Mitochondrial and Oxidative Stress-Mediated Activation of Protein Kinase D1 and Its Importance in Pancreatic Cancer

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Due to alterations in their metabolic activity and decreased mitochondrial efficiency, cancer cells often show increased generation of reactive oxygen species (ROS), but at the same time, to avoid cytotoxic signaling and to facilitate tumorigenic signaling, have mechanism in place that keep ROS in check. This requires signaling molecules that convey increases in oxidative stress to signal to the nucleus to upregulate antioxidant genes. Protein kinase D1 (PKD1), the serine/threonine kinase, is one of these ROS sensors. In this mini-review, we highlight the mechanisms of how PKD1 is activated in response to oxidative stress, so far known downstream effectors, as well as the importance of PKD1-initiated signaling for development and progression of pancreatic cancer.

Keywords: protein kinase D, oxidative stress, mitochondria, pancreatic cancer, signaling

INTRODUCTION

The Warburg effect in cancer cells is the product of two factors, a return of cells to glycolytic metabolism and increased production of mitochondrial reactive oxygen species (ROS), which is due to alterations in oxidative phosphorylation (1). In established tumors, increased levels of oxidative stress are often accompanied by upregulation of antioxidant systems (2, 3). The upregulation of antioxidant systems keeps ROS at levels where they are protumorigenic and promote cell survival and proliferation, but do not induce apoptosis or necrotic cell death. This mini-review focuses on a ROS-sensing signaling pathway that controls tumor cell detoxification, proliferation, and survival through activation of protein kinase D1 (PKD1).

Protein kinase D1 is one of three members of the PKD family of serine/threonine kinases. PKD1 consists of an *N*-terminal regulatory region and a *C*-terminal kinase domain. Main elements in the regulatory region are two cysteine-rich (C1) domains that are important for lipid binding, and a pleckstrin homology (PH) domain, needed for protein–protein and protein–lipid interactions [reviewed in Ref. (4)]. Dependent on upstream signaling and binding partners, PKDs can be located at various cellular compartments and facilitate Golgi transport processes, as well as mitochondrial, cytosolic, and nuclear signaling [reviewed in Ref. (5)]. An increased oxidative stress leads to PKD1 localization to the mitochondria, where it is activated (6). ROS-activated PKD1 has been shown not only to initiate cytosolic signaling pathways (6–8) but also to redistribute to the nucleus (9). The signaling pathway that leads to the activation of PKD1 by oxidative stress seems unique because it involves tyrosine phosphorylation of the molecule at several residues (8, 10, 11), which do not occur when PKD1 is activated by receptor-mediated signaling (7).

PKD ACTIVATION DOWNSTREAM OF ROS

Protein kinase D1 can be activated by an increase in intracellular oxidative stress levels, such as induced by glutathione depletion or ectopic addition of hydrogen peroxide (7, 8, 12). PKD1 activation also occurs in response to an increase in mitochondrial ROS (mROS) caused by inhibitors of the mitochondrial respiratory chain (13). These include rotenone, a mitochondrial complex I inhibitor, and diphenyleneiodonium, an inhibitor of the NADPH cytochrome P450 reductase (6). Moreover, PKD1 is activated by oncogenes that increase mROS levels such as mutant versions (G12D, G12V) of V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRas) (14).

Increases in mitochondrial (and cellular) ROS levels initiate a series of tyrosine phosphorylations (Y95, Y432, Y463, and Y502) in PKD1 (8, 10, 11), which are mediated either directly by the proto oncogene tyrosine protein kinase Src or downstream of Src (10, 11). The mechanism of how Src is activated downstream of ROS is not fully understood, and conformational changes due to direct oxidation of cysteine residues, tyrosine nitration, or redox inactivation of inhibitory protein tyrosine phosphatases could be a cause of its increased activity. In this context, it was shown that ROS-responsive receptor-like PTP alpha is required for the activation of PKD1 in response to hydrogen peroxide (15), but a detailed mechanism was not provided. For Src-mediated phosphorylations of PKD1 at Y432 and Y502, no functional consequences have been attributed, so far. Phosphorylation of PKD1 at Y95 is directly mediated by Src (10), whereas Y463 has been shown to be directly phosphorylated by Abelson murine leukemia viral oncogene homolog 1 (Abl), when activated through Src (11).

A sequential model for activation of PKD1 by ROS has been proposed (**Figure 1**). The phosphorylation of PKD1 at Y463 in PH domain seems to be an initiating step that leads to a conformational change, which initiates membrane anchoring at the mitochondria
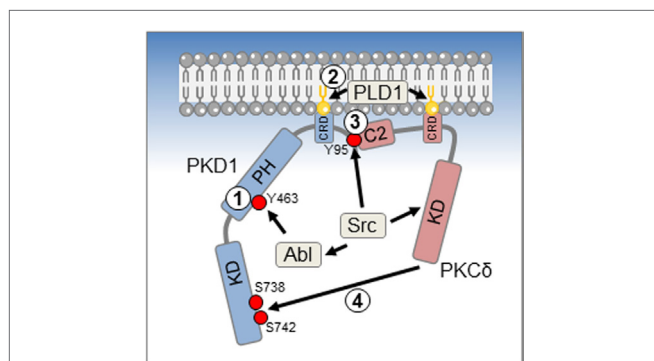
(16). This is mediated by binding to diacylglycerol that can be generated through activation of phospholipase D1 downstream of mROS (16). It should be noted that it was also shown that the multifunctional chaperone p32 can act as an adapter that associates PKD1 and PKCδ with mitochondrial membranes (17), but a role for p32 in ROS-initiated activation of PKD1 so far has not been investigated. A next step is the phosphorylation of PKD1 at Y95 by Src. This generates a binding motif for the C2 domain of PKCδ (10), another kinase that is activated downstream of oxidative stress and Src (18). PKCδ then phosphorylates the PKD1 activation loop serines (S738 and S742), resulting in a fully active kinase (7, 10).

SIGNALING THROUGH ROS-ACTIVATED PKD1 AND FUNCTIONAL CONSEQUENCES

Several signaling molecules that regulate cell survival and detoxification have been implicated downstream of oxidative stress-activated PKD1 (**Figure 2**). A main target is the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). After activation through the ROS/Src/Abl/PKCδ pathway, PKD1 induces canonical NF-κB signaling through IκB kinase β and subsequent downregulation of inhibitor of kappa-light-chain-enhancer of activated B cells alpha (8). However, the
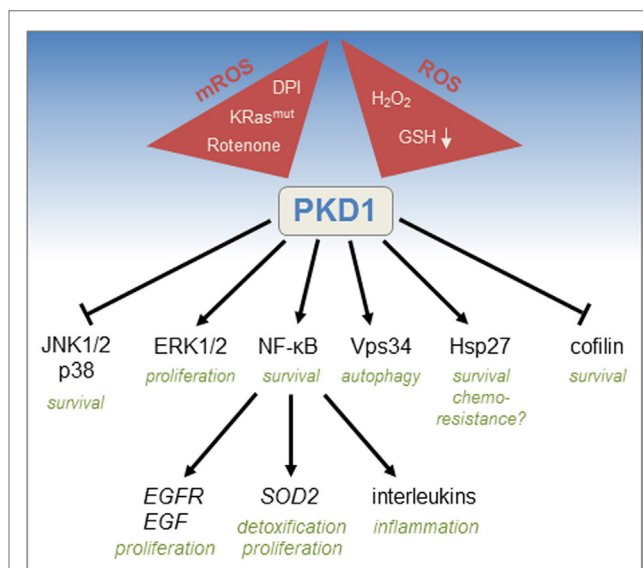
FIGURE 2 | **Mitochondrial reactive oxygen species (mROS)/reactive oxygen species (ROS)-induced activation of protein kinase D1 (PKD1) and downstream signaling**. Activation of PKD1 is mediated by increases in ROS as obtained after ectopically administered hydrogen peroxide (H_2O_2) or decrease of glutathione (GSH) or by increases in mROS as obtained by the expression of oncogenic KRas (KRas^mut), or inhibitors of the mitochondrial respiratory chain such as rotenone and diphenyleneiodonium. ROS-activated PKD1 promotes cell survival by inactivating c-Jun *N*-terminal kinase (JNK) 1/2 and p38 signaling, cofilin function, but also through phosphorylation of Hsp27 and activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). PKD1 also promotes proliferation by upregulating extracellular signal-regulated kinases 1/2 (ERK1/2) and epidermal growth factor receptor (EGFR) signaling. Other functions for ROS-activated PKD1 are upregulation of inflammatory cytokines, regulation of autophagy, and chemoresistance.

FIGURE 1 | **Reactive oxygen species (ROS)-induced activation mechanism for protein kinase D1 (PKD1)**. An initial event in activation of PKD1 in response to oxidative stress is the phosphorylation at Y463 by Abl (1). This leads to a conformational change in PKD1 that allows docking to membranes such as the outer mitochondrial membrane *via* binding to diacylglycerol (DAG) (2). For mitochondrial membrane anchoring, DAG is generated by ROS-activated phospholipase D1 (PLD1). A third activation step is the phosphorylation of PKD1 at Y95, which is mediated directly by Src (3). This leads to docking of PKCδ *via* its C2 domain and phosphorylation of the PKD1 activation loop serines S738 and S742, rendering PKD1 fully active (4).

exact molecular mechanisms of how this is facilitated are not known. NF-κB is a protein complex that controls inflammatory signaling, cytokine production, and cell survival. Downstream of PKD1, activation of NF-κB was linked to increased expression of *SOD2*, a gene encoding manganese superoxide dismutase (MnSOD) (6). MnSOD generates hydrogen peroxide, a *bona fide* signaling molecule that is important for tumor cell proliferation (2). PKD1-mediated activation of NF-κB also increases expression of epidermal growth factor receptor (EGFR) and its ligands transforming growth factor alpha (TGFα) and epidermal growth factor (EGF) (14). Besides activation of NF-κB, PKD1 is also involved in other signaling pathways to promote cell survival. For example, in response to oxidative stress, cofilin2 translocates to the mitochondria to interact with the proapoptotic molecule Bax (19). PKD1 inhibits the cofilin phosphatase Slingshot 1L (20), and such signaling attenuates cofilin2 translocation to mitochondria, preserves mitochondrial integrity after oxidative stress, and mediates cell survival (19). Another pathway of how PKD1 promotes cell survival is by activating extracellular signal-regulated kinases 1/2, which confers a protective response to chronic oxidative stress, and by downregulating c-Jun *N*-terminal kinase (JNK) signaling that promotes apoptosis (21, 22). Similarly, downregulation of p38 MAPK signaling by PKD1 in response to hydrogen peroxide has been demonstrated to protect cells from apoptosis (23). Another target for ROS-activated PKD1 is the small heat shock protein Hsp27, which is phosphorylated by PKD1 at S82 (24). PKD1-phosphorylated Hsp27 can bind apoptosis signal-regulating kinase 1 to prevent JNK-induced apoptosis (25). Hsp27 also has been implicated in chemoresistance of several cancers (26, 27). In addition, the tumor suppressor death-associated protein kinase phosphorylates and activates PKD1 in response to oxidative damage (28). Such signaling induces autophagy, due to PKD1-mediated phosphorylation of Vps34, which increases its lipid kinase activity and autophagosome formation (29).

ROS–PKD1 SIGNALING IN CANCER

Reactive oxygen species–PKD1 signaling has emerged to be important in the pathophysiology of neurodegenerative diseases (30, 31), cardioprotection against ischemia/reperfusion injury (19), tissue inflammation (32), and several cancers, including basal cell carcinoma (33) and pancreatic cancer (14, 34). We here focus on the role of above pathway in pancreatic cancer.

Almost all pancreatic ductal adenocarcinomas (PDAs) are initiated by acquisition of activating *KRAS* mutations (35). During development and progression of PDA, oncogenic KRas protein causes metabolic changes that increase levels of ROS (14, 36–40). KRas-induced suppression of respiratory chain complexes I and III can cause mitochondrial dysfunction and increased generation of mROS (14, 40, 41). Other sources for increased mROS in PDA are enhanced growth factor signaling (42). Oncogenic KRas also activate nuclear respiratory factor 2 to upregulate antioxidant systems to counterbalance the increases in oxidative stress (14, 43). This is accompanied by an upregulation of the *SOD2* gene, whose gene product, MnSOD, leads to formation of hydrogen peroxide (44). In sum, the upregulation of antioxidant enzymes keeps ROS at levels where they are protumorigenic (3, 14, 45, 46).

Further depletion of KRas-caused mROS decreases pancreatic tumorigenesis in genetic animal models (14, 45).

Although in normal fibroblast cells, the ROS/PKCδ/PKD1 pathway downstream of oncogenic KRas upregulates pro-inflammatory signaling (expression of interleukin-6 and interleukin-8) and may contribute to senescence (47), under pathophysiological conditions, this pathway drives initiation of PDA. For example, after pancreatic inflammation (pancreatitis), PKCδ/PKD1/NF-κB signaling is induced in pancreatic acinar cells (48) and contributes to acinar-to-ductal metaplasia, a process that leads to pancreatic lesions (34). In the presence of an oncogenic KRas mutation, these lesions can then further develop to pancreatic cancer. KRas/mROS/PKD1/NF-κB signaling contributes to tumor initiation by upregulating expression of EGFR and its ligands TGFα and EGF (14). EGFR signaling then elevates overall (oncogenic and wild-type) KRas activity to pathological levels (49–51). Another role for PKD1 during initiation of pancreatic cancer is the activation of Notch signaling downstream of mutant KRas (34). Although there is no direct evidence that PKD1/Notch signaling is due to production of mROS, Notch and NF-κB pathways have been shown to co-operate in processes that mediate development of PDA (52).

CONCLUSION

The occurrence of increased oxidative stress in tumor cells requires ROS-sensing signaling to upregulate antioxidant systems to counterbalance ROS. This opens an opportunity for targeting tumor cells (46). In response to ROS, PKD1 has been shown to regulate prosurvival and proliferation signaling through various factors (**Figure 2**). In addition, PKD1 signaling also determines the threshold of mitochondrial depolarization that leads to the production of ROS (53). Therefore, targeting PKD1 or PKD1 downstream signaling may be efficient to drive ROS to levels where they are toxic for cancer cells. In recent years, a variety of PKD inhibitors have been developed and successfully tested in preclinical models. For example, for othotopically implanted pancreatic cancer cells, the PKD inhibitor CRT0066101 showed promising effects on primary tumors (54). However, it is not known if this inhibitor can be used for late stage tumors, or if it will show efficacy in combination therapy with currently used chemotherapeutics. Clearly, additional studies are needed to fully evaluate the value of targeting ROS-PKD signaling for cancer therapy.

AUTHOR CONTRIBUTIONS

Both authors have made equal intellectual contributions to text and figures.

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