Protein kinase D1: a protein of emerging translational interest

Meena Jaggi [1], Cheng Du[2], Wenguang Zhang[3], Kethandapatti C. Balaji[2]

[1] Sanford Research/USD, Cancer Biology Research Institute, Department of OBGYN and Basic Biomedical Science, Sanford School of Medicine of The University of South Dakota and Sanford Health Systems, Sioux Falls, SD, USA 57105, [2] Division of Urology, Department of Surgery, University of Massachusetts Medical School, Worcester, MA 01655, [3]University of Nebraska Medical Center, Division of Urologic Surgery, Omaha, Nebraska, USA 68198-2360

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1. ABSTRACT

Protein kinases are of particular interest in treatment of human diseases because of their enzymatic activity and susceptibility to successful therapeutic targeting. Protein kinase D1 (PKD1), cloned from humans and mouse by two different groups and reported simultaneously in the 1990s, is emerging as a protein of translational value. Accumulating evidence in the literature demonstrates that PKD1 plays a role in several cellular processes such as apoptosis, immune regulation, cell proliferation, oxidative stress signaling, adhesion and motility. PKD1 mediates cellular functions through facilitating Golgi fission and protein transport by its domain specific interaction with several membranous, cytosolic and nuclear proteins and is regulated by several molecular mechanisms including autoinhibition, subcellular localization, phosphorylation and protein cleavage. PKD1 is down regulated in advanced prostate cancer human specimens and influences cell adhesion and motility of prostate cancer cells *in vitro*. In addition, PKD1 plays a role in several tissues including skin, immune cells, cardiac myocytes, thymus gland, vascular smooth muscle, osteoblasts and other cancer cells. In this review, while addressing the molecular aspects of PKD1, we will also highlight the potential translational implication of PKD1 function in human diseases.

2. INTRODUCTION

Protein kinase D1 (PKD1) is a widely distributed protein kinase present in eukaryotic cells and is involved in a multitude of functions in normal and diseased states in humans (1). PKD1 has been shown to play a role in proliferation of keratinocytes, endothelial and pancreatic cancer cells (2, 3), B and T lymphocytes and mast cell signaling (4-6), development of central tolerance in thymus gland (7, 8), cardiac myocyte contraction (9), osteoblast differentiation (10), aggregation of prostate cancer cells (11), the Golgi apparatus reorganization, and regulating the fission of vesicles from the trans-Golgi network (TGN) (12). PKD1 is conserved in mammalian cells and plays a variety of functions in human organs. This review article will broadly discuss PKD1 biological functions and regulation with specific emphasis on the role of PKD1 in human diseases and potential therapeutic implications, thereby highlighting the emerging translational value of PKD1 in management of human diseases.

3. PROTEIN KINASE D1 (PKD1) GENE

The PKD1 gene is evolutionarily well preserved in eukaryocytic cells with homologues present in mouse (*M. musculus*), rat (*R. norvegicus*), Drosophila (*D. melanogaster*), *C. Elegans* and yeast (*S. cerevisiae*)
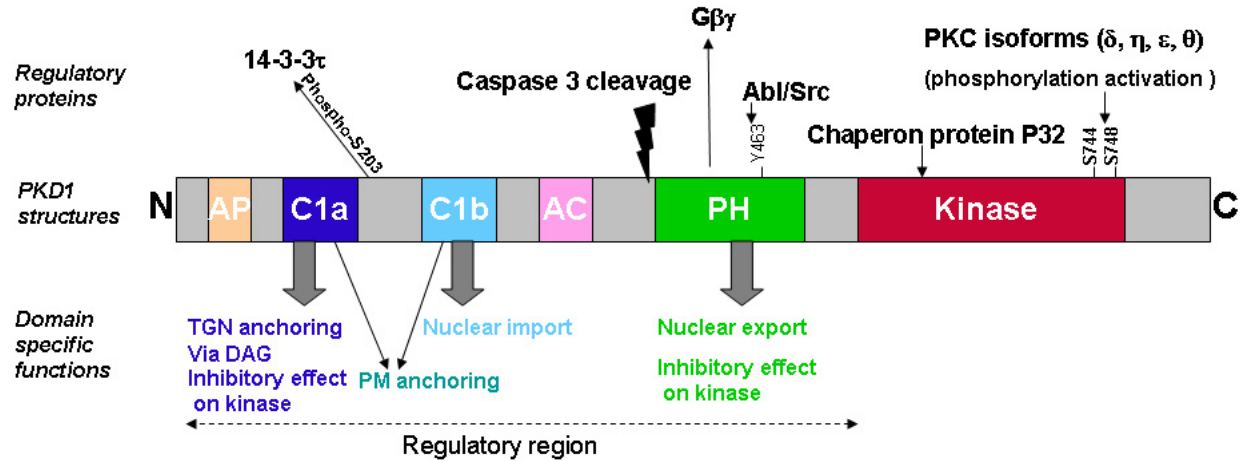
Figure 1. Regulation of Protein Kinase D1 (PKD1) activity and domain specific function. PKD1 remains inactive in cytosol partly through auto-inhibition of catalytic activity by pleckstrin homology (PH) domain. When PKD1 is stimulated (by engagement of G-protein coupled receptors (GPCRs), crosslink of antigen receptors, induction of cellular oxidative stress, or direct stimulation by phorbol esters or Bryostatin), DAG binds to C1b domain and induces PKD1 translocation to the plasma membrane, where it is phosphorylated and activated by PKC isoforms. Direct binding of G-protein subunits beta gamma to the PH domain releases the auto-inhibition of catalytic domain leading to PKD1 activation. Another known activation mechanism of PKD1 is Tyr-463 phosphorylation by Abl/Src kinase in response to oxidative stress. Binding to 14-3-3 or chaperon protein p32 lowers PKD1 activity. PKD1 undergoes cleavage releasing a 62 kD catalytic fragment by caspase 3 in response to apoptosis inducing agents.

(13). Complementary DNA clones encoding the human PKD1 (initially called atypical PKC mu) and the PKD1 from mouse were reported in 1994 by two different laboratories (14, 15). The PKD1 gene is located on human chromosome14q11 (homologues: Mouse Ch.6 and rat Ch.8m) and PKD1 mRNA is expressed in several normal organs with highest expression in brain, heart and lungs (1, 15). The gene spans over 351 kb and the transcript consists of 18 exons measuring about 3.8 kb in length, which encodes a protein with 912 amino acid residues.

PKD1 was initially described as PKC mu, an atypical member of the Protein Kinase C (PKC) family (14). The PKCs comprise a family of intracellular serine/threonine-specific kinases that are critical in signal transduction of a wide range of biological responses, including cellular proliferation and differentiation (16). While PKD1 is homologous to conventional PKCs in the duplex zinc-finger like cysteine rich motifs, it has a unique transmembrane and pleckstrin homology domain and an amino terminal signal peptide (1). In addition, PKD1 is constitutively localized to cellular membranes such as the Golgi apparatus, unlike other members of the PKC family which are constitutively cytosolic and localize to membranes on activation (17, 18). These unique structural characteristics suggest a novel function for PKD1 that is distinct from the PKC family of proteins.

Because of several distinct structural features of PKD1, it has been assigned to a new protein kinase subfamily called protein kinase D (PKD). The PKD family is comprised of three kinases: PKD1, PKD2 and PKD3 (PKDν) (14, 15, 19, 20). The PKD family members share a similar modular structure, which consists of alanine and proline rich (AP), cysteine-rich (C1, divided into two subdomains; C1a and C1b), pleckstrin homology (PH) and kinase domains (KD) and acidic-rich region (AC) (14, 15). In addition to the putative transmembrane region, diacylglycerol/phorbol ester binding cysteine rich domain and PH domain, the salient features of the PKD subfamily structure and function include a C-terminal catalytic kinase domain with a primary sequence and substrate specificity distinct from the PKC family of proteins. Unlike PKC kinase domains, the catalytic domain of PKDs (including PKD1) is distantly related to calcium calmodulin dependent kinases (21). Several protein interactions with PKD1 are domain-specific, and each domain of PKD1 plays a role in specific PKD1 function (Figure 1).

4. FUNCTIONS OF PKD1

PKD1 performs a variety of functions in cells and is target dependent. PKD1 has been shown to play a major role in multiple cellular functions including DNA synthesis, cell proliferation, apoptosis, oxidative stress response, immune response, cell motility and invasion, Golgi and membrane trafficking and other intracellular signal transduction pathways (Figure 2).

4.1. Role of PKD1 in cellular proliferation and DNA synthesis

PKD1 plays a direct role in proliferation of a variety of cells including keratinocytes, endothelial cells, T cells and pancreatic cancer cells (3, 8, 22). In endothelial cells, vascular endothelial growth factor (VEGF) activates PKD via the VEGFR2/PLCgamma/PKCalpha pathway,
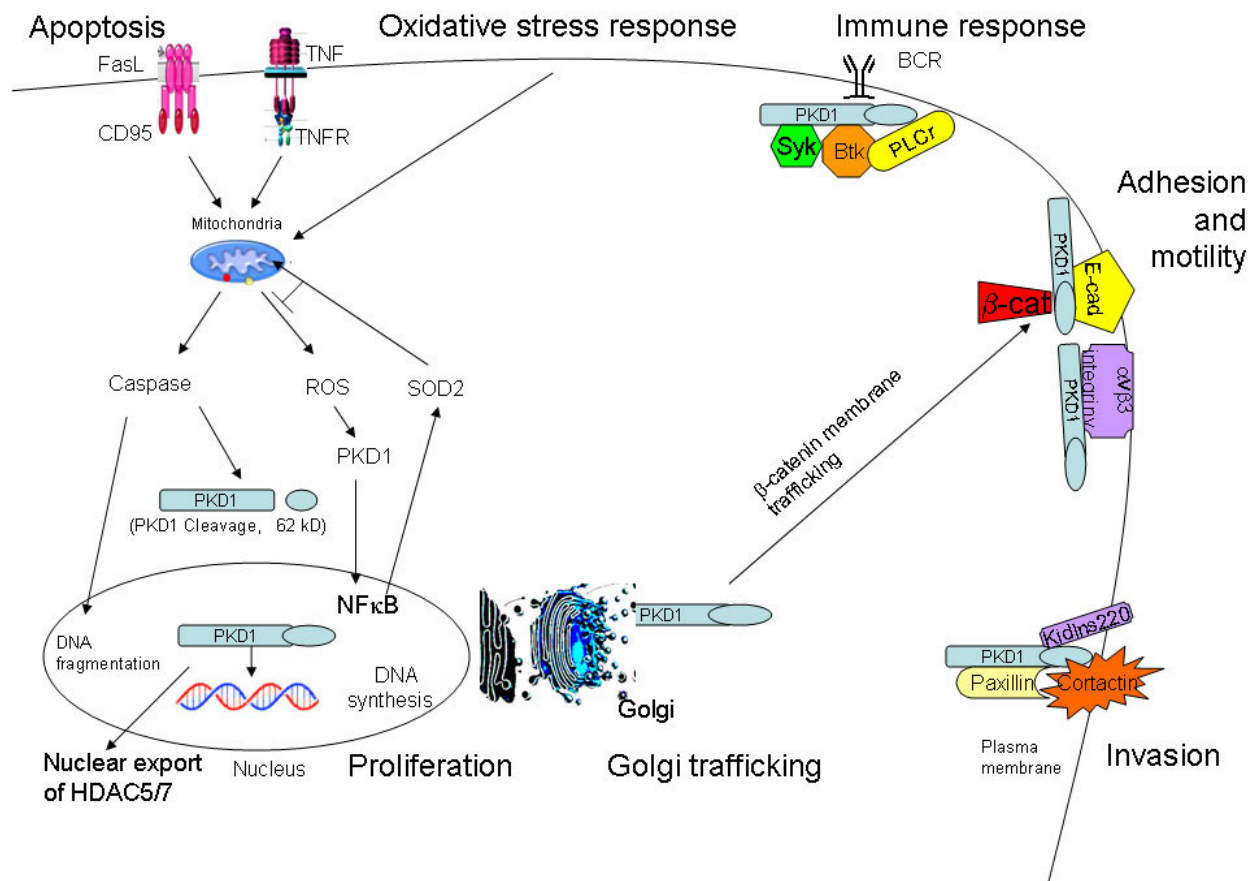
Figure 2. Schematic representation of cellular functions of Protein Kinase D1 (PKD1). Function detailed in text. Abbreviations used in the figure: FasL – Fas ligand; TNF – Tumor necrosis factor; TNFR – Tumor necrosis factor receptor; ROS – Reactive oxygen species; BCR – B cell receptor; SOD2 – Superoxide dismutase 2; E-cad – E cadherin; β-cat –beta-catenin; HDAC – Histone deacetylase; Btk – Burton's tyrosine kinase; Syk – spleen tyrosine kinase; PLC betagamma – Phospholipase Cgamma.

which induces ERK signaling and is associated with endothelial cell proliferation (2). PKD1 expression enhances keratinocyte proliferation in normal and neoplastic mouse epidermis and in cell culture (23). PKD1 overexpression in pancreatic tumor cells increases telomerase activity, and up regulates anti-apoptotic proteins c-FLIP and survivin (24). In intestinal epithelial cells arginine vasopressin (AVP) promotes PKD1 activation and in Swiss 3T3 cells PKD1 induced by bombesin, vasopressin, or PDBu, mediated by increasing the duration of MEK/ERK/RSK signaling, increases DNA synthesis and cell proliferation (25-27). Neurotensin induces protein kinase C-dependent PKD1 activation and DNA synthesis in human pancreatic carcinoma cell line PANC-1 (8). While the existing data in the literature suggest an association between PKD1, cell proliferation, and DNA synthesis, a direct causal relationship or downstream effectors of PKD1 on cellular proliferation remains to be investigated.

4.2. Role of PKD1 in apoptosis

In addition to cellular proliferation and DNA synthesis, PKD1 also plays an anti-apoptotic role in cells. PKD1 has been shown to reduce sensitivity to TNF-induced apoptosis, which correlates with the amount of

transfected PKD1 expression (22). Studies using other apoptotic agents demonstrate that, while sensitivity to apoptosis induced by the lipid mediator ceramide was unchanged in PKD1 transfectants, PKD1 may be specifically involved in H_2O_2-induced apoptotic effects through JNK activation. H_2O_2, but not TNF, induced phosphorylation of PKD1 and translocation of PKD1 from the endothelial cell membrane to the cytoplasm, where it associates with the JNK upstream activator, apoptosis signal-regulating kinase 1 (ASK1) (28, 29). PKD1 has also been shown to prevent CD95-mediated apoptosis and to enhance proliferation in pancreatic tumor cells. Overexpression of PKD1 strongly reduced CD95-mediated apoptosis and led to significant increase in cell growth and telomerase activity. Inhibition of PKD1 with Gö6983, a staurosporine derivative and differential PKC inhibitor, sensitized resistant cells to CD95-induced apoptosis (24). These studies indicate that PKD1 can play an anti-apoptotic role in a cell context and apoptosis-inducing agent dependent manner.

During caspase dependent apoptosis, PKD1 is proteolytically cleaved by caspase 3 at two distinct sites located between the acidic and pleckstrin homology (PH)

domains, generating a 62 kDa catalytic fragment of the kinase (Figure 1) (30). The *in vivo* caspase-dependent generation of the PKD1 fragments correlates with PKD1 kinase activation. The overexpression of anti-apoptotic Bcl-x(L) protein or the baculovirus p35 protein blocked the PKD1 cleavage. Cells stably expressing the catalytic fragment of PKD1 are more sensitive to apoptosis induced by genotoxic stress. The expression of the caspase-resistant PKD1 mutant partially inhibits DNA damage-induced apoptosis (31, 32). On the contrary, another study in literature demonstrates that the catalytic fragment of PKD1 does not have any apoptotic function (30).

4.3. PKD1 and Golgi Organization

Multiple protein kinases have been shown to play critical roles in regulating either Golgi function or structure. PKD1 localizes to Golgi and is involved in vesiculation of Golgi apparatus and membrane sorting of proteins (17). An intact C1b domain is necessary for Golgi localization of PKD1, mediated by interaction with diacylglycerol (33). A pivotal role of PKD1 is suggested in regulating the fission (detachment) of cargo-containing tubular elements from trans-Golgi network (TGN) to the cell surface (12). The Gbetagamma sub-units of G proteins can induce vesiculation of the Golgi apparatus. PKD1 is a direct Gbetagamma-activated target required for this vesiculation process and regulates the dynamics of Golgi membranes and protein secretion (34). Further studies have revealed a requirement for another kinase, PKCeta, and specific betagamma subunits, beta1gamma2, and beta 3 gamma2 in the PKD-dependent fission of Golgi membranes and that beta1gamma2, PKCeta, and PKD act in series to promote membrane fission to generate transport carriers from the TGN. The PKD isoforms may be differentially involved in cargo trafficking (35, 36). While non-polarized HeLa cells do not express PKD1, they do express the other PKD isoforms, PKD2 and PKD3, which may be involved in the transport of proteins that contain basolateral sorting signals. Studies using polarized MDCK kidney cells that express all three known PKD isoforms demonstrate that PKD1 and PKD2 are also involved in the transport of basolateral, but not apical cargo (37). However, in fibroblasts PKD1 mediated anterograde membrane trafficking from the TGN to the plasma membrane facilitating locomotion and localized at the leading edge with Rac1-dependent activity (38). Both PKD activity and the intact PH domain have been shown to be required for protein transport along the secretory pathway (34). Phosphatidylinositol 4-phosphate (PI (4) P) is regarded as an important lipid mediator in vesicular trafficking. Recent studies have shown that PKD-mediated phosphorylation of phosphatidylinositol 4-kinase IIIbeta (PI4KIIIbeta) stimulated lipid kinase activity of PI4KIIIbeta and enhanced vesicular stomatitis virus G-protein transport to the plasma membrane (39). Clearly, PKD1 plays a role in Golgi fission, which leads to membrane trafficking of proteins. Current investigations are focused on identifying the protein interactions involved in the process.

4.4. Role of PKD1 in immune system

PKD1 plays a role in transducing antigen receptor signaling to downstream effector proteins in cells with immune functions. PKD1 is activated by T cell receptor (TCR), B cell receptor (BCR), or mast cell high affinity IgE receptor activation. In B lymphocytes, activation of PKD1 is dependent on BCR stimulation and is regulated by co-receptor CD40 binding with TRAF2 protein (40, 41). Upon TCR stimulation, PKD robustly augments hematopoietic progenitor kinase 1 (HPK1) in Jurkat T cells and enhances HPK1-driven SAPK/JNK and NF-kappaB activation; conversely, antisense down-regulation of PKD results in reduced HPK1 activity. The activation of major lymphocyte signaling pathways via HPK1 results in relocation, autophosphorylation, and transphosphorylation of HPK1 by PKD (41). In addition, there is indirect evidence to suggest that PKD1 may play a role in T cell adhesion. Activation of T cells by the PKC activators use PKD1 as an effector kinase to influence beta1 integrin function through Rap1 activation. PKD1 is identified as a downstream target of the theta isoform of PKC in both COS-7 cells and lymphocytes. Although the above data suggest a role for PKD1 in signaling in lymphocytes, the exact functional role for PKD1 in immune regulation is currently unknown.

4.5. Role of PKD1 in cell adhesion and invasion

Recent studies in our laboratory suggest that PKD1 plays an important role in cell adhesion and motility in prostate cancer cells. We demonstrated that E-cadherin, a member of the cadherin-catenin protein complex that is dysregulated in prostate cancer and is known to be involved with altered cellular aggregation and adhesion in cancer cells (42, 43), interacts with PKD1 (11). Inhibition of PKD1 activity by the selective inhibitor Gö6976 in LNCaP prostate cancer cells resulted in decreased cellular aggregation and overexpression of PKD1 in C4-2 prostate cancer cells increased cellular aggregation and decreased cellular motility (11). In addition, our studies also demonstrated that the PKD1 kinase domain directly interacts with beta-catenin (44), a distinct member of the cadherin-catenin protein complex, which plays a dual role in cell adhesion as well as in the Wnt (Wingless type) signaling pathway associated with cell proliferation (45). Moreover, PKD1 is associated with membrane trafficking of beta-catenin from Golgi and activation of PKD1 by Bryostatin increases membrane beta-catenin and decreases nuclear beta-catenin, unveiling a novel potential mechanism by which PKD1 can contribute to prostate cancer progression by altering the subcellular distribution of beta-catenin (44).

PKD1 also seems to play a role in adhesion of other cell lines. PKD1 interacts directly with other important focal adhesion molecules, such as alphavbeta3 integrin. Down regulation of PKD1 inhibits PDGF dependent recycling of alphavbeta3 from early endosomes to the plasma membrane and blocks recruitment of alphavbeta3 to newly formed focal adhesions during cell spreading (46). There is also indirect experimental evidence that suggests an association between PKD1 and breast cancer cell adhesion. PKD1 translocates to the membrane upon stimulation with cis-polyunsaturated FA (cis-PUFA), which induced cell adhesion in a dose-dependent manner in the MDA-MB-435 breast cancer cell line (47). PKD1,

cortactin and paxillin have been co-immunoprecipitated from invadopodia of breast cancer cells. Cortactin and paxillin are actin associated proteins and have a well established role in cell invasion, suggesting the role of PKD1 in cancer cell invasion (48). PKD1 activation by insulin growth factor 1 (IGF-1) is associated with trans-endothelial cell migration of multiple myeloma cells *in vitro*, which was abrogated by phosphatidylinositol 3-kinase (PI-3K) inhibitors. This suggests that PKD1 activation by IGF-1 is mediated by PI-3K (49). Currently available *in vitro* experimental evidence strongly suggests that PKD1 plays a significant role in cell adhesion in multiple cell lines, although definitive *in vivo* data is lacking.

4.6. Role of PKD1 in oxidative stress signaling

Partial reduction of molecular oxygen can generate reactive oxygen species (ROS), including hydrogen peroxide, and the free radicals superoxide and hydroxyl ion, which are strongly associated with human disorders such as cancer, inflammation, degenerative and age related disorders (50). Effective scavenging of free radicals is an important defense mechanism against DNA damage that can result in disease. Release of mROS activates PKD1 leading to activation of NF-kappaB, which subsequently resulting in expression of superoxide dismutase2 (SOD2). SOD2 is involved in elimination of free radicals in mitochondria (51). Oxidative stress induces phosphorylation of PKD1 at Tyr463 by the tyrosine kinase Abl, and phosphorylation at the activation loop Ser738/Ser742 by PKCdelta, which results in fully active PKD1 that controls NF-kappaB activation through the IkappaB kinase (IKK) complex. Resveratrol, a potent antioxidant, blocks both PKD activation and NF-kappaB induction (52). Other mechanisms such as Src-dependent and -independent pathways of PKD1 activation by oxidative stress have also been demonstrated (53). While an important role for PKD1 in eliminating free radicals is being revealed, clearly much work needs to be done to establish a direct causal role, if any, of PKD1 dysregulation leading to oxidative damage in disease states.

4.7. Organ specific cellular functions of PKD1

In addition to its role in cellular processes, expression of PKD1 is of functional consequence in several organ systems including heart, nervous system, vascular system and bone cell differentiation. PKD1 interacts with several proteins of importance in cardiac myocytes; cardiac troponin I (cTnI), myosin-binding protein C (cMyBP-C), and telethonin, of which cTn1 undergoes substrate phosphorylation at ser 22 and ser 23 resulting in reduced myofilament calcium sensitivity (9). Stimulation with G-protein coupled receptor (GPCR) (norepinephrine, angiotensin II, and endothelin 1) and phorbol ester activates and translocates PKD1 to the Z-discs in neonatal rat cardiomyocytes in a PKC dependent manner (54). PKD1 phosphorylates Kidins 220, which is a downstream target of p75 neurotrophin receptor, plays a critical role in regulating neuronal plasticity, survival, and differentiation in the nervous system (55). The capsaicin receptor VR1 is a polymodal nociceptor activated by multiple stimuli in neural cells. PKD1 directly phosphorylated rVR1 or N-

terminal fragment (amino acids 1-118) of rVR1 and mutation of S116A in rVR1 blocked both the phosphorylation of rVR1 by PKD1 and the enhancement by PKD1 of the rVR1 response to capsaicin (56). Postsynaptic P2X1 ATP-gated channels are expressed in smooth muscle cells of the vascular and genitourinary systems. Intracellular calcium and a subset of diacylglycerol-dependent protein kinases including PKD1 seem to be involved in regulation of P2X1 receptor channels by modulating their recovery from desensitization (57). PKD1 is activated in freshly isolated salivary gland cells by P2X (7) receptors, which are ligand-gated non-selective cation channels, which could be inhibited by PKC inhibitors (58). Thrombin induces activation of PKD through PKCdelta in vascular smooth muscle cells (59). PKD1 is required along with PKC delta for PMA- and DAG-induced MARCKS phosphorylation and hyperpermeability in pulmonary microvascular endothelial cells (60). In mesenchymal stem cells, bone morphogenic protein 2 (BMP-2) and insulin growth factor I (IGF-I) induced a selective phosphorylation of PKD1, and PKD1 was required for mineralization (61). BMP-2 induces activation of PKD1 by Serine 916 phosphorylation in osteoblastic cells by a PKC-independent mechanism and is involved in activation of JNK and p38 induced by BMP-2 (10). Data to date suggests that PKD1 plays a role in key functions of several organ systems, which makes PKD1 a potential therapeutic target in several disease states.

4.8. Role of PKD1 in other signal transduction pathways

PKD1 has also been demonstrated to interact with several other proteins that are involved in other signal transduction pathways but whose functional significance remains to be studied. Novel PKD1 substrates or protein interactors include metallothionein 2A (62), Bruton's tyrosine kinase (Btk), considered an essential signal transducer in B- cells (63), heat shock protein27 (Hsp27) and RIN1 (64). The exact role of PKD1 interaction with several of these proteins in cells is currently being studied. The normal localization and function of RIN1, as well as its ability to compete with RAF, are regulated in part by 14-3-3 binding, which in turn is controlled by PKD1 phosphorylation of serine 351 *in vitro* and *in vivo* (65). By binding to RIN1, PKDs release Ras protein resulting in an activation of the Raf-MEK-ERK pathway (66). PKD1 seems to play a role in influencing oncogenic signaling. PKD1 phosphorylation of epidermal growth factor (EGF) receptors and its binding to JNK blocks c-Jun phosphorylation (67) and in addition it also plays a role in MEK/ERK/RSK signaling. The stimulatory effect of PKD1 on GPCR-induced cell proliferation (26) has been linked to its ability to increase the duration of the MEK/ERK/RSK pathway activation, leading to accumulation of immediate gene products including c-Fos that stimulates cell cycle progression (27). PKD1 also interacts with anchor protein AKAP-Lbc, a protein kinase A (PKA)-anchoring protein, which contributes to PKD1 activation by recruiting an upstream kinase PKCeta and coordinates PKA phosphorylation events that release activated protein kinase D1 (68). The other less investigated function of PKD1 includes its effect on ion transport channels. PKD1 has

been shown to inhibit Na(+)/H(+) exchanger activity through indirect mechanisms using COS7 cells (69). Fundamentally, PKD1 is a central transducer of several signal transduction pathways.

5. REGULATION OF PKD1 FUNCTION

As discussed above, PKD1 is involved in multiple biological processes and, not surprisingly, PKD1 activity is subjected to several regulatory mechanisms including autoinhibition, subcellular localization, phosphorylation and protein cleavage. The PKD1 kinase activity is autoinhibited by the PH domain (70). This autoinhibition of PKD1 by the PH domain can be abrogated by several types of protein interactions with the PH domain. Oxidative stress induced phosphorylation of Tyr463 in the PH domain by Src and Abl kinases results in PKD1 activation, leading to downstream NF-kB activation (71, 72). GPCR activation causes G protein interaction with the PH domain and activates PKD1 without the substrate phosphorylation that influences membrane trafficking of proteins, suggesting that alteration in protein structure possibly leads to release of autoinhibition (70).

Upon stimulation, PKD1 rapidly moves to the plasma membrane through its C1b domain that binds to DAG and/or through binding to Gq (73). At the same time, novel PKCs, in response to DAG generation, are also recruited to the plasma membrane, where mouse PKD1 is transphosphorylated at Serine744 and 748 by PKC (74). The phosphorylated PKD1 rapidly transduces DAG-PKC signals from the cell surface by quickly dissociating from the plasma membrane, relocating to the cytosol, and eventually moving to the nucleus via the C1b domain for a short time period. Nuclear export of PKD1 is mediated by the PH domain (75). Prolonged association of PKD1 at the plasma membrane is achieved by recruitment of PKD1 with C1a and C1b domains (22).

A major mediator of PKD1 activation in cells is the PKC family of signal transduction proteins. Addition of PKC stimulators such as DAG or phorbol esters/phosphatidylserine directly activates PKD1 catalytic activity *in vitro*. Cells treated with phorbol esters (PKC activator) or Bryostatin, a small molecular activator of PKCs, induced accumulation of active PKD1 (73, 76, 77). Subsequent studies showed that a broad range of external signals, including engagement of GPCR, tyrosine kinase receptors, crosslink of B- or T-cell antigen receptors, or oxidative stress response induced PKD1 activation. Although PKD1 activation can be blocked by PKC selective inhibitors, these inhibitors do not inhibit PKD1 catalytic activity directly, implying that PKD1 activation is mediated by PKC.

The activation is phosphorylation-dependent, and serine738 and 742 residues in human PKD1 (corresponding to serine744 and 748 in mouse) have been identified as crucial phosphorylation sites. These serine residues are located in the activation loop of the PKD1 catalytic domain

(aa residues 730-750). Novel PKC isoforms, PKCtheta, PKCeta, PKCepsilon and PKCdelta have been involved in PKD1 activation (78, 79). Activation loop phosphorylation of PKD1 in response to oxidative stress and GPCR agonists is mediated by PKCdelta and PKCepsilon respectively (52, 80). The phosphorylation of Tyr463 in the PH domain also promotes PKD1 activation, specifically in response to oxidative stress, but not in response to other PKD1 activating stimuli such as PDGF (platelet-derived growth factor) or bradykinin (71). The PKD1 phosphorylation at Tyr463 by the tyrosine kinase Abl, and phosphorylation at the activation loop serine738/742 by the PKCdelta results in fully active PKD1 that controls NF-kappaB activation through the IkappaB kinase (IKK) complex in response to oxidative stress (81). Thrombin induced activation of PKD1 is mediated through PKCdelta in vascular smooth muscle cells (59) and cellular transgene experiments in lymphocytes demonstrate that PKD1 could be a downstream target of PKCtheta (82).

A PKD1 mutant with alanine substituted serine744 and 748 completely blocked PKD1 activation induced by phorbol ester treatment (83). Conversely, replacement of both serine residues with glutamic acid (PKD-S744E/S748E) markedly increased activity without phorbol ester stimulation (83). Phorbol ester treatment also induced transphosphorylation at serine255 by an unidentified PKC isoform (84). The S255E mutant is not constitutively active, and its activation is still dependent on phorbol esters treatment. However, unlike the wild type PKD1, the S255E mutant can be activated when treated with a combination of phorbol ester and a PKC inhibitor, suggesting mechanisms other than PKC-dependent phosphorylation is involved in PKD1 activation (84).

Phosphorylation of other serine residues also affects PKD1 activity and plays a role in modulation of PKD1 function *in vivo*. The C-terminal serine916 residue has been identified as an autophosphorylation site in PKD1 (84). One study demonstrated that activation of PKD1 by the antigen receptor ligation of T and B lymphocytes caused serine916 autophosphorylation, which was negated by the FcgammaRIIB receptor that mediates a negative feedback signal to the B cell antigen receptor (85). Although this phosphorylation is not required for PKD1 activation, it may play a structural role. The S916A mutant showed an increased resistance to proteolysis and dephosphorylation, a potential process for inactivation of PKD1 (84). Other proteins such as p32, a multifunctional chaperon protein, may play a kinase regulatory function *in vitro* possibly by stearic hindrance of substrate within the kinase domain in a compartment-specific manner (86). Activity of phosphorylated PKD1 can be attenuated by binding of the 14-3-3 to the C1a regulatory domain, suggesting yet another mechanism of PKD1 regulation (87). Alternatively, cleavage of PKD1 by caspase-3 releases a catalytic fragment, which plays a pro-apoptotic role (32). The data in literature demonstrates multiple regulatory mechanisms that are involved in PKD1 regulation, several of which are cell context dependent.

Table 1. Clinical Implications of PKD1 cellular functions

Functions	Cell Type	Clinical Implications
Cellular proliferation	Keratinocytes, endothelial cells, T cells and pancreatic cancer cells	Proliferatve disorders
DNA synthesis	Pancreatic and gastric epithelial cells	Solid tumors
Antigen receptor signaling	T cell receptor (TCR), B cell receptor (BCR)	Immunomodulator
Cell adhesion and motility	Prostate cancer cells	Cancer progression
Phosphorylation and nuclear export of HDAC5	Myocytes	Cardiac hypertrophy
Oxidative stress signaling	Epithelial cell lines	Aging, Degenerative disorders and cancer
Signal transduction	Several cell types	Potential therapeutic target

6. EMERGING TRANSLATION VALUE OF PKD1

PKD1 plays a role in multiple cellular processes that are of consequence in both benign and malignant human diseases (Table 1). Currently, two major promising areas where PKD1 could possibly play a critical role in disease progression are prostate cancer and cardiac failure. Prostate cancer is the most frequently diagnosed non-cutaneous cancer in American men, and about 30,000 men die from metastatic prostate cancer each year in the United States (88, 89). While most patients with metastatic prostate cancer will initially respond to androgen deprivation treatment, eventually almost all patients develop androgen independent prostate cancer leading to death (90, 91). We have identified that PKD1 is down-regulated in advanced prostate cancer (92). Further studies demonstrated that PKD1 is associated with cadherin-mediated cell adhesion and migration in prostate cancer cells (11). While the exact biological effect of PKD1 down regulation in patients with advanced prostate cancer remains to be confirmed, it is conceivable that PKD1 may prove to be a useful therapeutic target to influence disease progression. Bryostatin, PKD activator, has undergone Phase I and Phase II clinical trials in solid tumors with minimal response (93, 94). UCN-01, initially thought to be a PKC inhibitor and later discovered to work through other mechanisms including cyclin-dependent kinase inhibition has undergone Phase I and II clinical trials and has shown activity in cancer (95). Although neither of these drugs is specific for PKD1, they nevertheless establish the proof of principle that PKD1 modulation either alone or in conjunction with other proteins can be used to influence disease progression in humans. Given the increasing wealth of knowledge on the role of PKD1 in various cellular processes and recent data on PKD1 dysregulation in prostate cancer, PKD1 is a viable therapeutic target for small molecular modulators (96). Because of its presence on the plasma membrane, PKD1 may be amenable to monoclonal antibody targeting as well (97). Although PKD1 protein domain structure is predicted (14, 15, 97), the exact crystal structure remains to be established. Such studies in the future may facilitate designing small molecular modulators that would specifically target PKD1 with improved the specificity. Because PKD1 is widely distributed in tissues and its function is cell context dependent, future challenges would include designing drugs that would be tissue or disease specific.

Coronary heart disease is the most common cause of death in Western countries and is commonly associated with heart failure (98). Cardiac failure is usually preceded by cardiac hypertrophy that is mediated by altered gene expression involved in myocyte contraction, calcium handling and metabolism (99). Transcriptional regulation of gene expression is tightly coupled to histone deacetylases (HDAC) and histone acetyltransferases (HAT) that modify the access of transcription factors to DNA binding sites (100). PKD1 has been shown to participate in nuclear export of HDAC5. HDAC5 is phosphorylated by PKD1, which results in the binding of 14-3-3 protein to the phosphoserine motif on HDAC5, thus leading to nuclear export through a CRM1-dependent mechanism. This results in increased transcriptional activity of hypertrophy mediating genes in myocytes (99). PKD1 specific inhibitors may be of benefit in limiting cardiac hypertrophy, although this strategy is fraught with difficulties which include redundant signaling pathways and side effects secondary to ubiquitous distribution of PKD1.

There is some early promise that PKD1 may be a useful target for immunomodulation. Immunotherapy including monoclonal antibodies and vaccines is being successfully used to treat several human diseases (101). Current data in the literature suggests that PKD1 is involved in both T and B lymphocyte and mast cell signaling, although the exact role in immune response in humans remains to be elucidated. Several clinically used predominantly immunomodulatory drugs such as cyclosporine and FK-506 are also known to influence signal transduction in other organs, contributing to their side effects (102). It may be conceivable that drugs that influence PKD1 activity may be used to directly modify immune function. At the very least, they need to be considered for their effects on immune function if used in a different organ or disease context.

7. PERSPECTIVE

Since the identification and cloning of PKD1 in the early 1990s, significant advances have been made in understanding the possible role of PKD1 in cellular functions, including apoptosis, immune regulation, cell proliferation, adhesion, motility, oxidative stress response and signal transduction. PKD1 functions as an effector protein for cellular processes including Golgi fission and protein transport through its domain specific interaction with several membranous, cytosolic and nuclear proteins. PKD1 is regulated by protein interaction through several molecular mechanisms including auto-inhibition, subcellular localization, phosphorylation and protein cleavage. PKD1 is down regulated in advanced prostate cancer and influences cell adhesion and motility. While a definitive role for

PKD1 in humans, both in normal and diseased states, remains to be unequivocally established, current published literature suggests PKD1 is a novel protein of interest with emerging translational value, especially in management of prostate cancer, cardiac failure and immune function.

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Send correspondence to: Meena Jaggi, PhD or K.C. Balaji, M.D., FRCS, Professor and Chief, Division of Urology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA, Tel: 508-856-5821, Fax: 508 856 3134, E-mail: kcbalaji@umassmed.edu