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Review

Phosphorylation, protein kinases and ADPKD ☆

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease characterized by renal cyst formation and caused by mutations in the *PKD1* and *PKD2* genes, which encode polycystin-1(PC-1) and -2 (PC-2) proteins, respectively. PC-1 is a large plasma membrane receptor involved in the regulation of several biological functions and signaling pathways including the Wnt cascade, AP-1, PI3kinase/Akt, GSK3β, STAT6, Calcineurin/NFAT and the ERK and mTOR cascades. PC-2 is a calcium channel of the TRP family. The two proteins form a functional complex and prevent cyst formation, but the precise mechanism(s) involved remains unknown. This article is part of a Special Issue entitled: Polycystic Kidney Disease.

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Excess cAMP has been suggested to play a central role in the pathogenesis of ADPKD. Elevated cAMP appears to contribute to the hyperproliferation of cystic epithelium by stimulating Erk1/2 via Ras and B-Raf. Excess cAMP also drives cyst growth by activating the cystic fibrosis transmembrane conductance regulator (CFTR). Recently, a novel type-I cAMP-dependent protein kinase X (PRKX), which is functionally distinct from protein kinase A (PKA), has been demonstrated to play a role in ADPKD. PRKX over-expression has been shown to stimulate the migration of human fetal collecting tubule epithelia in culture and stimulate ureteric bud branching as well as glomerular induction in an embryonic kidney organ culture system. Using a viral vector gene transduction approach, PRKX has been demonstrated to counteract the adverse effects of PKD1 mutation. This review focuses on recent advances in our understanding of the functions of polycystins, as well as the roles of cAMP/cAMP-dependent kinase associated phosphorylation in the pathogenesis of ADPKD.

1. ADPKD and the polycystins

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder characterized by cyst formation and progressive enlargement of both kidneys, leading to end-stage renal disease (ESRD) [1,2]. ADPKD has an incidence of approximately 1 in 700 live births and is the leading cause of ESRD in the US. ADPKD is primarily characterized by renal cysts; however, it is also a systemic disorder, resulting in epithelial cysts in multiple organs including the liver and pancreas [3,4]. Other clinical manifestations include hypertension, cardiac valve abnormalities, and intracranial aneurysms [5]. Two genes have been shown to cause ADPKD when mutated: *PKD1*, which accounts for 85%, and *PKD2*, which is responsible for the remaining 15%. The PKD1 gene is located on chromosome 16 and encodes polycystin-1 (PC-1) [6], whereas the PKD2 gene is located on chromosome 4 and encodes polycystin-2 (PC-2) [7,8]. PC-1 and PC-2 assemble through coiled-coil domains present in their intracellular C-termini to form a functional complex, the activity of which is required to regulate pathways that when perturbed result in renal cystogenesis [9–12]. Most of their activities have been attributed to the complex of PC-1 and PC-2 complex, which explains some common symptoms observed in ADPKD1 and ADPKD2 patients [9]. However, these two proteins also exhibit independent functions.

PC-1 is a large integral membrane glycoprotein (4303 aa, MW ~460 kDa), which includes a long N-terminal extracellular domain (~3000 aa), 11 transmembrane domains and a short intracellular C-terminal domain (~200 aa) [13–16]. The extracellular portion has two leucine-rich repeats, a C-type lectin domain, 16 PKD (IgG-like) repeats, an REJ (receptor for egg jelly) domain, and a proteolytic GPS domain (G protein-coupled receptor [GPCR] proteolytic site) that was shown to be functionally active[17]. The intracellular C-tail of PC-1 contains a coiled-coil domain that is responsible for mediating interaction with PC-2 and other proteins [10–12] and a consensus site for interaction with heterotrimeric G proteins [18]. PC-1 has been

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shown to interact with the Gαi/Gα0 subunit of heterotrimeric G proteins, suggesting that PC-1 itself might be a GPCR [17]. The C-tail has also been shown to be cleaved at a minimum of two different sites, generating two distinct products: a 28- to 34-kDa product containing the entire intracellular C-tail of PC-1 (CTT) [19,20] and a second ~16-kDa product (p112) [21]. Both of these products interact with transcriptional factors β-catenin and STAT6 respectively to translocate into the nucleus [20,21]. The extent of the cleavage of CTT and its transcriptional activity depend on PC-2 and the regulation of intracellular calcium stores [22].

The subcellular localization of PC-1 is controversial, partially due to antibody specificity. However, evidence from several different studies seems to reach a consensus on its localization to cell–cell junctions where it modulates cell adhesion[23,24], and at sites of cell–matrix interactions [25]. PC-1 has also been localized to the primary cilium of renal epithelial cells, where it is thought to be involved in ciliary mechanotransduction [26].

2. Polycystins and phosphorylation

2.1. PC-1: Phosphorylation and function

Although a growing number of studies suggest that PC-1 is associated with several signaling pathways, the direct link of PC-1 with phosphorylation is not well elucidated. It is an area that has received comparatively little attention for a disease that is likely due to defective developmental signaling. The intracellular C-tail of PC-1 contains putative phosphorylation sites. There are four tyrosines presented, one in a consensus sequence for an SH2 domain-containing protein binding site [28]. In addition, among the several serines and threonines contained within this 200 amino acid portion, there are two consensus RSSR sequences in the human protein at 4162–4167 and 4250–4253, which are consistent with target sites for phosphorylation by protein kinase A (PKA) or C (PKC) [27,28]. Other putative domains known to be involved in signaling functions include a proline-rich sequence consistent with a putative WW site [82] and a heterotrimeric G protein activation sequence [27]. The C-terminal of PC-1 has been demonstrated to be phosphorylated by c-src at Y4237, by protein kinase A at S4252, and protein kinase X (PRKX) at S4166

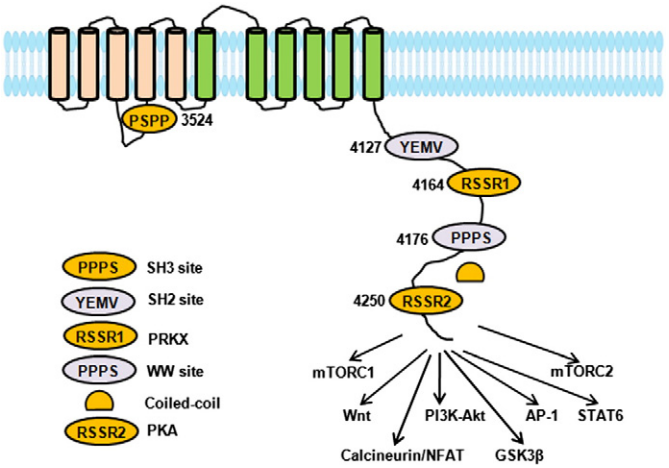

Fig. 1. The phosphorylation sites within PC-1 and the associated signaling pathways. The intracellular C-tail of PC-1 contains four tyrosines, one in a consensus sequence for an SH2 domain-containing protein binding site (Y4127, YEMV). There are two consensus RSSR sequences at 4162–4167 (RSSR1)and 4250–4253 (RSSR2), which are consistent with target sites for phosphorylation by cAMP-dependent kinases. S4164 is shown to be phosphorylated by PRKX and S4250 is phosphorylated by PKA. The other putative domains known to be involved in signaling functions include a proline-rich sequence consistent with a putative WW site (4176, PPPS). PC-1 has been shown to regulate several signaling cascades including the Wnt cascade, AP-1, PI3kinase/Akt, GSK3β, STAT6, the calcineurin/NFAT pathway, mTORC1 and mTORC2.

[63]. These findings suggest a role for PC-1 in phosphorylation activities and cell signaling. PC-1 has been shown to regulate several signaling cascades in line with its role as a receptor including the Wnt cascade [20,29], AP-1 [30], PI3kinase/Akt [31,32], GSK3β [31], STAT6 [21], the calcineurin/NFAT [33] pathway, and the ERK and mTOR cascades [34, Fig. 1]. A number of studies have demonstrated that the PC-1 C-terminal tail associates with β-catenin and inhibits canonical Wnt signaling, suggesting a novel mechanism through which PC1 cleavage may impact upon Wnt-dependent signaling and thereby modulate both developmental processes and cystogenesis [20]. PC-1 has also been demonstrated to activate c-Jun N-terminal kinase and AP-1, and this activation can be mediated by heterotrimeric G proteins [30]. PC-1 can induce resistance to apoptosis through the phosphatidylinositol 3-kinase/Akt signaling pathway [31,32]. PC-1 can also induce cell migration by regulating PI3kinase-dependent cytoskeletal rearrangements and GSK3-β dependent cell–cell mechanical adhesion [31]. In addition, studies suggested that PC-1 signaling could lead to a sustained elevation of intracellular Ca(2+) mediated by PC-1 activation of Galpha(q) followed by PLC activation, release of Ca(2+) from intracellular stores, and activation of store-operated Ca(2+) entry, thus activating calcineurin and NFAT [33]. Recently, accumulating evidence suggests that PC-1 might have a functional link to mTOR signaling cascade, which regulates protein synthesis and cell growth [34–38]. Studies have shown aberrant activation of mTOR in several rodent models of polycystic kidney disease [35,39,40] and treatment with rapamycin has been shown to alleviate cyst enlargement in murine models [41–44]. Recently, Cai et al. used a human model system to elucidate the mechanism by which PC-1 regulates the mTOR pathway and found that PC-1 modulates phoshatidylinositol 3-kinase (PI3K)/AKT signaling to TSC2 to repress mTOR [45]. In addition, Dere et al. [46] also demonstrated that the intracellular C-tail of PC-1 regulates mTOR signaling by altering the subcellular localization of the tuberous sclerosis complex 2 (TSC2) tumor suppressor. It has been shown that phosphorylation of TSC2 at S939 by AKT causes partitioning of TSC2 away from the membrane, its GAP target Rheb, and its activating partner TSC1 to the cytosol via 14-3-3 protein binding. In addition, they found that TSC2 and a C-terminal PC-1 peptide (CP1) directly interact and that a membrane-tethered CP1 protects TSC2 from AKT phosphorylation at S939, retaining TSC2 at the membrane to inhibit the mTOR pathway. These data identify a unique mechanism for modulation of TSC2 repression of mTOR signaling via membrane retention of this tumor suppressor, and identify PC-1 as a regulator of this downstream component of the PI3K signaling cascade. Taken together, all these findings suggest that the phosphorylation of PC-1 may be an important part of its function and play a significant role in the pathogenesis of ADPKD. However, the ligands initiate the phosphorylation/activation of PC-1 are not well known. Previously, immunocytochemistry, sucrose density gradient sedimentation, co-immunoprecipitation analyses and in vitro binding assays showed that PC-1 associates with the focal adhesion proteins talin, vinculin, p130Cas, FAK, alpha-actinin, paxillin and pp60c-src in subconfluent normal human fetal collecting tubule (HFCT) epithelia when cell–matrix interactions predominate. PC-1 also forms higher S value complexes with the cell–cell adherens junction proteins E-cadherin, beta- and gamma-catenins in confluent cultures when cell–cell interactions are predominant [47,48]. In addition, using primary human kidney epithelial cells, Roitbak et al. [49] showed the polycystins and their associated proteins E-cadherin and β-catenin distributed in a complex with the raft marker flotillin-2, but not caveolin-1, in high-density gradient fractions. Based on co-association of signalling molecules, such as Src kinases and phosphatases, they proposed that the polycystin multiprotein complex is embedded in a cholesterol-containing signalling microdomain specified by flotillin-2, which is distinct from classical light-buoyant-density, detergent-resistant domains. However whether these associated proteins are the ligands that can activate PC-1 and how they interact with PC-1 are remained to be further studied.