 from the junctional membrane, as determined by immuno-electron microscopy, is 25 nm (Pulimeno et al., 2010). Similarly to afadin and differently from E-cadherin, α-catenin, β-catenin and p120-catenin, PLEKHA7 is not detected along lateral contacts of epithelial cells (Pulimeno et al., 2010), thereby defining two pools of p120-catenin, belonging to two distinct E-cadherin complexes, one comprising and one lacking PLEKHA7 (Kourtidis et al., 2015) (Fig. 2A). However, exogenously expressed PLEKHA7 localizes at lateral contacts (Paschoud et al., 2014), suggesting different affinities for zonular and lateral complexes. PLEKHA7 is detected at zonular junctions in all epithelial tissues investigated so far, and in endothelial cells and tissues (Pulimeno et al., 2010; Guerrera et al., 2016; Lee et al., 2014). In human eye tissues expression of PLEKHA7 was detected in epithelial, smooth muscle and endothelial cells, and within anterior cavity structures, such as iris and ciliary body (Lee et al., 2014). The expression of the PLEKHA7 homolog in zebrafish, Hadp1, was determined only by mRNA in situ hybridization in the embryonic myocardium (Wythe et al., 2011). The unique subcellular localization and tissue distribution of PLEKHA7 distinguish it from both ZO-1 and E-cadherin (Pulimeno et al., 2010), suggesting that the PLEKHA7/afadin-associated protein complex bridges the ZA to tight junctions (TJ) (Citi et al., 2012).

In relationship to expression in human normal and cancer tissues, PLEKHA7 was detected at junctions of normal epithelial breast tubular structures and in low grade ductal carcinomas, but not in high grade and lobular carcinomas (Tille et al., 2015). Mislocalization or loss of PLEKHA7 was independently observed in human breast and renal cancers (Kourtidis et al., 2015).

4. Biological functions

PLEKHA7 is emerging as a multifunctional adaptor protein with important roles both in the structural organization of AJ, and in their signaling functions.

The complex between PLEKHA7, nezha and the minus-end directed motor kinesin KIFC3 allows PLEKHA7 to tether the E-cadherin complex to the minus end of noncentrosomal microtubules, thus stabilizing E-cadherin-based junctions (Meng et al., 2008) (Fig. 2B). By binding to afadin, PLEKHA7 is also indirectly linked to microfilaments (Fig. 2B). In MDCK cells, PLEKHA7 is required for the junctional localization of the zonular protein paracingulin, through their direct interaction (Pulimeno et al., 2011) (Fig. 2B). By recruiting PDZD11 to AJ, PLEKHA7 stabilizes nectins, the second major class of transmembrane adhesion receptors of AJ (Guerrera et al., 2016) (Fig. 2B). Thus, by binding to the E-cadherin complex through redundant direct and indirect interactions (p120-ctn, paracingulin, afadin), and to the nectin complex through PDZD11 and afadin, PLEKHA7 connects these two protein complexes within the ZA (Guerrera et al., 2016) (Fig. 2B). Although PLEKHA7 is not required for the formation of functional TJ (Kurita et al., 2013; Paschoud et al., 2014), overexpression experiments show that PLEKHA7 stabilizes TJ barrier function in a microtubule- and AJ-dependent manner (Paschoud et al., 2014).

In colon epithelial carcinoma cells (Caco2) the interaction with PLEKHA7 differentiates two pools of p120-catenin: an apical complex (unphosphorylated inactive p120-catenin), bound to PLEKHA7; and a basolateral complex (p120-catenin phosphorylated by activated Src), which signals to promote cell cycle progression and cancer. Indeed, Caco2 cells depleted of PLEKHA7 show increased anchorage independent growth and expression of transformation and mesenchymal markers (Kourtidis et al., 2015). Analysis of PLEKHA7 immunoprecipitates reveals the presence of components of the miRNA processing complex (DROSHA and DGCR8), allowing the processing and regulation of miR-24, miR-30a, miR30-b and let7-g, which suppress the expression of growth promoting proteins SNAIL, MYC and cyclin D1 at junctions (Kourtidis et al., 2015; Kourtidis and Anastasiadis, 2016). Consequently, in cells depleted of PLEKHA7 the junctional localization of the microprocessor complex is lost, and there is a decrease in the levels of the above mentioned miRNAs. Since reintroduction