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PRKX, a Novel cAMP-Dependent Protein Kinase Member, Plays an Important Role in Development

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ABSTRACT

The human protein kinase X gene (PRKX) and cAMP-dependent protein kinase (PKA) are both c-AMP-dependent serine/threonine protein kinases within the protein kinase AGC subgroup. Of all the protein kinases in this group, PRKX is the least studied. PRKX has been isolated from patients with chondrodysplasia punctate and is involved in numerous processes, including sexual differentiation and fertilization, normal kidney development and autosomal dominant polycystic kidney disease (ADPKD), blood maturation, neural development, and angiogenesis in vitro. Although the role of PRKX in development and disease has been reported recently, the underlying mechanism of PRKX activity is largely unknown. In addition, based on the expression pattern of PRKX and the extensive role of PKA in disease and development, PRKX might have additional crucial functions that have not been addressed in the literature. In this review, we summarize the characteristics and developmental functions of PRKX that have been reported by recent studies. In particular, we elucidate the structural and functional differences between PRKX and PKA, as well as the possible roles of PRKX in development and related diseases. Finally, we propose future studies that could lead to important discoveries of more PRKX functions and the underlying mechanisms involved. J. Cell. Biochem. 117: 566–573, 2016.
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AGC kinase is a subgroup of Ser/Thrprotein kinases composed of 60 members that include PKA, cGMP-dependent protein kinase (PKG), and protein kinase C (PKC) [Pearce et al., 2010; Taylor et al., 2012]. Like PKA, PRKX is a cAMP-dependent protein kinase, and free cAMP is required for the activation of PRKX [Taylor et al., 2012]. The structure and function of PKA, as well as PKA substrates, have been studied extensively, whereas in contrast, studies of PRKX are quite limited. Human PRKX was first isolated in 1995, and sequence analysis suggested that cAMP-dependent protein kinase is distinct from PKA [Klink et al., 1995]. Recently, several studies have demonstrated a crucial role of PRKX in the regulation of sexual differentiation and fertilization, blood maturation, neural development, and kidney development and disease, but the underlying working mechanisms are unclear. In this review, we discuss the differences between PRKX and PKA and review current studies of PRKX, including its roles in human developmental diseases.

PRKX, A cAMP-DEPENDENT PROTEIN KINASE DISTINCT FROM PKA

IDENTIFICATION AND CHARACTERIZATION OF PRKX

In addition to phosphorylation, protein kinases of the AGC family play multiple roles in cell growth and development [Pearce et al., 2010]. The recently discovered protein kinase X, PRKX, which is a novel cAMP-dependent protein kinase [Klink et al., 1995; Blaschke et al., 2000; Li et al., 2002], was initially isolated from a candidate region for chondrodysplasia punctata on the human X chromosomal subregion Xp22.3. Sequence analysis found that PRKX has a predicted 358 amino acid open reading frame (ORF) [Klink et al.,

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1995]. In addition, PRKX shares high sequence similarity scores with the *Drosophila melanogaster* DC2 kinase [Kalderon and Rubin, 1988], the fungus *Blastocladiella emersonii* PKA catalytic subunit [Marques Mdo et al., 1992], and the slime mold *Dictyostelium discoideum*[Anjard et al., 1993]. However, sequence similarity with the human PKA catalytic subunits is rather low (Fig. 1). Thus, PRKX is regarded as a novel type of human Ser/Thr protein kinase that is related to the catalytic subunit of the cAMP-dependent protein kinases, but distinct from the PKA catalytic subunit. In addition, PRKX is part of a family of four genes or pseudogenes. Toder et al. [1997] have confirmed this characterization and note that the existence of pseudogenes is the result of chromosome rearrangement during primate evolution. PRKX alleles have been isolated from the Y chromosome (i.e., protein kinase Y, PrKY), and analysis showed 94% sequence similarity to PRKX, but the PrKY sequence is shortened by 81 amino acids at the C terminus compared with the homologous PRKX [Schiebel et al., 1997].

After isolation of human PRKX, the mouse homolog of human PRKX, Pkare, was isolated using race-PCR later in 2000 by Blaschke. The cDNA sequence of Pkare contains a 1,065 bp ORF and is predicted to encode a protein with 355 amino acids [Blaschke et al., 2000]. Like human PRKX, the isolated Pkare protein exhibits all characteristics of a serine/threonine protein kinase that is closely related to the cAMP-dependent subtype (Fig. 1). However, unlike human PRKX, Pkare does not have a Y chromosomal homolog [Blaschke et al., 2000]. Although the role of human PRKX has been demonstrated in human development and disease in vitro [Li et al., 2002; Li, 2011], isolation of the mouse homolog Pkare may prove valuable for studying the mechanisms through which PRKX functions.

STRUCTURAL AND FUNCTIONAL DIFFERENCES BETWEEN PRKX AND PKA

Using sequence and phylogenetic analysis, human PRKX and mouse Pkare are both predicted to be cAMP-dependent Ser/Thr protein kinases. Although PRKX and PKA both belong to the group of AGC protein kinases, which modulate many biological functions and diseases, including diabetes, cancer, schizophrenia, cardiac disease, stroke, Huntington's disease, and Alzheimer's disease [Pearce et al., 2010], these two protein kinases appear to have a large functional diversity based on their different structures and expression patterns.

As a cAMP-dependent protein kinase, PRKX (Pkare in the mouse) belongs to an ancient subfamily containing *Dictyostelium* KAPC-DICDI and consists of a single regulatory subunit and a single catalytic subunit [Li et al., 2002; Kaletta et al., 2003], which is unlike PKA, whose holoenzyme consists of two regulatory subunits and two catalytic subunits. In addition, only one gene encodes the catalytic subunit of PRKX/PRKY, whereas three genes encode the catalytic subunits of PKA. Alignment of the PRKX and PKA catalytic domains reveals an extensive conservation of sequence in multiple regions [Li et al., 2002], including the following: (i) the Mg-AMP binding domain, (ii) kinase residues, (iii) the PKA catalytic loop, (iv) the PKA D^{184}FG β8–β9 loop, (v) the PKA P+1 peptide recognition residues, and (vi) the PKA autophosphorylation site. In contrast, several residues that are crucial for specific binding of the regulatory subunit are not conserved. These residues are PRKXQ92 and PKA E86; PRKX N140 and PKA R134; and PRKX D199 and PKA G193 [Li et al., 2002 #789]. PKA isozymes are classified according to their regulatory subunits as either type I or type II. Crystal structures and solution scattering data provide evidence of the differences between the global structure of PKA type I and type II holoenzymes [Takegoshi et al., 2001]. In humans, 4PKA regulatory (R) subunit isoforms (RIα, RIβ, RIIα, and RIIβ) and 3PKA catalytic (C) subunits (Cα, Cβ, and Cδ) have been identified. In PRKX, only RIα binds to the PRKX regulatory domain. This interaction causes a conformational change in PRKX to an inactive conformation [Zimmermann et al., 1999; Diskar et al., 2007], which is called autoinhibition and is an evolutionarily conserved feature of PRKX and four of its orthologs, including mouse Pkare, *D. melanogaster* DC2, *Trypanosoma brucei* PKAC3, and human PRKY [Zimmermann et al., 1999; Diskar et al., 2010]. Taken together, previous studies have suggested the structural differences between PRKX and PKA, and these differences probably contribute to the distinct distribution and biological functions of PRKX and PKA.

THE PARTNER OF PRKX AND POSSIBLE SUBSTRATE

The PKA holoenzyme consists of two regulatory and two catalytic subunits and appears inactive in the absence of cAMP. When cAMP binds to the regulatory subunits, a conformational change occurs that releases the active catalytic subunits, which phosphorylate the downstream substrates of PKA or stimulate gene transcription [Pearce et al., 2010]. Some of the substrates of the protein kinase AGC group, including PKA, have been identified and extensively studied, whereas the substrates of PRKX have not been well studied and remain unknown (Table I), although most of the residues or regions involved in PRKX substrate binding are extensively conserved in comparison to PKA catalytic subunits. Early studies showed that PRKX could phosphorylate the peptidekemptide [Zimmermann et al., 1999] and the tau-protein [Boom Hvd, 2003], which is also a substrate of TTBK2 [Tomizawa et al., 2001, #798]. In addition, we found that polycystin 1 could be one of the PRKX substrates in kidney development and autosomal dominant polycystic kidney disease (ADPKD) [Li et al., 2008; Li et al., 2009]. Recently, Glesne and Huberman carried out yeast two-hybrid studies and identified that the regulatory subunit Iαof PKA, T54 and Smad6, are the partners of PRKX. In vitro phosphorylation assays demonstrated that increased PRKX expression during macrophage differentiation leads to increased serine phosphorylation of Smad6, and PRKX can phosphorylate Smad6 at a serine residue [Glesne and Huberman, 2006]. These results indicated that Smad6 is one of the substrates of PRKX. The MBD4 protein has previously been described as a phosphor-protein that contains both an N-terminal methyl-CpG binding domain (MBD) and a C-terminal DNA glycosylase domain [Giovinazzo et al., 2013]. A yeast two-hybrid system also identified MBD4 as a partner of PRKX and suggested that MBD4 could be a potential substrate for PRKX. However, more work needs to be done to confirm this hypothesis [Lin and Li, 2011]. While studying the role of PRKX in kidney development, we also found that PRKX binds toBAG-3, PIN-1, and MAGI-1 through its WW domain as determined by TransSignal domain arrays. The interaction between PRKX and Pin-1 is further confirmed by a co-immunoprecipitation assay in

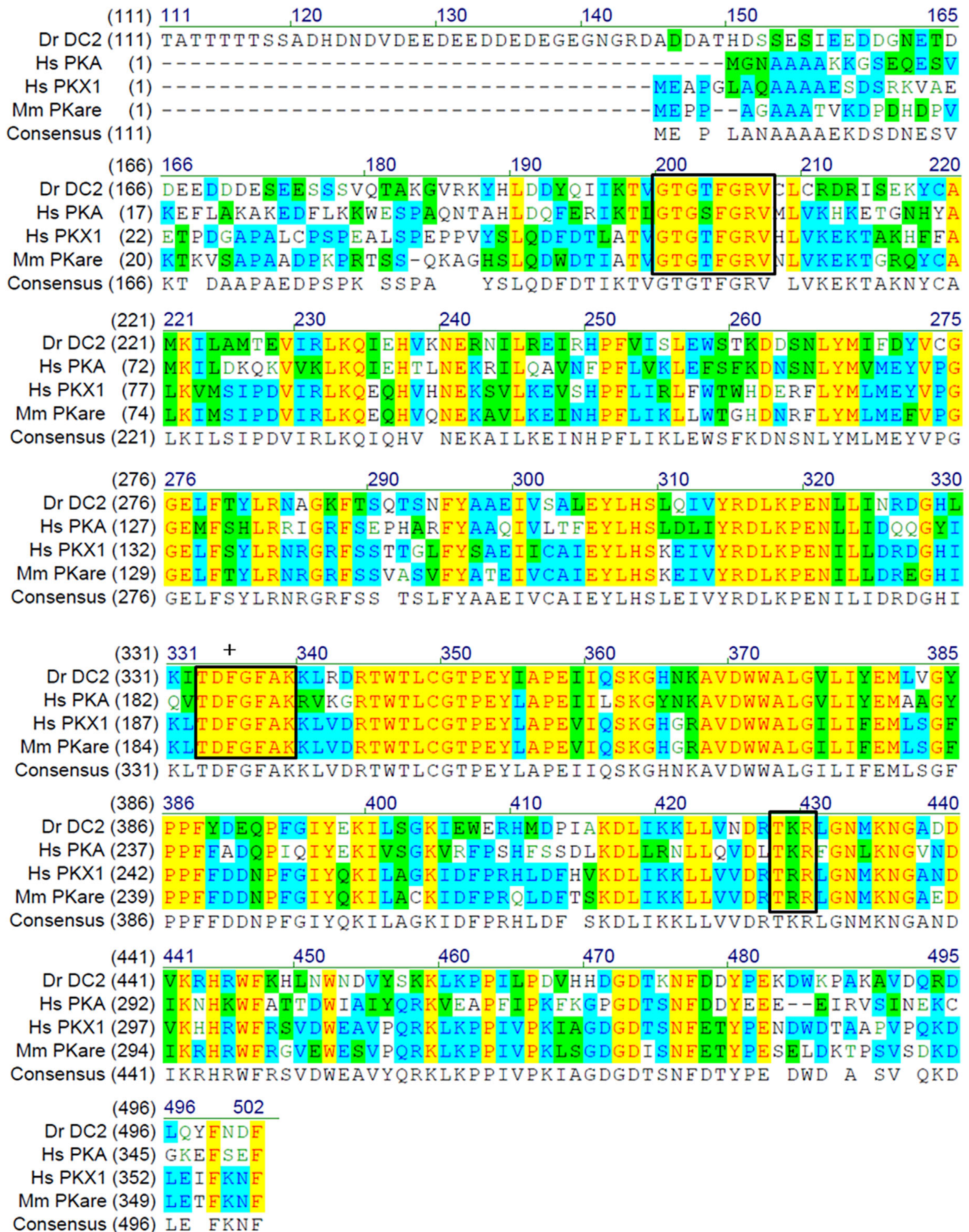

Fig. 1. Comparison of the human PRKX with closely related protein kinase catalytic subunits. The amino acid (AA) alignment is shown for PRKX, the DC2 kinase from *Drosophila melanogasler* (Dros), the human PKA catalytic subunitα (PRKACA), and mouse Pkare. Regions with identical AA are highlighted in yellow, and regions with high similarity are highlighted in green and blue. The three consensus sequences are labeled with black squares. The end of the alignment corresponds to the C-termini of all four proteins. + indicates the ATP binding domain.

TABLE I. Substrates of PRKX and PKA, and the validation methods.

Kinase	Substrates/partners	Validation method	Interacting regions	References
PRKX	Polycystin 1	Phosphorylation studies, co-immunoprecipitation	N/A	Li et al. [2008]; Li et al. [2009]
	Kemptide	Kinetic assays	N/A	Zimmermann et al. [1999]
	T54, Smad6	Yeast two-hybrid interaction screen, co-immunoprecipitation, in vitro phosphorylation assays	PPX/VYSI/LK/QV/XFD	Glesne and Huberman [2006]
	MBD4	Yeast two-hybrid	N/A	Lin and Li [2011]
	BAG-3, PIN-1, and MAGI-1	TransSignal domain arrays, co-immunoprecipitation	WW domain	Li et al. [2002]; Li et al. [2009]
	AAV Rep78	Yeast two-hybrid	Rep78 PKI-like domain	Di Pasquale and Chiorini [2003]
PKA	AID, bAD, calpain 2, CFTR, CREb, GRK2, GSK3, HSL, KCNN2, NFAT2, and VASP	Yeast two-hybrid, co-immunoprecipitation, et al.	Arg–X–Ser/Thr or Arg–Arg/Lys–X–Ser/Thr	Pearce et al. [2010]

HFCT cell lysates [Li et al., 2009], which suggested that PIN-1, BAG-3, and MAGI-1 are likely substrates of PRKX, although more studies are needed to substantiate this hypothesis. In human cells affected by the AAV virus, the AAV Rep78 protein was identified to interact with PRKX, using the yeast two-hybrid approach, and this interaction was reported to modulate viral interference between AAV and the adenovirus [Di Pasquale and Chiorini, 2003]. Hence, AAV Rep78 might also be a substrate of PRKX in the AAV virus.

In conclusion, although earlier studies have identified substrates of PRKX [Zimmermann et al., 1999; Glesne and Huberman, 2006], several recent studies have also identified several other partners of PRKX in cells. However, most of these candidates need to be investigated further to confirm whether they are substrates of PRKX. In addition, the question of whether some of the known substrates of PKA are also substrates for PRKX is of importance for further investigation. Identification of PRKX substrates will be beneficial for studying the mechanisms via which PRKX exert its biological functions.

THE EXPRESSION OF PRKX IN TISSUES AND CELL LINES

The sequence and structure of PRKX are different from PKA [Li et al., 2002]. The expression and distribution pattern of PRKX in both tissues and cells are also distinct compared to PKA. Several studies reported that human PRKX is abundantly expressed in a wide range of developing tissues, but is much less expressed or not detectable in mature tissues [Schiebel et al., 1997; Zimmermann et al., 1999; Li et al., 2002; Li et al., 2005a,b]. These results are consistent with the expression pattern of Pkare observed in mice [Blaschke et al., 2000; Li et al., 2005a]. Transcript level detection through Northern blot analysis revealed that PRKX mRNA is highly expressed in human fetal kidneys, brains, and lungs [Li et al., 2002; Aguiari et al., 2009]. However, PRKX mRNA is much less expressed in adult tissues, such as placenta [Aguiari et al., 2009]. In situ hybridization studies further showed that PRKX mRNA is abundantly expressed in fetal kidney ureteric bud epithelial cells and ADPKD epithelial cells, but not in normal adult kidney cells [Li et al., 2002]. Protein level detection through Western blot analysis and immunohistochemistry revealed that PRKX was ubiquitously distributed and highly expressed across development in the murine central nervous system and heart [Li et al., 2005a], but very low levels of expression were detected in the collecting duct epithelia of 32-week fetal kidneys and no expression was detected in 4- or 16-year-old human kidney sections [Li et al., 2005b]. Abundant levels of PRKX were also detected in fetal human liver but not adult liver [Li et al., 2005a,b]. In vascular cells, we demonstrated that PRKX is expressed in the cytoplasm and nuclei of umbilical vein endothelial cells (HUVECs) and mouse aorta endothelial cells (MAEs) [Li et al., 2011].

Mouse Pkare expression has also been detected in a variety of organs, including the kidney, liver, spleen, testis, ovary, lung, heart, and brain [Blaschke et al., 2000; Li et al., 2005a]. In situ hybridization on staged mouse embryos revealed a highly distinctive expression pattern during neuronal development, with elevated Pkare expression observed only in differentiating neurons within the first ganglion, the dorsal root ganglia, and the mantle layer of the telencephalon [Blaschke et al., 2000]. In the adult brain, Pkare expression was

observed in the brain stem, hypothalamus, hippocampus, cerebellum, olfactory bulbs, and cerebral hemispheres. Pkare expression, therefore, represents a marker for neuronal differentiation and could be a novel component of the cAMP-regulated pathways involved in the development of neurons [Li et al., 2005a,b].

Proper subcellular localization and distribution are required for protein kinases to fulfill their functions in development and disease [Pearce et al., 2010]. Targeting of PKA to specific sites in the cell is largely achieved by A kinase anchoring proteins (AKAPs) [Perrichot et al., 1999], and the functional motif in AKAPs is an amphipathic helix that docks with high affinity to PKA regulatory subunits [Somlo and Markowitz, 2000]. Although, thus far, no kinase anchoring proteins have been reported for PRKX, and some of the proteins associated with PRKX might help anchor PRKX to the appropriate subcellular region [Glesne and Huberman, 2006; Li et al., 2009; Giovinazzo et al., 2013] .

Taken together, previous findings indicate that PRKX (Pkare in mice) expression is age related and has specificity in tissues and cells or cell lineages. Furthermore, PRKX may be localized in appropriate subcellular areas through the action of anchoring proteins.

THE ROLE OF PRKX IN DEVELOPMENT AND DISEASES

THE ROLE OF PRKX IN KIDNEY DEVELOPMENT, ADPKD, AND KIDNEY CANCER

KAPC-DICDI is highly homologous to PRKX. Several studies have shown that KAPC-DICDI plays an important role in cell-shape changes, morphogenetic cell migration, and transcriptional regulation of cellular differentiation in *D. discoideum* development [Cantiello, 2004; Li et al., 2005c; Sun et al., 2005], which implies that PRKX may have a critical role in the regulation of morphogenesis and transcription in higher eukaryotes.

ADPKD is a genetically determined developmental disorder of the kidney, which affects 500,000 patients in the United States. Our previous work has demonstrated that overexpression of PRKX strongly activates cellular morphogenesis and the formation of epithelial tubular structures in vitro [Li et al., 2002]. Using an embryonic kidney organ culture system and a technique for viral vector gene transduction [Li et al., 2009], we also demonstrated that PRKX kinase expression stimulates ureteric bud branching and induction of glomeruli [Li et al., 2009]. These results suggest that PRKX might play an important role during normal kidney development, and abnormal activation of PRKX could result in thepathologic cystic tubular phenotype in ADPKD [Li et al., 2005b; Li et al., 2009]. This hypothesis is further supported by other studies [Li et al., 2008], wherein we observed an increased tubule epithelial cell–matrix adhesion and a decreased migration in ADPKD epithelia. In a cultured kidney system with PKD1 mutation, we observed less ureteric bud branching and aberrant renal tubule dilation. However, expression of constitutively active PRKX in human ADPKD epithelial cell lines can rescue adhesion and migration defects [Li et al., 2008]. In addition, co-injection of constitutively active PRKX with inhibitory pMyr-EGF-PPKD1 into the ureteric buds of mouse embryonic kidneys in an organ culture resulted in restoration of normal branching morphogenesis[Li et al., 2008]. This function of PRKX coincides with two facts. One is the specific structure of PRKX, which has two putative proline-rich WW domain binding sites, phospho-SP/-TP and PPxY. The WW domain is one of the most versatile protein–protein interaction modules that is involved in a variety of cellular processes, including cell adhesion and migration [Le et al., 2005; Witzgall, 2005]. The second is that PRKX was shown to bind Pin-1, Magi-1, and Bag-3 via the WW domain in vitro [Li et al., 2009]. Pin-1, Magi-1, and Bag-3 have also been implicated in a variety of cellular processes, including cell proliferation, apoptosis, development, differentiation, and tumorigenesis [Lantinga-van Leeuwen et al., 2004; Sessa et al., 2004]. The interaction of PRKX with Pin-1, Magi-1, and Bag-3 through the WW domain could contribute to the role of PRKX in the regulation of kidney development and the pathogenesis of ADPKD.

In addition to its developmental role, PRKX has also been implicated to be oncogenic. Bender and Ullrich found that PRKX was highly expressed in the kidney carcinoma lines WM115^{SDes} and WM266-4^{SDes}. Reduction of PRKX, TTBK2 or RSK4 triggers sunitinib sensitization and increases sunitinib inhibition of cancer cell migration [Bender and Ullrich, 2012]. Mechanistically, PRKX was shown to be capable of activating CREB-dependent transcription in vitro [Di Pasquale and Stacey, 1998], and the transcription factor CREB induces expression of the Microphthalmia-associated transcription factor (MITF) [Saha et al., 2006]. Therefore, PRKX expression increases MITF expression and enhances sunitinib resistance. This result implicates a critical role of PRKX in kidney carcinoma development and formation. Thus, PRKX might represent a target for the development of novel strategies to overcome resistance in sunitinib therapy.

OTHER PROPOSED ROLES OF PRKX IN DEVELOPMENT AND DISEASE

Aside from its role in normal kidney development and kidney disease, several studies have demonstrated that PRKX is also involved in angiogenesis, sex reverse disorder, and virulence. A recent study showed that PRKX stimulates endothelial cell proliferation, migration, and vascular-like structure formation [Li et al., 2011], and this functionality could be exerted through the interaction of PRKX with Pin-1, Magi-1, and Bag-3 [Li et al., 2011]. Sex reversal is a disorder caused by an abnormal interchange between the X and Y chromosomes. Schiebel et al. [1997] first isolated PRKY, the homolog to PRKX, and found that abnormal interchange frequently occurs between PRKX and PRKY, which causes sex reversal disorder in patients. Recently, Nakashima et al. [2013] identified a Japanese male infant with an apparent 45,X karyotype. Further analysis showed that a simple Xp terminal deletion, a complex Yp translocation with the middle Yp breakpoint, and a translocation fusion point resided within a 246 bp X-Y homologous segment at "hot spot A" in the 5′ region of PRKX/PRKY. These results suggest a critical role of a common paracentric Yp inversion in the occurrence of PRKX/PRKY-mediated unbalanced Xp; Yp translocation [Nakashima et al., 2013]. In addition to interchange of PRKX/PRKY, other susceptible regions for X-Y translocation have also been identified that are located close to PRKX/PRKY [Beaulieu Bergeron et al., 2011]. These results suggest that the stability of PRKX or PRKY might be crucial for normal sex development and fertilization. However, PRKX/PRKY interchange

has not appeared in every sex reversal patient. During differentiation of myeloid precursor cells, PKC isoforms α, β, and δ have been implicated in the induction of macrophage differentiation in normal progenitor cells and in human and murine myeloid cell lines [Badenas et al., 2000]. The Macrophage-colony-stimulating factor (M-CSF) induces rapid catalytic activation of PKCδ and membrane translocation of the tyrosine phosphorylated PKC δ. M-CSF and PKC δ also induce expression of the PRKX murine homolog, Pkare [Junttila et al., 2003]. These facts suggest that PRKX might lie downstream of PKCδ and that PRKX is involved in the regulation of myeloid precursor cell differentiation. In the *Drosophila* neuron, SWS interacts specifically with the C3 catalytic subunit of cAMP-activated protein kinase (PKA-C3), and overexpression of PKA-C3 induces degeneration and enhances neurodegeneration in *sws* mutants [Bettencourt da Cruz et al., 2008]. PKA-C3, together with orthologs in mice (Pkare) and humans (PRKX), constitutes a novel class of catalytic subunits [Zimmermann et al., 1999; Blaschke et al., 2000; Diskar et al., 2007]. Because PRKX is expressed in the human brain, it is likely that PRKX plays an important role in some neurodegeneration disorders. It is well known that AAV2 is involved in the cell differentiation processes in affected human cells. Several studies reported that AAV2 Rep78 interacts with PRKX, and this interaction inhibits the kinase activities of PRKX and blocks the induction of CREB-dependent transcription in HeLa cells. Thus, AAV2 may utilize Rep78 to subvert cAMP-dependent regulatory pathways during the differentiation of infected human cells [Di Pasquale and Stacey, 1998]. In addition, Faucher et al. [2008] per-] performed a number of experiments using the mutant of the prpZ gene cluster and found that prpZ, prkY, and prkX are virulence genes that may be part of a signaling pathway controlling the long-term survival of *S. Typhi* in host cells.

Taken together, all of these findings suggest that PRKX not only plays an important role in the development of the kidney and ADPKD but also is critically involved in the development of other organs and tissues, including the brain, blood vessels, and blood cells, as well as related developmental disorders. In addition, because RIα functions as the only regulatory subunit for PRKX, PRKX may be involved in regulating R1α-dependent diseases such as Carney complex (CNC) [Carney et al., 1986 #791]. In this disease, the patient appears with spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas [Bossis et al., 2004 #796; Sahut-Barnola, #797]. PRKX is a relatively novel kinase that has been identified only recently and has not been extensively studied. Future studies will reveal more critical functions of PRKX in the development of other human organs and tissues, as well as related diseases.

CONCLUSION AND PROPOSED FUTURE WORK

In summary, published research shows that the structure of PRKX is distinct from that of PKA, particularly with respect to some critical residues in the catalytic subunit. The function of PRKX is also significantly different from that of PKA, as evidenced by the diversity of PRKX and PKA expression patterns and substrates. Although PRKX is involved in normal kidney development, ADPKD, angiogenesis and other developmental processes and diseases, additional possible functions of PRKX and the mechanisms underlying these functions are largely unknown. For example, Pkare, the mouse homolog of human PRKX, is expressed in the brain and heart. However, whether PKare is involved in the development of the heart and neural system in vivo remains a key topic for further study. In addition, the role of human PRKX in normal kidney development has only been demonstrated in cultured kidney systems. The role of PRKX has not yet been studied for kidney development in vivo and in kidney progenitor cell fate and migration. Because some G-protein-coupled receptors are involved in regulating early development processes, such as gastrulation cell movement, and cAMP regulation by G-protein-coupled receptors is required for the activation of PRKX, another key question is whether PRKX is involved in the regulation of gastrulation in early cellular development. In addition, further investigation of the substrates of PRKX is particularly important. The identification of PRKX substrates and investigation of the role of these substrates would significantly contribute to the understanding of the mechanism through which PRKX exerts its critical biological functions in development and disease.

To learn more about the role of PRKX and its underlying mechanisms, animal models should be used. The homolog of PRKX in mouse has been isolated, and its expression pattern suggests a possible role in heart, neural, and kidney development and disease [Blaschke et al., 2000; Li et al., 2005a]. Thus, the construction of Pkare mutant mice would facilitate exploration of the developmental role of Pkare in vivo, as well as the underlying pathomechanism of PRKX-related disease. In addition, a predicted homolog of PRKX in zebrafish has been found. Additional studies performed in zebrafish will further clarify the role of PRKX during embryonic development because this animal model offers unique advantages, such as transparent embryos and facile genetic manipulation, e.g., construction of mutant and transgenic zebrafish lines, and transient over-expression and knockdown of specific genes. In conclusion, our review suggests that PRKX may be involved in regulating many developmental processes, such as heart and neural development. However, more studies are needed to clearly establish the role PRKX plays during the development of these organs.

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