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REVIEW

Protein Kinase Cδ in Apoptosis: A Brief Overview

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Abstract Protein kinase C-delta (PKCδ), a member of the lipid-regulated serine/threonine PKC family, has been implicated in a wide range of important cellular processes. In the past decade, the critical role of PKCδ in the regulation of both intrinsic and extrinsic apoptosis pathways has been widely explored. In most cases, over-expression or activation of PKCδ results in the induction of apoptosis. The phosphorylations and multiple cell organelle translocations of PKCδ initiate apoptosis by targeting multiple downstream effectors. During apoptosis, PKCδ is proteolytically cleaved by caspase-3 to generate a constitutively activated catalytic fragment, which amplifies apoptosis cascades in nucleus and mitochondria. However, PKCδ also exerts its anti-apoptotic and pro-survival roles in some cases. Therefore, the complicated role of PKCδ in apoptosis appears to be stimulus and cell type dependent. This review is mainly focused on how PKCδ gets activated in diverse ways in response to apoptotic signals and how PKCδ targets different downstream regulators to sponsor or restrain apoptosis induction.

Keywords Protein kinase C-delta · Apoptosis · Signaling molecules

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Introduction

Protein kinase C (PKC) is a family of serine/threonine protein kinase which plays key roles in cell proliferation and apoptosis. Over 26,000 research articles have been published on PKCs in the past decade. According to their domain structures and regulation mechanisms, a dozen subspecies in PKC family are classified into three subgroups: conventional PKCs (cPKC), novel PKCs (nPKC), and atypical PKCs (Bertram and Ley 2011). Protein kinase C-delta (PKCδ) is the first identified member of the nPKC subfamily, which is calcium-insensitive but can be activated by diacylglycerol (DAG) or phorbol esters (Kikkawa et al. 2002). Unlike the other cell type-specific members of nPKC, PKCδ is ubiquitously expressed in mammalian tissues, including epidermis, placenta, uterus, brain, lung, hematopoietic system, and kidney (Leibersperger et al. 1991). PKCδ is composed of the N-terminal regulatory and the C-terminal catalytic domains, which are connected by a short link region (Fig. 1). The regulatory domain contains a C1 region and a C2-like region. The former region includes two cysteine-rich sequences (C1A and C1B) that are able to bind DAG and phorbol esters to trigger the activation of PKCδ. Mechanistically, DAG binding changes the configuration of PKCδ and hence exposes the substrate binding region, allowing the downstream signal transduction of PKCδ (Newton 2009). The C2-like region structurally resembles the calcium binding C2 motif of cPKC isoforms yet lacks the amino acids necessary for Ca^{2+} coordination. Consequently, the region is unable to trigger PKCδ activation in response to the increase of Ca^{2+} concentration. There is a pseudosubstrate motif between the C2-like and C1 regions, and this motif is believed to occupy the substrate recognition site in the catalytic domain. Therefore, the kinase is maintained in an inactive form until the

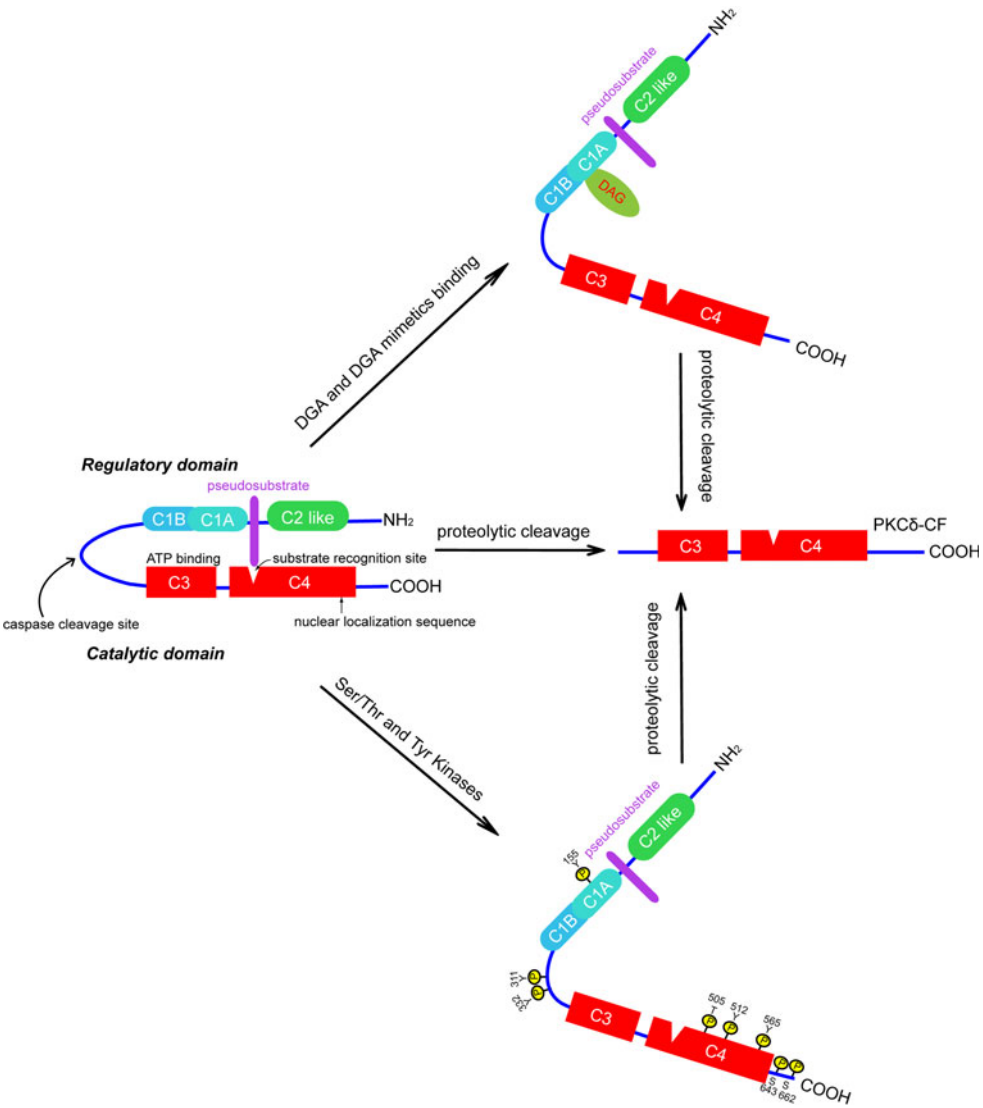

Fig. 1 Diagram of the activation mechanisms of PKCδ. PKCδ binds to DAG and its mimetics or is phosphorylated by Ser/Thr and Tyr kinases, which changes the conformation and exposes the substrate binding site of PKCδ to get activated. PKCδ is proteolytically cleaved by caspase-3, which releases the catalytic fragment, a constitutively active form

conformational change is induced. The catalytic domain of PKCδ contains two conserved regions, C3 and C4, which are essential for the kinase activity and the binding of adenosine-5′-triphosphate (ATP)/substrate. The catalytic domain tails with a turn motif and a hydrophobic motif, which are important for the catalytic competence and binding interactions between PKCδ and its partners (Kikkawa et al. 2002). The hinge region harbors a caspase cleavage site. During apoptosis, caspase-3 proteolytically cleaves PKCδ into a 41-kDa catalytic fragment and a 38-kDa regulatory fragment. This has been confirmed both in vivo and in vitro (Ghayur et al. 1996), and thus the C-terminal catalytic fragment is released from inhibition of the N-terminal regulatory domain (Emoto et al. 1995, 1996).

PKCδ is activated by a variety of apoptosis stimuli such as DNA damaging agents, ultraviolet (UV) radiation, phorbol 12-myristate12-acetate (PMA) and reactive oxygen species (ROS) (Anantharam et al. 2002; Chen et al. 1999; Konishi et al. 2001; Majumder et al. 2001; Reyland et al. 1999; Song et al. 2005). In PKCδ null mice, the mitochondrial functions are compromised, which caused more ROS generation and a shift from glucose to lipid metabolism in murine hearts (Mayr et al. 2004a, b), which indicated the role of PKCδ in mitochondria-related apoptosis. And in PKCδ null mice the smooth muscle cells were resistant to apoptosis induced by UV, tumor necrosis factor (TNF)α, or hydrogen peroxide (Leitges et al. 2001), whereas embryonic fibroblasts (MEFs) cells were resistant to UV radiation-induced G_2/M arrest, Cdk1 phosphorylation, and apoptosis (LaGory et al. 2010). Loss of PKCδ also protected salivary glands against γ-irradiation-induced apoptosis in vivo (Humphries et al. 2006). Furthermore, it has been verified that the overexpression of PKCδ catalytic fragment alone was sufficient to induce apoptosis (DeVries et al. 2002; Leverrier et al. 2002; Zhao et al. 2009). These

studies clearly demonstrated the pro-apoptotic role of PKCδ in vivo in response to diverse apoptotic stimuli. In addition, this pro-apoptotic role of PKCδ has also been proved by the apoptosis inhibition functions of dominant-negative PKCδ mutant, chemical inhibitor as well as RNAi against PKCδ in different cell types (Fujii et al. 2000; Gonzalez-Guerrico and Kazanietz 2005; Song et al. 2005). Recently, Kim et al. (2011) identified another alternative splice form of PKCδ which lacks the N-terminal regulatory domain and the ATP binding region and thus functions as a natural inhibitor to reduce PKCδ-induced apoptosis. The activation of PKCδ in prostate cancer cells caused apoptosis via the release of death receptor ligands and the activation of the extrinsic apoptotic cascade (Gonzalez-Guerrico and Kazanietz 2005), which indicated the role of PKCδ in both intrinsic and extrinsic apoptosis pathways. Overall, these studies suggest the pro-apoptosis functions of PKCδ in diverse cell contexts and apoptosis stimuli. Conversely, it was reported that under TNFα induction, PKCδ targeted the nucleus and formed a complex with RelA/p65, which was important for NFκB activity and the cell survival protection in response to TNFα (Lu et al. 2009). This review aims to provide an overview of PKCδ regarding regulation and function in response to apoptosis stimuli.

PKCδ Activation and Expression Regulation in Response to Apoptosis

PKCδ is a self-inhibited kinase, in which the substrate recognition site in C-terminal catalytic domain is occupied by a pseudosubstrate motif in N-terminal regulatory domain. PKCδ can be activated by multiple mechanisms, including binding of lipid second messenger DAG and its mimetics, phosphorylations, and proteolytic cleavage (Fig. 1). According to the three-dimensional structure of PKCδ C1 domain and phorbol ester complex, the binding of C1 domain with DAG or PMA forms a contiguous hydrophobic surface and promotes PKCδ binding to cell membranes (Thamilselvan et al. 2009; von Burstin et al. 2010; Zhang et al. 1995). In addition, the binding of C1 to DAG or PMA changes the conformation and exposes the catalytic domain of PKCδ, allowing the substrates to bind (Kanthasamy et al. 2003).

Several phosphorylation sites in PKCδ are modulated in cell type and stimulus specific manner, and thus have different contributions on PKCδ activation (Johnson et al. 1996). Three conserved serine/threonine resides have been identified in the C-terminal catalytic domain of PKCδ including Thr-505 in the activation loop, Ser-643 in the turn motif, and Ser-662 in the hydrophobic motif (Konishi et al. 2001; Newton 2001; Steinberg 2004). Ser-643 is an auto-phosphorylation site and the site-directed mutation of Ser-643 markedly decreased PKCδ activity (Li et al. 1997). This phosphorylation site was activated in response to interferon (IFN) and PMA treatment (Zhao et al. 2004, 2005), as well as H_2O_2-induced apoptosis (Lee et al. 2005). The phosphorylation of Ser-662 is concomitant with Thr-505 phosphorylation (Le Good et al. 1998). However, serum starvation-induced Ser-662 phosphorylation was independently controlled by a pathway involving mammalian TOR (Parekh et al. 1999). Although Thr-505 did not affect the kinase activity of PKCδ in bacteria expressed recombinant protein (Stempka et al. 1997), its phosphorylation is believed to be important for PKCδ activation under PMA and other stimuli (Kambhampati et al. 2003; Li et al. 2005; Liu et al. 2010) and is required for the stability of PKCδ in embryonic stem cells mediated by the 3-phosphoinositide-dependent protein kinase-1 (Balendran et al. 2000). Moreover, the phosphorylation of Ser-662/Thr505 has also been detected in response to H_2O_2- or PMA-induced apoptosis (Konishi et al. 2001; Lee et al. 2005; Sabri et al. 2002).

PKCδ is the most efficiently tyrosine-phosphorylated kinase among the PKC family. Phosphorylations of Tyr-52, Tyr-64, Tyr-155, Tyr-187, Tyr-311, Tyr-332, Tyr-512, Tyr-523, and Tyr-565 have been identified in PKCδ. These phosphorylations are associated with tyrosine kinases such as Src, Fyn, Lyn, Abl, PYk2, and Lck (Kikkawa et al. 2002). The tyrosine phosphorylation context regulates the catalytic activity of PKCδ. For example, the tyrosine phosphorylations of PKCδ induced by Ras oncogene, transforming growth factor α, or epidermal growth factor in keratinocytes are associated with a reduction of PKCδ catalytic activity (Denning et al. 1993, 1996; Joseloff et al. 2002). Increasing number of studies has showed that the tyrosine phosphorylations are important for PKCδ translocation, activation, and downstream signal transduction (Kikkawa et al. 2002; Kronfeld et al. 2000). Tyr-64 and Tyr-155 phosphorylations in the N-terminal regulatory domain were important for the nuclear translocation and apoptosis induction in salivary epithelial cells (Humphries et al. 2008). Moreover, the translocation of PKCδ into the nucleus required C2 domain tyrosine phosphorylations, which were associated with Src kinase activity induced by G-protein-coupled receptor agonist uridine triphosphate (UTP) (Kajimoto et al. 2010). The etoposide-induced tyrosine phosphorylation of PKCδ also contributed to nuclear translocation and caspase-dependent cleavage of PKCδ. In addition, overexpressing PKCδ mutants in Tyr-64 and Tyr-187 lead to decreased apoptosis in response to etoposide compared to the overexpression of wild type PKCδ (Blass et al. 2002). Mutating Tyr-52, Tyr-155, and Tyr-565 to phenylalanine enhanced etoposide-induced apoptosis in glial cells; in contrast, the mutations of Tyr-64

and Tyr-187 to phenylalanine inhibited caspase-3-dependent PKCδ activation and reduced etoposide-induced apoptosis (Blass et al. 2002). In parallel, the tyrosine phosphorylations of PKCδ identified in ceramide-induced apoptosis also showed different functions. Ceramide, the basic structural unit of the sphingolipids, is an intracellular lipid second messenger (Senchenkov et al. 2001), and its accumulation is related to various apoptosis stimuli (Dbaibo et al. 1998; Haimovitz-Friedman et al. 1994; Rotolo et al. 2005). Both chemical inhibitor and siRNA knock-down experiments support that PKCδ is important for ceramide-induced apoptosis in Hela cells, in which Tyr-311 and Tyr-332 phosphorylations were observed. However, the function of these phosphorylation sites in apoptosis induction could not be verified by site-directed mutations (Kajimoto et al. 2004; Kikkawa et al. 2002). No uniform pattern and consequence of PKCδ phosphorylations has been established from the published literature, because the catalytic activity of phosphorylated PKCδ is highly cell type and stimulus dependent as described above. One possible reason is that most studies relied on in vitro kinase assays to address the functional changes in PKCδ phosphorylations. It is predictable that more than one tyrosine phosphorylation would be generated simultaneously in vivo in response to stimulus. The phosphorylation context and the resulting conformational change determine the particular activity and function of PKCδ. Overall, it is believed that phosphorylation of the catalytic domain increases the kinase activity, whereas phosphorylation of the regulatory domain is associated with cellular actions of PKCδ (Steinberg 2004).

PKCδ can be activated by caspase-mediated proteolysis. Emoto et al. (1995) reported that PKCδ is proteolytically activated by cysteine protease caspase-1 also called interleukin-1β converting enzyme in response to ionizing radiation-induced apoptosis. Thereafter, the proteolytic cleavage of PKCδ has been observed in various cell types in response to multiple apoptosis stimuli, including TNFα or Fas ligand and DNA damage agents, such as etoposide, cisplatin, camptothecin and UV, γ-radiation. Following studies showed that PKCδ is an endogenous substrate for caspase-3, and caspase-3 cleaves PKCδ at ^{324}DILD^{327} in mouse, ^{324}DIPD^{327} in rat, and ^{326}DMQD^{329} in human. This process has been well reviewed previously (Brodie and Blumberg 2003; Kanthasamy et al. 2003; Kikkawa et al. 2002). After cleavage, the pseudosubstrate motif in the regulatory domain is released from the substrate recognition site of the catalytic domain, which enables PKCδ to become constitutively activated. This unique mechanism makes the activation of PKCδ irreversible. The conformational changes made by DAG binding and phosphorylation modifications are reversible, which hold PKCδ at a prime status for the auto-inhibition release and downstream targeting. For example, CD45, a receptor-type tyrosine phosphatase, is shown to be involved in dephosphorylation of PKCδ (Deszo et al. 2001). However, when cells are destined for apoptosis, caspase-3-mediated proteolytic cleavage activates PKCδ in a one-way direction, and this amplified signal is more sufficient for apoptosis induction. This has been verified by the study which showed that overexpression of a caspase-resistant PKCδ mutant (D330A) protected keratinocytes from UV-induced apoptosis (D'Costa and Denning 2005). This is also supported by the observation that overexpression of PKCδ catalytic fragment alone was sufficient to induce apoptosis, and overexpression of PKCδ kinase dead mutants protected cells from apoptosis (DeVries et al. 2002; Zhao et al. 2009). In addition, the regulatory domain of PKCδ is also required for etoposide-induced apoptosis in C6 glioma cells (Blass et al. 2002). In contrast, the proteolytic cleavage of PKCδ also involved caspase-3-independent mechanisms, such as other caspases like caspase-2 which can also cleave PKCδ during apoptosis (Panaretakis et al. 2005). This is also supported by the study which proved the pro-apoptosis role of PKCδ in caspase-3 null MCF-7 cells in response to UV (Zeidan et al. 2008). Our study also showed that the proteolytic cleavage of PKCδ occurred prior to caspase-3 activation in a camptothecin analog-induced apoptosis (Song et al. 2005), indicating the function of PKCδ in an early stage of apoptosis.

Besides the kinase activation regulation, PKCδ expression also fluctuates during apoptosis. Sp protein transcriptionally up-regulates PKCδ, facilitating oxidative stress-induced apoptosis in neuron cells (Jin et al. 2011a). NFκB P50/p60 forms a complex with p300 and α-synuclein and binds to PKCδ promoter. This complex down-regulated PKCδ and protected dopaminergic neuronal cells against oxidative stress-induced apoptosis (Jin et al. 2011b). After activation by DAG or 12-*O*-tetradecanoylphorbol-13-acetate (TPA), PKCδ was ubiquitinated and targeted to proteasome for degradation in rat fibroblasts (Lu et al. 1998). This negative feedback protects cells from the following apoptosis execution induced by PKCδ activation.

The Early Effect of PKCδ in Apoptosis Induction

Although activated caspase-3 has an amplifying effect on PKCδ proteolysis, in some cases PKCδ-mediated apoptosis does not involve its proteolytic cleavage by caspase-3. This suggests that allosteric activation of PKCδ is also sufficient to trigger apoptosis. For example, caspase-3-dependent cleavage of PKCδ was not required for the cell death in LNCap cells, CHO cells and HaCaT cells treated respectively with phorbol esthers, UV and H_2O_2 (Fujii et al. 2000; Fukunaga et al. 2001; Konishi et al. 1997). Our group has

also demonstrated a positive feedback loop between caspase activation and PKCδ activation using that NSC606985, a rarely studied camptothecin analog, in leukemic model. Inhibition of PKCδ activity by chemical inhibitor rottlerin and later on by the overexpression of PKCδ dominant-negative mutant completely blocked NSC606985-induced mitochondrial ΔΨm loss and caspase-3 activation, while the inhibition of caspase-3 by z-DEVD-fluoromethyl ketone only partially attenuated PKCδ activation and apoptosis (Song et al. 2005; Zhao et al. 2009). It is worthwhile to note that rottlerin, the widely used PKCδ inhibitor, has been shown to have many off-target effects (Davies et al. 2000; Soltoff 2007). So the results from this inhibitor raise the question if PKCδ is truly responsible for those observations, or the off-target effects on other pathways may influence the results. A more specific approach to inhibit PKCδ activity such as overexpression of dominate negative mutant or siRNA knock-down would be a better way to investigate the function of PKCδ. A number of studies showed that PKCδ is able to target mitochondria to initiate apoptosis (Lasfer et al. 2006; Liu et al. 2003a; Majumder et al. 2001). Moreover, the vascular smooth muscle cells isolated from PKCδ null mice were defective in caspase-3 activation in response to oxidative stress-induced apoptosis (Bowles et al. 2007; Kato et al. 2009). All these findings indicated that PKCδ activation could be an early event upstream of mitochondrial ΔΨm loss and caspase-3 activation during apoptosis, whereas how PKCδ affects mitochondrial membrane potential during apoptosis requires further study. In other words, PKCδ is able to initiate apoptosis signals before caspase-3 activation or in a caspase-3-independent way, which was also supported by the fact that PKCδ increased autocrine release of death receptor ligands to trigger extrinsic apoptotic cascade (Gonzalez-Guerrico and Kazanietz 2005). On the contrary, PKCδ is able to activate caspase-3 and caspase-9 directly. PKCδ-phosphorylated caspase-3 and increased its activity during spontaneous monocyte apoptosis and in monocyte-induced apoptosis (Voss et al. 2005). The role of PKCδ in the activation of caspase-9 was demonstrated in response to etoposide, taxol, and UV radiation (Matassa et al. 2001), possibly via the activation of AKT pathway (Cardone et al. 1998; Martins et al. 1998). Moreover, the phosphorylation of PKCδ may function before the proteolytic cleavage of PKCδ to initiate the apoptotic cascade. The phosphorylations of PKCδ on Tyr-64/187 are regarded to be essential for the cleavage of PKCδ by caspase-3 and the pro-apoptotic effect of PKCδ in response to etoposide treatment (Blass et al. 2002), while the phosphorylations of PKCδ on Tyr-52/64/155 are associated with the anti-apoptotic effect of PKCδ in response to Sindbis virus-induced glioma cell death (Zrachia et al. 2002).

PKCδ in Mitochondria and Apoptosis

Another feature of PKCδ during apoptosis is the multi-subcellular organelle translocations. In response to diverse apoptosis stimuli, PKCδ is localized in almost all the subcellular organelles, including mitochondria, nucleus, cell membrane, Golgi apparatus, endoplasmic reticulum (ER), and recently reported lysosomes. Those subcellular translocalizations of PKCδ are cell type and apoptotic stimulus dependent, which indicates that the downstream targets of PKCδ are distributed among different subcellular organelles for apoptosis induction.

The first well-studied subcellular organelle targeted by PKCδ in apoptosis is mitochondria, which is the apoptosis signal integrator (Brenner and Kroemer 2000). In PMA-induced apoptosis, the mitochondrion translocation of the endogenous and ectopic-expressed PKCδ was found in U937 cells (Majumder et al. 2000) and mouse keratinocyte SP-1 cells (Li et al. 1999). A similar translocation of PKCδ has also been shown in oxidative stress- and DNA damage agents-induced apoptosis (Majumder et al. 2001). Recently, it was reported that the expanded AUUCU repeated intronic RNA within ATXN1, the major pathogenic mechanism of spinocerebellar ataxia type 10 (SCA10), strongly bound to heterogeneous nuclear ribonucleoprotein K (hnRNPK) facilitating massive translocation of PKCδ to mitochondria and activation of caspase-3 in SCA10 cells (White et al. 2010).

Several targets of PKCδ in mitochondria during apoptosis have been reported. PKCδ enhanced ceramide formation in mitochondria in prostate cancer cells during multi anti-cancer drug treatment, which stimulated cytochrome *c* release and caspase-9 activation (Sumitomo et al. 2002). PKCδ also targeted phosphorylated phospholipid scramblase 3, which is a critical regulator of mitochondrial structure and respiration, in UV radiation or PMA treated Hela and HEK293 cells (Liu et al. 2003a, b). More interestingly, it was found that PKCδ directly phosphorylated myeloid cell leukemia protein 1 and promoted its proteolytic degradation during UV-induced apoptosis (Sitailo et al. 2006). In response to DNA damage, PKCδ associated with checkpoint point protein Rad9 to target Bcl-2 and facilitated apoptosis (Yoshida et al. 2003). Moreover, the active form of PKCδ stimulated the up-regulation and activation of Bax in response to UV radiation (Sitailo et al. 2004). In addition, PKCδ has been shown to be related to the dephosphorylation of BAD (Murriel et al. 2004). Recently another PKCδ mitochondrial target Drp1, a major mitochondrion fission protein, has been identified in SH-SY5Y neuronal cells. PKCδ phosphorylated Drp1 at Ser 579 and facilitated Drp1 translocation to mitochondria under oxidative stress, thus increasing mitochondrial fragmentation, which is associated with apoptosis (Frank

et al. 2001; Qi et al. 2011).Overall, those studies prove the existence of a positive feedback loop between caspase activation and PKCδ which in turn triggers more caspase activation. Our report showed that the cleavage of PKCδ prior to caspase-3 and block of caspase-3 activity only partially blocked PKCδ activation (Song et al. 2005). This indicated that PKCδ may trigger apoptosis initiating signals in mitochondria prior to caspase-3 activation. In addition, over-expression of kinase inactive mutant PKCδ instead of the wide type failed to target mitochondria and was not able to induce apoptosis in salivary epithelial cells (Matassa et al. 2001). This indicated that the kinase activity of PKCδ is essential for the apoptosis initiation signals in mitochondria.

PKCδ in Nucleus and Apoptosis

The nucleus translocation of PKCδ to amplify apoptosis process has been reported in a wide range of cells in response to various apoptosis stimuli (Blass et al. 2002; DeVries et al. 2002; Scheel-Toellner et al. 1999; Yuan et al. 1998). In particular, the proteolytically cleaved catalytic fragment (PKCδ-CF) has been found to be dominantly localized in nucleus (Blass et al. 2002; DeVries et al. 2002) due to the following mechanisms. First, a nucleus import sequence of PKCδ is identified in the catalytic fragment of PKCδ, which partially explains the dominant localization of PKCδ-CF in nucleus during apoptosis. Furthermore, using site direct mutation DeVries et al. (2002) proved that this nucleus import sequence is necessary for the pro-apoptosis role of PKCδ and only nuclear, but not cytoplasmic, expression of PKCδ dominant-negative mutant inhibits etoposide-induced apoptosis. Second, it was reported that full-length PKCδ also transiently accumulates in the nucleus and is cleaved by activated caspase-3 in response to apoptotic signals (DeVries-Seimon et al. 2007), which has also been verified by the reports that the tyrosine phosphorylation of PKCδ preceded caspase-3 cleavage and was important for PKCδ nuclear accumulation (Blass et al. 2002; Humphries et al. 2008). These indicate that PKCδ-CF is at least partially generated in nucleus during apoptosis.

Accumulating nuclear targets have been identified and most of them function in apoptosis, such as DNA-dependent protein kinase (Bharti et al. 1998), lamin B (Cross et al. 2000), and Rad9 (Yoshida et al. 2003). For example, PKCδ interacted with topoisomerase IIα and attenuated DNA damage-induced activation of topoisomerase IIα and apoptosis (Yoshida et al. 2006a). It was also shown that PKCδ interacted with and phosphorylated P53 at Ser-15 and Ser-46, which potentiated p53-dependent apoptosis in response to genotoxic stress in MCF-7 and HEK293T cells as well as nitric oxide-induced apoptosis in dopaminergic cells (Lee et al. 2006; Yoshida et al. 2006b). Ecotropic viral integration site 1 and promyotic leukemic zinc finger, which are associated with DNA damage-induced apoptosis, also have been identified as PKCδ targets (Hew et al. 2011).

Our recent studies demonstrated that PKCδ is involved in proteasome-dependent degradation of nuclear proteins, which contributed to PKCδ-mediated apoptosis. First, we reported that during apoptosis induction of leukemic cells, PKCδ was able to stimulate proteasome-dependent degradation of CCAAT/enhancer-binding protein α (C/EBPα), a key regulator of differentiation and proliferation in various cell types. More importantly, ectopic expression of PKCδ-CF stimulated the ubiquitination of C/EBPα protein, while the chemical inhibition or over-expression of PKCδ dominant-negative mutant significantly suppressed the ubiquitination of C/EBPα protein under apoptosis induction. In addition, the anti-apoptotic function of C/EBPα has also been proved by siRNA silencing and inducible over-expression in leukemic cells (Zhao et al. 2009). More interestingly, we found another nuclear protein, hnRNP K protein, which was also down-regulated by PKCδ in the proteasome-dependent manner (Gao et al. 2009). Although we are still working on whether PKCδ directly phosphorylates C/EBPα and hnRNP K, these studies suggest that PKCδ may affect the ubiquitin–proteasome machinery, which is important for the control of apoptosis induction (Xie 2010).

PKCδ in Other Cell Organelles and Apoptosis

The diverse and varied subcellular localization patterns of PKCδ in apoptosis make PKCδ more intricate. The cell membrane translocation of PKCδ was observed in UV radiation and PMA stimulation in various cell types (Chen et al. 1999; Hendey et al. 2002; Park et al. 2009; von Burstin et al. 2010). Using membrane-targeted PKCδ construct, this peripheral localization of PKCδ has been shown to be required for apoptosis initiation (von Burstin et al. 2010). Recently, a new approach to detect the spatial and temporal PKCδ activation was developed by Kajimoto et al. (2010). Using this Fluorescence resonance energy transfer-based new reporter technology, they observed that phorbol esters triggered fluorescence at the plasma membrane and also in small amounts in the Golgi apparatus and mitochondria in a Src-independent manner (Kajimoto et al. 2010). In parallel, the translocations to ER and Golgi apparatus have also been reported in response to apoptotic signal (Kajimoto et al. 2001; Zrachia et al. 2002). The PKCδ tyrosine phosphorylation and ER translocation were associated with the complex formation between PKCδ and

tyrosine kinase Abl, which was required for ER-stress-mediated apoptosis. Accordingly, the chemical inhibitor rottlerin blocked the translocation of the PKCδ–Abl complex from the ER to the mitochondria and conferred protection against apoptosis, which indicated that the PKCδ–Abl complex is important for the communication between ER and mitochondria in apoptosis response (Qi and Mochly-Rosen 2008). Also, another ER protein P23 (Tmp21) was found to interact with PKCδ and retain PKCδ in the ER. This limited the activation and pro–apoptotic function of PKCδ in LNCaP cells (Wang et al. 2011). Recently, the lysosomal translocation of PKCδ has been reported in camptothecin lactone (CPT)-treated U937 cells. It was shown that PKCδ targeted lysosomal acidic sphingomyelinase to increase ceramide content, inducing the labilization of lysosomal membranes and activating lysosomal pathway of apoptosis under CPT treatment (Parent et al. 2011). Translocation of PKCδ to Golgi apparatus was found under ceramide or IFNγ stimulus (Kajimoto et al. 2004). This translocation was associated with PKCδ tyrosin phosphorylation, but whether it is related to apoptosis induction is still not clear (Kajimoto et al. 2004).

Other PKCδ-Related Downstream Signals During Apoptosis

It has been well studied that STAT1 activation under IFN stimulation is dependent on the signal transduction between PKCδ and c-Jun NH2-terminal kinase (JNK) (Zhao et al. 2005). In response to apoptosis induction, the JNK/STAT1 pathway has been proven as a functional downstream target of PKCδ contributing to apoptosis. When cells were treated with PMA or etoposide, JNK kinase was activated in a PKCδ-dependent manner. Blocking PKCδ by the chemical inhibitor rottlerin significantly inhibited JNK activation, and suppressing the JNK pathway also reduced PKCδ-related galectin-1-induced T cell apoptosis (Brandt et al. 2010) and arsenic-induced hepatic cell death (Das et al. 2010). JNK was activated by PKCδ possibly through MKK7 (MAPK kinase 7) during DNA damage–response (Yoshida et al. 2002). At the bottom of PKCδ–JNK signal pathway, STAT1 transactivation has also been proven to be important for PKCδ-related apoptosis. Etoposide treatment resulted in PKCδ-dependent phosphorylation of STAT1 on ser-727 in Hela cells, and PKCδ-induced apoptosis was suppressed in STAT1 deficient U3A cells (DeVries et al. 2004). All this evidence indicates that PKCδ–JNK–STAT1 might be a major signal pathway for apoptosis inducing role of PKCδ.

Directly targeting or not, activated PKCδ also affects other signaling cascades in apoptosis regulation, including PI3-kinase/AKT pathway (Murriel et al. 2004), extracellular signal-regulated kinase (ERK), and P38 pathway (Efimova et al. 2004; Lee et al. 2002). For example, PKCδ formed a complex with ShcA to induce phosphorylation of ERK in MEF cells in response to H_2O_2-induced apoptosis (Hu et al. 2007). Recently serine/threonine kinase vaccinia-related kinase 1 (VRK1) was identified as a PKCδ target. PKCδ activated VRK1 and P53 in nucleus, thus was involved in DNA damage-induced apoptosis (Park et al. 2011).

Conclusions and Future Perspectives

The mechanisms by which PKCδ regulate apoptosis as illustrated in Fig. 2 still remain elusive. A positive loop between mitochondrial-mediated caspase activation and PKCδ cleavage and activation supported the pro-apoptotic role of PKCδ in cytoplasm. The nucleus may be another major target for PKCδ to amplify apoptosis signal. The nucleus translocation of PKCδ has been proven to be essential for apoptosis induction, and the proteolytically cleaved constitutive active catalytic fragment of PKCδ accumulates in nucleus (DeVries et al. 2002). Therefore, more and more studies are revealing new nuclear targets of PKCδ, including the recently identified C/EBPα and hnRNPK (Gao et al. 2009; Zhao et al. 2009) which function in apoptosis. PKCδ is an early and essential regulator of apoptosis in diverse cell types and under various stimuli. For example, PKCδ functions upstream of the mitochondria as an integrator for various death signals, and the cytoplasm-localized PKCδ may function at the initial stage of apoptosis. Phosphorylations, especially the tyrosine phosphorylations of PKCδ, which usually occur at the beginning of apoptosis, are responsible for its translocations to cell membrane, mitochondria, ER, and lysosome. However, a detailed understanding of the intracellular actions of PKCδ will require the knowledge of their distinct cellular substrates in response to apoptosis.

Although some kinase signals have been proven to activate PKCδ at an early stage of apoptosis, the exact mechanisms are still largely unknown. In order to address this question, we performed quantitative proteomic analysis before PKCδ activation under NSC606985 treatment in U937 cells and found that over 30 proteins are regulated. The down-regulation of NDRG1 was proved to be involved in proteolytic activation of PKCδ during apoptosis induction (Zheng et al. 2009). Yet, it is still difficult to identify the direct effectors which initiate PKCδ activation during apoptosis. Using a structural or functional approach to identify PKCδ complex and the interaction binding sites in PKCδ would be a more practical way. Addressing all the above queries will lead to a thorough understanding of how the complex interactions retain PKCδ in the cytoplasm or translocate PKCδ into other

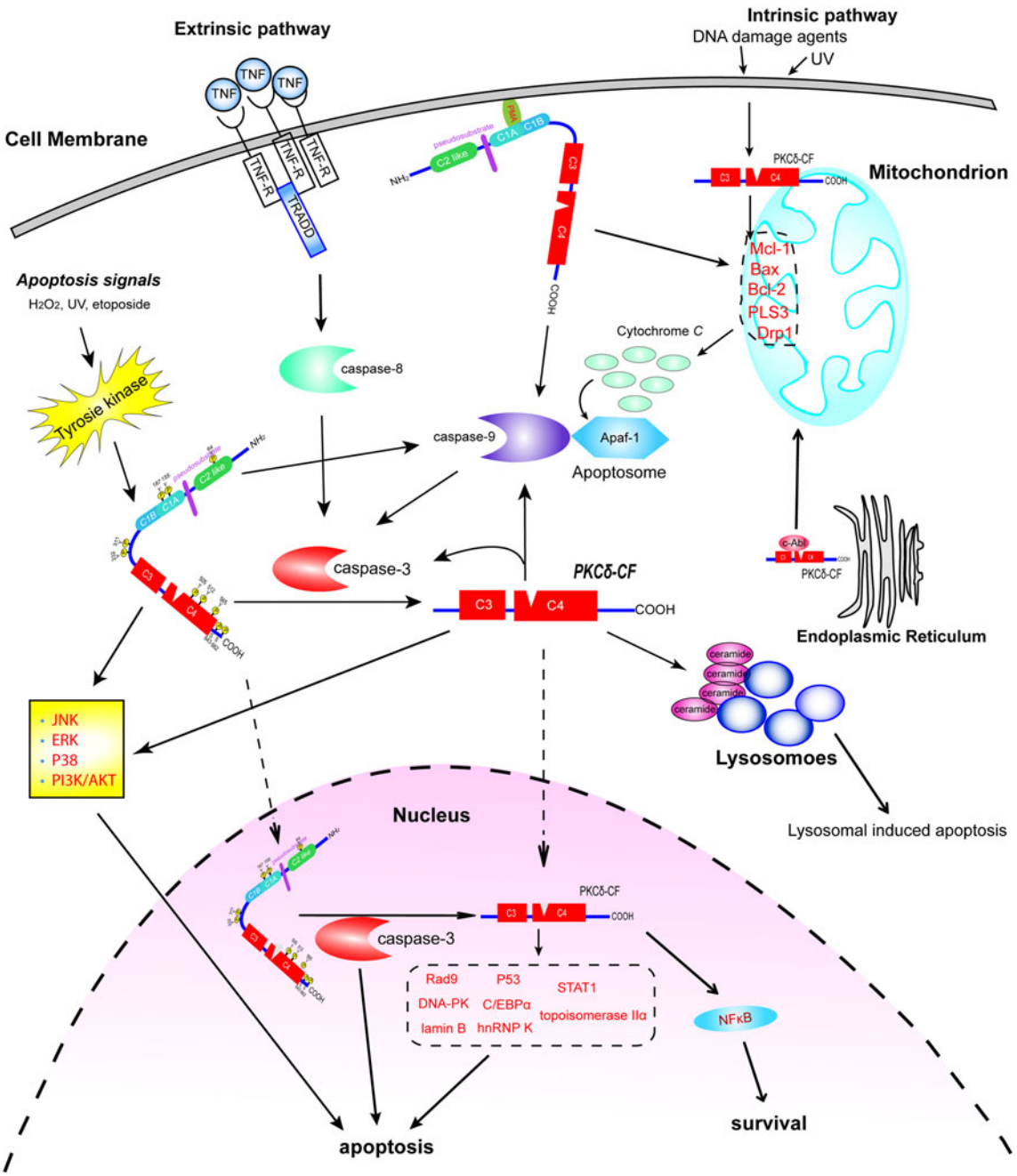

Fig. 2 Major PKCδ signals in response to apoptosis. Death receptor apoptosis signal triggers activation of caspase-8, which in turn prompts caspase-3 activation and then initiates proteolytic cleavage of PKCδ. In parallel, the intrinsic apoptosis signals from mitochondria release Cytochrome *c*, active caspase-9 and Apaf-1 to form apoptosome and then trigger the activation of caspase-3, which initiates the proteolytic cleavage of PKCδ. The PKCδ with PMA binding on cell membrane functions on apoptosome to facilitate apoptosis. The cleaved PKCδ constitutively active fragment (PKCδ-CF) feeds back on caspase-3 and caspase-9 to amplify apoptosis. Moreover, PKCδ-CF translocates into (1) mitochondria to enhance the intrinsic apoptosis signal, (2) endoplasmic reticulum to form complex with c-Abl and communicate with mitochondria to enhance intrinsic apoptosis signal, (3) lysosomes to increase the accumulation of ceramide and then initiate the lysosomal-induced apoptosis, (4) nucleus to target the downstream effectors and induce apoptosis. On the other hand, the activated PKCδ in nucleus targets NFκB to induce survival signals. In addition, apoptosis agents such as H_2O_2, UV, or etoposide activate tyrosine kinases which phosphorylate PKCδ, targeting either apoptosome or other kinase such as PI3K or MAP kinase to induce apoptosis. Furthermore, the phosphorylated PKCδ translocates into different cell organelles including nucleus and is proteolytically cleaved by caspase-3. *TNF* tumor necrosis factor, *TNF-R* TNF receptor, *TRADD* TNF-R-associated death domain, *DNA-PK* DNA-dependent protein kinase

organelles and initiate PKCδ activation at the early stages of apoptosis.

The ability to evade apoptosis is a common feature of cancer, and genetic disruptions of the apoptotic pathway are responsible for neoplasia. As an initiator of apoptosis, PKCδ is suspected as a tumor suppressor gene, and some PKCδ-targeted molecules have shown significant apoptosis induction ability in vitro and in vivo (Hampson et al. 2005; Liu et al. 2007; Podar et al. 2007). However, in some cases PKC also enhanced tumorigenesis, evidenced by that PKCδ deficient mice were protected from urethane-induced lung tumor (Symonds et al. 2011). Maybe this is not related to the pro-apoptotic function of PKCδ. Therefore, elucidating the role of PKCδ in tumor development and therapy would be another promising research direction.

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References

Anantharam V, Kitazawa M, Wagner J et al (2002) Caspase-3-dependent proteolytic cleavage of protein kinase C delta is essential for oxidative stress-mediated dopaminergic cell death after exposure to methylcyclopentadienyl manganese tricarbonyl. J Neurosci 22:1738–1751

Balendran A, Hare GR, Kieloch A et al (2000) Further evidence that 3-phosphoinositide-dependent protein kinase-1 (PDK1) is required for the stability and phosphorylation of protein kinase C (PKC) isoforms. FEBS Lett 484:217–223

Bertram A, Ley K (2011) Protein kinase C isoforms in neutrophil adhesion and activation. Arch Immunol Ther Exp 59:79–87

Bharti A, Kraeft SK, Gounder M et al (1998) Inactivation of DNA-dependent protein kinase by protein kinase C delta: implications for apoptosis. Mol Cell Biol 18:6719–6728

Blass M, Kronfeld I, Kazimirsky G et al (2002) Tyrosine phosphorylation of protein kinase C delta is essential for its apoptotic effect in response to etoposide. Mol Cell Biol 22:182–195

Bowles DK, Maddali KK, Dhulipala VC et al (2007) PKC delta mediates anti-proliferative, pro-apoptic effects of testosterone on coronary smooth muscle. Am J Physiol Cell Physiol 293:C805–C813

Brandt B, Abou-Eladab EF, Tiedge M et al (2010) Role of the JNK/c-Jun/AP-1 signaling pathway in galectin-1-induced T-cell death. Cell Death Dis 1:e23

Brenner C, Kroemer G (2000) Apoptosis. Mitochondria—the death signal integrators. Science 289:1150–1151

Brodie C, Blumberg PM (2003) Regulation of cell apoptosis by protein kinase c delta. Apoptosis 8:19–27

Cardone MH, Roy N, Stennicke HR et al (1998) Regulation of cell death protease caspase-9 by phosphorylation. Science 282:1318–1321

Chen N, Ma W, Huang C et al (1999) Translocation of protein kinase Cepsilon and protein kinase C delta to membrane is required for ultraviolet B-induced activation of mitogen-activated protein kinases and apoptosis. J Biol Chem 274:15389–15394

Cross T, Griffiths G, Deacon E et al (2000) PKC-delta is an apoptotic lamin kinase. Oncogene 19:2331–2337

Das J, Ghosh J, Manna P et al (2010) Protective role of taurine against arsenic-induced mitochondria-dependent hepatic apoptosis via the inhibition of PKC delta-JNK pathway. PLoS ONE 5:e12602

Davies SP, Reddy H, Caivano M et al (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J 351(Pt 1):95–105

Dbaibo GS, Pushkareva MY, Rachid RA et al (1998) p53-dependent ceramide response to genotoxic stress. J Clin Invest 102: 329–339

D'Costa AM, Denning MF (2005) A caspase-resistant mutant of PKC-delta protects keratinocytes from UV-induced apoptosis. Cell Death Differ 12:224–232

Denning MF, Dlugosz AA, Howett MK et al (1993) Expression of an oncogenic rasHa gene in murine keratinocytes induces tyrosine phosphorylation and reduced activity of protein kinase C delta. J Biol Chem 268:26079–26081

Denning MF, Dlugosz AA, Threadgill DW et al (1996) Activation of the epidermal growth factor receptor signal transduction pathway stimulates tyrosine phosphorylation of protein kinase C delta. J Biol Chem 271:5325–5331

Deszo EL, Brake DK, Cengel KA et al (2001) CD45 negatively regulates monocytic cell differentiation by inhibiting phorbol 12-myristate 13-acetate-dependent activation and tyrosine phosphorylation of protein kinase C delta. J Biol Chem 276:10212–10217

DeVries TA, Neville MC, Reyland ME (2002) Nuclear import of PKC delta is required for apoptosis: identification of a novel nuclear import sequence. EMBO J 21:6050–6060

DeVries TA, Kalkofen RL, Matassa AA et al (2004) Protein kinase C delta regulates apoptosis via activation of STAT1. J Biol Chem 279:45603–45612

DeVries-Seimon TA, Ohm AM, Humphries MJ et al (2007) Induction of apoptosis is driven by nuclear retention of protein kinase C delta. J Biol Chem 282:22307–22314

Efimova T, Broome AM, Eckert RL (2004) Protein kinase C delta regulates keratinocyte death and survival by regulating activity and subcellular localization of a p38delta-extracellular signal-regulated kinase 1/2 complex. Mol Cell Biol 24:8167–8183

Emoto Y, Manome Y, Meinhardt G et al (1995) Proteolytic activation of protein kinase C delta by an ICE-like protease in apoptotic cells. EMBO J 14:6148–6156

Emoto Y, Kisaki H, Manome Y et al (1996) Activation of protein kinase C delta in human myeloid leukemia cells treated with 1-beta-D-arabinofuranosylcytosine. Blood 87:1990–1996

Frank S, Gaume B, Bergmann-Leitner ES et al (2001) The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev Cell 1:515–525

Fujii T, Garcia-Bermejo ML, Bernabo JL et al (2000) Involvement of protein kinase C delta (PKC delta) in phorbol ester-induced apoptosis in LNCaP prostate cancer cells. Lack of proteolytic cleavage of PKC delta. J Biol Chem 275:7574–7582

Fukunaga M, Oka M, Ichihashi M et al (2001) UV-induced tyrosine phosphorylation of PKC delta and promotion of apoptosis in the HaCaT cell line. Biochem Biophys Res Commun 289:573–579

Gao FH, Wu YL, Zhao M et al (2009) Protein kinase C-delta mediates down-regulation of heterogeneous nuclear ribonucleoprotein K protein: involvement in apoptosis induction. Exp Cell Res 315:3250–3258

Ghayur T, Hugunin M, Talanian RV et al (1996) Proteolytic activation of protein kinase C delta by an ICE/CED 3-like protease induces characteristics of apoptosis. J Exp Med 184: 2399–2404

Gonzalez-Guerrico AM, Kazanietz MG (2005) Phorbol ester-induced apoptosis in prostate cancer cells via autocrine activation of the

extrinsic apoptotic cascade: a key role for protein kinase C delta. J Biol Chem 280:38982–38991

Haimovitz-Friedman A, Kan CC, Ehleiter D et al (1994) Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. J Exp Med 180:525–535

Hampson P, Chahal H, Khanim F et al (2005) PEP005, a selective small-molecule activator of protein kinase C, has potent antileukemic activity mediated via the delta isoform of PKC. Blood 106:1362–1368

Hendey B, Zhu CL, Greenstein S (2002) Fas activation opposes PMA-stimulated changes in the localization of PKC delta: a mechanism for reducing neutrophil adhesion to endothelial cells. J Leukoc Biol 71:863–870

Hew HC, Liu H, Lu ZG et al (2011) Identification of Evi-1 as a novel effector of PKC delta in the apoptotic response to DNA damage. Biochim Biophys Acta 1809:285–294

Hu Y, Kang C, Philp RJ et al (2007) PKC delta phosphorylates p52ShcA at Ser29 to regulate ERK activation in response to H2O2. Cell Signal 19:410–418

Humphries MJ, Limesand KH, Schneider JC et al (2006) Suppression of apoptosis in the protein kinase C delta null mouse in vivo. J Biol Chem 281:9728–9737

Humphries MJ, Ohm AM, Schaack J et al (2008) Tyrosine phosphorylation regulates nuclear translocation of PKC delta. Oncogene 27:3045–3053

Jin H, Kanthasamy A, Anantharam V et al (2011a) Transcriptional regulation of Pro-apoptotic protein kinase C-delta: implications for oxidative stress-induced neuronal cell death. J Biol Chem 286:19840–19859

Jin H, Kanthasamy A, Ghosh A et al (2011b) Alpha-synuclein negatively regulates protein kinase C delta expression to suppress apoptosis in dopaminergic neurons by reducing p300 histone acetyltransferase activity. J Neurosci 31:2035–2051

Johnson LN, Noble ME, Owen DJ (1996) Active and inactive protein kinases: structural basis for regulation. Cell 85:149–158

Joseloff E, Cataisson C, Aamodt H et al (2002) Src family kinases phosphorylate protein kinase C delta on tyrosine residues and modify the neoplastic phenotype of skin keratinocytes. J Biol Chem 277:12318–12323

Kajimoto T, Ohmori S, Shirai Y et al (2001) Subtype-specific translocation of the delta subtype of protein kinase C and its activation by tyrosine phosphorylation induced by ceramide in HeLa cells. Mol Cell Biol 21:1769–1783

Kajimoto T, Shirai Y, Sakai N et al (2004) Ceramide-induced apoptosis by translocation, phosphorylation, and activation of protein kinase C delta in the Golgi complex. J Biol Chem 279:12668–12676

Kajimoto T, Sawamura S, Tohyama Y et al (2010) Protein kinase C {delta}-specific activity reporter reveals agonist-evoked nuclear activity controlled by Src family of kinases. J Biol Chem 285:41896–41910

Kambhampati S, Li Y, Verma A et al (2003) Activation of protein kinase C delta by all-trans-retinoic acid. J Biol Chem 278: 32544–32551

Kanthasamy AG, Kitazawa M, Kanthasamy A et al (2003) Role of proteolytic activation of protein kinase C delta in oxidative stress-induced apoptosis. Antioxid Redox Signal 5:609–620

Kato K, Yamanouchi D, Esbona K et al (2009) Caspase-mediated protein kinase C-delta cleavage is necessary for apoptosis of vascular smooth muscle cells. Am J Physiol Heart Circ Physiol 297:H2253–H2261

Kikkawa U, Matsuzaki H, Yamamoto T (2002) Protein kinase C delta (PKC delta): activation mechanisms and functions. J Biochem 132:831–839

Kim JD, Seo KW, Lee EA et al (2011) A novel mouse PKC delta splice variant, PKC deltaIX, inhibits etoposide-induced apoptosis. Biochem Biophys Res Commun 410:177–182

Konishi H, Tanaka M, Takemura Y et al (1997) Activation of protein kinase C by tyrosine phosphorylation in response to H2O2. Proc Natl Acad Sci USA 94:11233–11237

Konishi H, Yamauchi E, Taniguchi H et al (2001) Phosphorylation sites of protein kinase C delta in H2O2-treated cells and its activation by tyrosine kinase in vitro. Proc Natl Acad Sci USA 98:6587–6592

Kronfeld I, Kazimirsky G, Lorenzo PS et al (2000) Phosphorylation of protein kinase C delta on distinct tyrosine residues regulates specific cellular functions. J Biol Chem 275:35491–35498

LaGory EL, Sitailo LA, Denning MF (2010) The protein kinase C delta catalytic fragment is critical for maintenance of the G2/M DNA damage checkpoint. J Biol Chem 285:1879–1887

Lasfer M, Davenne L, Vadrot N et al (2006) Protein kinase PKC delta and c-Abl are required for mitochondrial apoptosis induction by genotoxic stress in the absence of p53, p73 and Fas receptor. FEBS Lett 580:2547–2552

Le Good JA, Ziegler WH, Parekh DB et al (1998) Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. Science 281:2042–2045

Lee YJ, Soh JW, Dean NM et al (2002) Protein kinase C delta overexpression enhances radiation sensitivity via extracellular regulated protein kinase 1/2 activation, abolishing the radiation-induced G(2)-M arrest. Cell Growth Differ 13:237–246

Lee YJ, Lee DH, Cho CK et al (2005) HSP25 inhibits protein kinase C delta-mediated cell death through direct interaction. J Biol Chem 280:18108–18119

Lee SJ, Kim DC, Choi BH et al (2006) Regulation of p53 by activated protein kinase C-delta during nitric oxide-induced dopaminergic cell death. J Biol Chem 281:2215–2224

Leibersperger H, Gschwendt M, Gernold M et al (1991) Immunological demonstration of a calcium-unresponsive protein kinase C of the delta-type in different species and murine tissues predominance in epidermis. J Biol Chem 266:14778–14784

Leitges M, Mayr M, Braun U et al (2001) Exacerbated vein graft arteriosclerosis in protein kinase C delta-null mice. J Clin Invest 108:1505–1512

Leverrier S, Vallentin A, Joubert D (2002) Positive feedback of protein kinase C proteolytic activation during apoptosis. Biochem J 368(Pt 3):905–913

Li W, Zhang J, Bottaro DP et al (1997) Identification of serine 643 of protein kinase C-delta as an important autophosphorylation site for its enzymatic activity. J Biol Chem 272:24550–24555

Li L, Lorenzo PS, Bogi K et al (1999) Protein kinase C delta targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector. Mol Cell Biol 19: 8547–8558

Li J, O'connor KL, Greeley GH Jr et al (2005) Myristoylated alanine-rich C kinase substrate-mediated neurotensin release via protein kinase C-delta downstream of the Rho/ROK pathway. J Biol Chem 280:8351–8357

Liu J, Chen J, Dai Q et al (2003a) Phospholipid scramblase 3 is the mitochondrial target of protein kinase C delta-induced apoptosis. Cancer Res 63:1153–1156

Liu J, Dai Q, Chen J et al (2003b) Phospholipid scramblase 3 controls mitochondrial structure, function, and apoptotic response. Mol Cancer Res 1:892–902

Liu W, Zhu YS, Guo M et al (2007) Therapeutic efficacy of NSC606985, a novel camptothecin analog, in a mouse model of acute promyelocytic leukemia. Leuk Res 31:1565–1574

Liu X, Yuan J, Wu AW et al (2010) Phorbol ester-induced human cytomegalovirus major immediate-early (MIE) enhancer activation through PKC-delta, CREB, and NF-kappaB desilences MIE gene expression in quiescently infected human pluripotent NTera2 cells. J Virol 84:8495–8508

Lu Z, Liu D, Hornia A et al (1998) Activation of protein kinase C triggers its ubiquitination and degradation. Mol Cell Biol 18: 839–845

Lu ZG, Liu H, Yamaguchi T et al (2009) Protein kinase C delta activates RelA/p65 and nuclear factor-kappaB signaling in response to tumor necrosis factor-alpha. Cancer Res 69:5927–5935

Majumder PK, Pandey P, Sun X et al (2000) Mitochondrial translocation of protein kinase C delta in phorbol ester-induced cytochrome *c* release and apoptosis. J Biol Chem 275:21793–21796

Majumder PK, Mishra NC, Sun X et al (2001) Targeting of protein kinase C delta to mitochondria in the oxidative stress response. Cell Growth Differ 12:465–470

Martins LM, Kottke TJ, Kaufmann SH et al (1998) Phosphorylated forms of activated caspases are present in cytosol from HL-60 cells during etoposide-induced apoptosis. Blood 92:3042–3049

Matassa AA, Carpenter L, Biden TJ et al (2001) PKC delta is required for mitochondrial-dependent apoptosis in salivary epithelial cells. J Biol Chem 276:29719–29728

Mayr M, Chung YL, Mayr U et al (2004a) Loss of PKC-delta alters cardiac metabolism. Am J Physiol Heart Circ Physiol 287:H937–H945

Mayr M, Metzler B, Chung YL et al (2004b) Ischemic preconditioning exaggerates cardiac damage in PKC-delta null mice. Am J Physiol Heart Circ Physiol 287:H946–H956

Murriel CL, Churchill E, Inagaki K et al (2004) Protein kinase C delta activation induces apoptosis in response to cardiac ischemia and reperfusion damage: a mechanism involving BAD and the mitochondria. J Biol Chem 279:47985–47991

Newton AC (2001) Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. Chem Rev 101:2353–2364

Newton AC (2009) Lipid activation of protein kinases. J Lipid Res 50(Suppl):S266–S271

Panaretakis T, Laane E, Pokrovskaja K et al (2005) Doxorubicin requires the sequential activation of caspase-2, protein kinase C delta, and c-Jun NH2-terminal kinase to induce apoptosis. Mol Biol Cell 16:3821–3831

Parekh D, Ziegler W, Yonezawa K et al (1999) Mammalian TOR controls one of two kinase pathways acting upon nPKC delta and nPKC epsilon. J Biol Chem 274:34758–34764

Parent N, Scherer M, Liebisch G et al (2011) Protein kinase C-delta isoform mediates lysosome labilization in DNA damage-induced apoptosis. Int J Oncol 38:313–324

Park SK, Hwang YS, Park KK et al (2009) Kalopanaxsaponin A inhibits PMA-induced invasion by reducing matrix metalloproteinase-9 via PI3 K/Akt- and PKC delta-mediated signaling in MCF-7 human breast cancer cells. Carcinogenesis 30:1225–1233

Park CH, Choi BH, Jeong MW et al (2011) Protein kinase C delta regulates vaccinia related kinase 1 in DNA damage induced apoptosis. Mol Biol Cell 22:1398–1408

Podar K, Raab MS, Zhang J et al (2007) Targeting PKC in multiple myeloma: in vitro and in vivo effects of the novel, orally available small-molecule inhibitor enzastaurin (LY317615.HCl). Blood 109:1669–1677

Qi X, Mochly-Rosen D (2008) The PKC delta-Abl complex communicates ER stress to the mitochondria—an essential step in subsequent apoptosis. J Cell Sci 121(Pt 6):804–813

Qi X, Disatnik MH, Shen N et al (2011) Aberrant mitochondrial fission in neurons induced by protein kinase C{delta} under oxidative stress conditions in vivo. Mol Biol Cell 22:256–265

Reyland ME, Anderson SM, Matassa AA et al (1999) Protein kinase C delta is essential for etoposide-induced apoptosis in salivary gland acinar cells. J Biol Chem 274:19115–19123

Rotolo JA, Zhang J, Donepudi M et al (2005) Caspase-dependent and -independent activation of acid sphingomyelinase signaling. J Biol Chem 280:26425–26434

Sabri A, Wilson BA, Steinberg SF (2002) Dual actions of the Galpha(q) agonist Pasteurella multocida toxin to promote cardiomyocyte hypertrophy and enhance apoptosis susceptibility. Circ Res 90:850–857

Scheel-Toellner D, Pilling D, Akbar AN et al (1999) Inhibition of T cell apoptosis by IFN-beta rapidly reverses nuclear translocation of protein kinase C-delta. Eur J Immunol 29:2603–2612

Senchenkov A, Litvak DA, Cabot MC (2001) Targeting ceramide metabolism—a strategy for overcoming drug resistance. J Natl Cancer Inst 93:347–357

Sitailo LA, Tibudan SS, Denning MF (2004) Bax activation and induction of apoptosis in human keratinocytes by the protein kinase C delta catalytic domain. J Invest Dermatol 123:434–443

Sitailo LA, Tibudan SS, Denning MF (2006) The protein kinase C delta catalytic fragment targets Mcl-1 for degradation to trigger apoptosis. J Biol Chem 281:29703–29710

Soltoff SP (2007) Rottlerin: an inappropriate and ineffective inhibitor of PKC delta. Trends Pharmacol Sci 28:453–458

Song MG, Gao SM, Du KM et al (2005) Nanomolar concentration of NSC606985, a camptothecin analog, induces leukemic-cell apoptosis through protein kinase C delta-dependent mechanisms. Blood 105:3714–3721

Steinberg SF (2004) Distinctive activation mechanisms and functions for protein kinase C delta. Biochem J 384(Pt 3):449–459

Stempka L, Girod A, Muller HJ et al (1997) Phosphorylation of protein kinase C delta (PKC delta) at threonine 505 is not a prerequisite for enzymatic activity. Expression of rat PKC delta and an alanine 505 mutant in bacteria in a functional form. J Biol Chem 272:6805–6811

Sumitomo M, Ohba M, Asakuma J et al (2002) Protein kinase C delta amplifies ceramide formation via mitochondrial signaling in prostate cancer cells. J Clin Invest 109:827–836

Symonds JM, Ohm AM, Carter CJ et al (2011) Protein Kinase C delta Is a Downstream Effector of Oncogenic K-ras in Lung Tumors. Cancer Res 71:2087–2097

Thamilselvan V, Menon M, Thamilselvan S (2009) Oxalate-induced activation of PKC-alpha and -delta regulates NADPH oxidase-mediated oxidative injury in renal tubular epithelial cells. Am J Physiol Renal Physiol 297:F1399–F1410

Von Burstin VA, Xiao L, Kazanietz MG (2010) Bryostatin 1 inhibits phorbol ester-induced apoptosis in prostate cancer cells by differentially modulating protein kinase C (PKC) delta translocation and preventing PKC delta-mediated release of tumor necrosis factor-alpha. Mol Pharmacol 78:325–332

Voss OH, Kim S, Wewers MD et al (2005) Regulation of monocyte apoptosis by the protein kinase C delta-dependent phosphorylation of caspase-3. J Biol Chem 280:17371–17379

Wang H, Xiao L, Kazanietz MG (2011) p23/Tmp21 associates with protein kinase C{delta} (PKC{delta}) and modulates its apoptotic function. J Biol Chem 286:15821–15831

White MC, Gao R, Xu W et al (2010) Inactivation of hnRNP K by expanded intronic AUUCU repeat induces apoptosis via translocation of PKC delta to mitochondria in spinocerebellar ataxia 10. PLoS Genet 6:e1000984

Xie Y (2010) Structure, assembly and homeostatic regulation of the 26S proteasome. J Mol Cell Biol 2:308–317

Yoshida K, Miki Y, Kufe D (2002) Activation of SAPK/JNK signaling by protein kinase C delta in response to DNA damage. J Biol Chem 277:48372–48378

Yoshida K, Wang HG, Miki Y et al (2003) Protein kinase C delta is responsible for constitutive and DNA damage-induced phosphorylation of Rad9. EMBO J 22:1431–1441

Yoshida K, Yamaguchi T, Shinagawa H et al (2006a) Protein kinase C delta activates topoisomerase IIalpha to induce apoptotic cell death in response to DNA damage. Mol Cell Biol 26:3414–3431

Yoshida K, Liu H, Miki Y (2006b) Protein kinase C delta regulates Ser46 phosphorylation of p53 tumor suppressor in the apoptotic response to DNA damage. J Biol Chem 281:5734–5740

Yuan ZM, Utsugisawa T, Ishiko T et al (1998) Activation of protein kinase C delta by the c-Abl tyrosine kinase in response to ionizing radiation. Oncogene 16:1643–1648

Zeidan YH, Wu BX, Jenkins RW et al (2008) A novel role for protein kinase C delta-mediated phosphorylation of acid sphingomyelinase in UV light-induced mitochondrial injury. FASEB J 22:183–193

Zhang G, Kazanietz MG, Blumberg PM et al (1995) Crystal structure of the cys2 activator-binding domain of protein kinase C delta in complex with phorbol ester. Cell 81:917–924

Zhao KW, Li X, Zhao Q et al (2004) Protein kinase C delta mediates retinoic acid and phorbol myristate acetate-induced phospholipid scramblase 1 gene expression: its role in leukemic cell differentiation. Blood 104:3731–3738

Zhao KW, Li D, Zhao Q et al (2005) Interferon-alpha-induced expression of phospholipid scramblase 1 through STAT1 requires the sequential activation of protein kinase C delta and JNK. J Biol Chem 280:42707–42714

Zhao M, Duan XF, Zhao XY et al (2009) Protein kinase C delta stimulates proteasome-dependent degradation of C/EBPalpha during apoptosis induction of leukemic cells. PLoS ONE 4:e6552

Zheng Y, Wang LS, Xia L et al (2009) NDRG1 is down-regulated in the early apoptotic event induced by camptothecin analogs: the potential role in proteolytic activation of PKC delta and apoptosis. Proteomics 9:2064–2075

Zrachia A, Dobroslav M, Blass M et al (2002) Infection of glioma cells with Sindbis virus induces selective activation and tyrosine phosphorylation of protein kinase C delta. Implications for Sindbis virus-induced apoptosis. J Biol Chem 277:23693–23701