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Review

The isoform-specific regulation of apoptosis by protein kinase C

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Abstract. The process of apoptosis is regulated at several levels through phosphorylation by many different protein kinases. The protein kinase C (PKC) family, which comprises at least 10 isoforms with distinct means of regulation and tissue distribution patterns, have been shown to exert both inhibitory and stimulatory influences on apoptosis. This review details recent progress made in determining the roles played by individual PKC isoforms in the control of apoptosis, with reference to their target substrates and actions in different cell types. Although notable exceptions exist, the weight of evidence indicates that the α, β, ε and atypical isoforms are anti-apoptotic in their action, whereas the δ and θ isoforms are usually involved in the promotion of apoptosis.

Key words. Caspase; mitochondria; Bcl-2; cell death; phosphorylation; phorbol ester; diacylglycerol.

Introduction

Apoptosis is the physiological process of cell suicide that occurs normally during development or as a stress response, for example to agents that damage DNA beyond the capacity of repair mechanisms [1]. Deregulation of apoptosis is a hallmark of many diseases such as cancer, acquired immunodeficiency syndrome (AIDS) and some neurodegenerative disorders [2]. The biochemical mechanisms involved in the initiation and execution of apoptosis have been extensively studied and are now extremely well understood at the molecular level. During the course of these studies it has emerged that components of apoptotic signalling pathways are regulated by phosphorylation, and in many cases the protein kinases responsible for these modifications have been identified. For example, early studies with tumour-promoting phorbol esters highlighted a major role for protein kinase C (PKC) in regulating apoptosis. More recently, the roles played by specific members of the PKC family have begun to emerge. The purpose of this article is to review recent information regarding the pro- and anti-apoptotic characteristics of individual PKC isoforms with reference to substrates that participate in the apoptotic process. We begin with brief overviews on the biochemistry of apoptosis and the PKC family. For more detailed appraisals many excellent reviews have recently been published [3–8].

Apoptosis

Apoptosis can be initiated by various stimuli, including genotoxic agents that cause DNA damage, loss of extracellular survival factors or activation of death receptors [9]. Despite this diversity in the ways in which apoptosis can be initiated, the ultimate morphological and biochemical characteristics of the process are identical. Thus, apoptotic cells are distinguished by disruption and blebbing of their cell membranes, a decrease in cell volume, nuclear condensation and intranucleosomal cleavage of DNA. The final stage of apoptosis is characterised by degradation of DNA into 180-bp mononucleosomal

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fragments, condensation of organelles into apoptotic bodies and the loss of lipid asymmetry in the plasma membrane. Apoptotic bodies are phagocytosed by neighbouring cells or professional phagocytes to prevent secondary necrosis and inflammation.

Central to the execution of the suicide program are the cysteine-dependent aspartate-directed proteases, so called caspases [4]. Caspases are synthesised as inactive zymogens that are activated by cleavage of the pro-form to generate smaller fragments which dimerise to form the active enzyme. As well as cleaving other proteins, caspases activate one another, resulting in a cascade of caspase activation. In general, initiator caspases, such as caspases-8 and -10, which act at the apex of the cascade contain long pro-domains, whilst effector caspases, such as caspases-3, -7 and -6 contain short prodomains. At present, there are two well-understood mechanisms of caspase activation: activation following ligation of cell surface death receptors such as CD95 (also known as Fas) or tumour necrosis factor receptor 1 (TNF-R1) and caspase activation via the mitochondrial pathway.

Like other members of the TNF-receptor superfamily, CD95 is a transmembrane protein containing a region in the cytoplasmic tail known as the death domain (DD) [10]. This region is required for interaction with the adaptor molecule Fas-associated death domain (FADD) protein, which also contains a DD. FADD binds to the DD of caspase-8, thus recruiting this caspase to the CD95 receptor complex. The physiological ligand for CD95 is CD95 ligand (CD95L). CD95L expression is restricted mainly to activated cytotoxic T cells and sites of immune privelege such as the eye and testis. When CD95L binds CD95, both molecules trimerise. This is an absolute requirement for CD95-induced apoptosis, since CD95 monomers and dimers are inactive. CD95 ligation causes FADD and caspase-8 to associate and form the so-called death-inducing signalling complex (DISC). Upon recruitment to the DISC, pro-caspase-8 undergoes cleavage by a neighbouring caspase-8, releasing active caspase-8 into the cytosol. Active caspase-8 then activates caspase-3, either by direct processing or through the mitochondrial pathway (see fig. 1).

Early work did not point to a role for the mitochondria in apoptosis, since mitochondrial changes were more apparent during necrosis, when they became swollen. In addition, cells that lack mitochondrial DNA are capable of undergoing apoptosis. However, it is now recognised that mitochondria play a key role in the apoptotic program and contain many apoptogenic factors, including cytochrome-c, caspases-2, -3 and -9 and apoptosis protease-activating factor-1 (Apaf-1) [10, 11]. Mitochondria release these apoptogenic products upon mitochondrial membrane depolarisation. This occurs when pro-apoptotic members of the Bcl-2 family, such as Bid, form pores in the mitochondrial membrane [2, 11]. The Bcl-2 family of proteins consist of both pro-apoptotic and anti-apoptotic members [11]. The actions of pro-apoptotic members, such as Bax, Bad, Bak and Bim, are counteracted by the presence of anti-apoptotic members such as Bcl-2 and Bcl-X_L. Pro- and anti-apoptotic Bcl-2 proteins are able to heterodimerize and thus suppress each other's function, and it is a widely held belief that the relative expression of pro- and anti-apoptotic Bcl-2 proteins is an important determinant of cell fate. Both pro- and anti-apoptotic proteins are also subject to posttranslational modifications, which affects their death or survival-promoting function. For example, phosphorylation of Bad by protein kinases such as PKB/Akt, which are activated in response to survival stimuli, suppresses its pro-apoptotic function by causing its sequestration away from the mitochondrial membrane [11]. Caspases activated as a result of death receptor stimulation also modify Bcl-2 family protein function. For example, cleavage of Bid by caspase-8 promotes its interaction with Bax and the formation of a channel in the mitochondrial membrane. This leads to the release of Apaf-1, cytochrome-c and caspase-9, and formation of the apoptosome, which in turn activates caspase-9, allowing it to proteolytically activate the effector caspase-3 (fig. 1). Caspase-3 then cleaves a host of cytosolic and nuclear substrate proteins, which are responsible for maintaining cell survival and DNA integrity [3].

The PKC family

Isoform classification

PKC is a family of serine/threonine protein kinases that regulate various cellular functions, including proliferation, differentiation and apoptosis. The 10 identified PKC isoforms are divided into three subfamilies based on their structure and the cofactors required for optimal activation [8]. Phosphatidylserine (PS), a negatively charged membrane phospholipid, is required for activation of all isoforms. The atypical (aPKC) isotypes (ζ and λ/ι) are dependent solely on PS for their activation, although other lipids and cofactors may modulate their function. The conventional or classical PKCs (cPKCs: α, β_I, β_{II} and γ) and the novel PKCs (nPKC: δ, ε, η and θ) are optimally activated by the additional binding of diacylglyerol (DAG), a lipid that is produced from the hydrolysis of membrane inositol phospholipids by phospholipase C. However, cPKC and nPKC isoforms differ by binding DAG in a calcium-dependent and -independent manner, respectively. In addition to the conventional, novel and atypical PKC isoforms, a number of proteins are sometimes classified as PKCs, such as PKCμ (also known as PKD) and PKCυ, although these enzymes have structural or functional characteristics that distinguish them from all other PKCs [12].

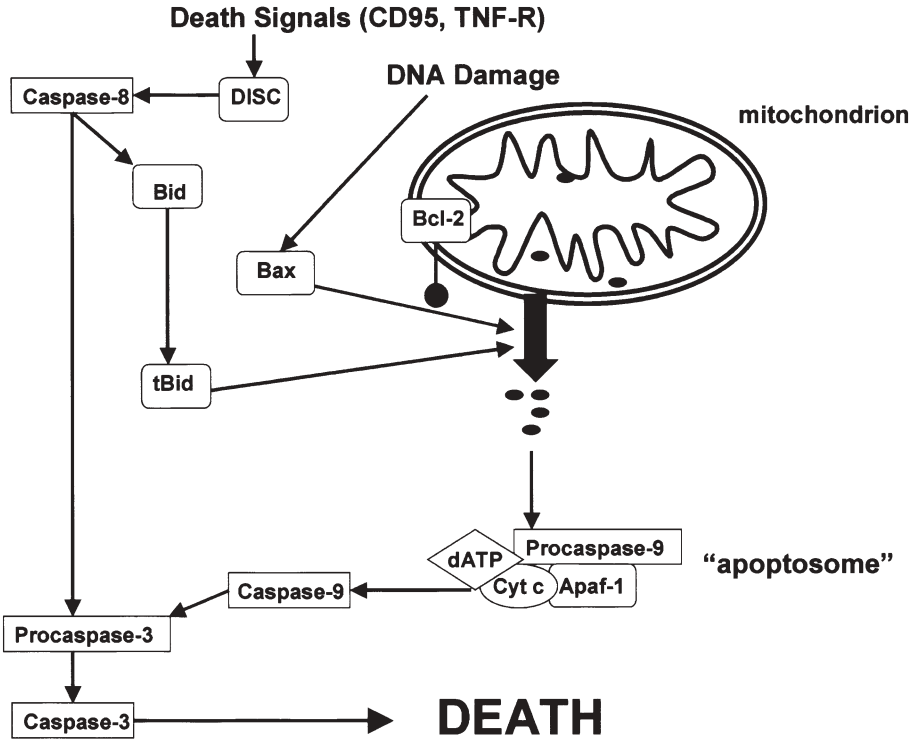

Figure 1. Caspase activation by mitochondrion-dependent and -independent pathways. The two major routes leading to the activation of effector caspases in apoptosis. Pro-apoptotic Bcl-2 family members such as Bax or Bid promote apoptosis via the mitochondrial pathway. This involves mitochondrial membrane depolarisation and release of proteins, which subsequently form a complex known as the apoptosome. As well as promoting mitochondrial-dependent apoptosis, death signals that act via cell surface receptors such as CD95 or the TNF-receptor activate caspases directly. Only the major pathways and events are shown for clarity. *DISC*, death-inducing signalling complex.

Structural features

The differential dependence on cofactors for optimal activation can be explained in terms of differences in the protein structures of the subfamilies (fig. 2). The overall PKC isoform structure comprises four conserved domains (C1–C4) and five variable domains (V1–V5). The regulatory domain includes the C1 and C2 regions as well as a pseudo-substrate region that interacts with the catalytic domain, thereby maintaining the enzyme in an inactive state within the cytosol [13, 14]. The C1 domain contains two cysteine-rich zinc fingers that constitute the binding site for DAG and DAG mimics such as phorbol esters and bryostatin-1. aPKCs have only one zinc finger present in the C1 domain, thus explaining their inability to be activated by DAG or phorbol esters. The C2 domain binds calcium and is absent in nPKC and aPKC isozymes. The regulatory domain is also responsible for the binding of receptors for activated C kinases (RACKs), anchoring proteins that determine the ultimate sub-cellular locations of PKCs after activation [15]. The catalytic segment contains the C3 and C4 domains, which function as the ATP binding site and the kinase catalytic centre, respectively. The variable domain, V3, is the hinge region where the PKC isoforms are cleaved by caspases into separate catalytic and regulatory domains.

PKC activation

In order for PKC to become activated by DAG, the protein must first be primed by phosphorylation on a number of critical residues, a process that occurs during or after translocation to the plasma membrane [16]. Three conserved sites in the catalytic domain are phosphorylated. The first phosphorylation step, in which a residue in the activation loop of the carboxy terminus is modified, is catalysed by 3-phosphoinositide-dependent kinase (PDK-1). Phosphorylation of this conserved turn motif triggers autophosphorylation on a site in the hydrophobic region, conserved in cPKC and nPKC isoforms, an event that is most likely coupled to kinase activation [6]. The final phosphorylation step releases the enzyme into the cytosol in a conformation in which the pseudo-substrate region masks catalytic activity.

Activation of phospholipase C (PLC), of which there are several subtypes, through the actions of extracellular stimuli acting on cell surface receptors leads to hydrolysis of inositol-containing phospholipids and the generation of DAG in the plasma membrane (fig. 3). This provokes the translocation of inactive PKC to the membrane, where it binds PS and DAG (in the case of cPKCs and nPKCs). The energy that is produced from the binding of PKC to these molecules is sufficient for the displacement

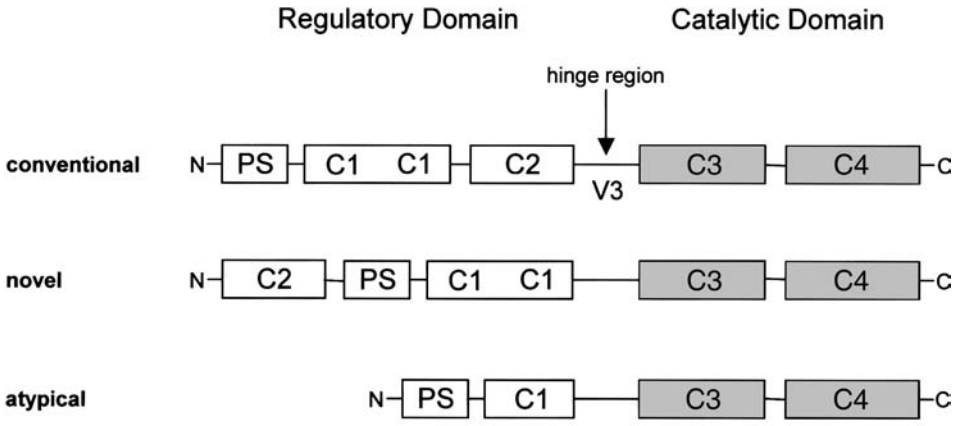

Figure 2. Main structural features of the PKC family. All PKC family members comprise four conserved domains (C1–4) and five variable (V) domains. Only the V3 domain, which is the site of proteolytic cleavage, is shown for clarity. Immediately N terminal to the C1 region all isoforms contain a pseudosubstrate domain (PS) that maintains PKC in a catalytically inactive form. The C1 domains bind diacylglycerol (and calcium in the case of conventional PKCs). Atypical PKCs have only one C1 domain and are unable to bind diacylglycerol. The catalytic domain comprises the ATP binding site (within C3) and substrate binding site (within C4). Full and further details are provided within the text.

of the pseudo-substrate and the consequential activation of the PKC isoform [8]. After activation, PKCs bind to the isoform-specific RACKs that determine PKC localisation and, accordingly, this distribution partly controls which molecules are ultimately targeted by PKC.

PKC and apoptosis

The initial results of studies that aimed to establish the role of PKC in apoptotic signalling pathways were very contradictory. Activation of PKC was capable of producing apoptosis in particular cell types while preventing it in others, thereby making it difficult to predict a specific role for PKC in the apoptotic process. For example, DNA fragmentation and apoptosis induced by calcium ionophore or glucocorticoids in thymocytes was prevented on activation of PKC by phorbol ester [17], suggesting that PKC stimulation inhibits apoptosis. On the other hand, activation of PKC by phorbol esters, also in thymocytes, enhanced the DNA fragmentation induced by tyrosine-kinase inhibitors, implying that PKC promotes apoptosis [18]. The contradictory nature of these results occurred primarily from the use of phorbol esters as PKC activators. Phorbol esters can stimulate both cPKC and nPKC isoforms, but prolonged treatment of cells with these agents results in PKC downregulation and cellular depletion. In addition, the use of different cell types with widely divergent PKC isoform expression profiles confounded simple interpretation of results from studies of this type. It is now clear that there are isoform-specific roles for PKC in the regulation of apoptosis. Thus, the PKC-dependent promotion or suppression of apoptosis occurs as a function of the isoform(s) that are activated and the cell type under investigation.

With the discovery and development of more isoform-specific activators and inhibitors, increased precision in the targeting of PKC isoforms has become possible [6]. The results of studies employing such agents have led to a generalized consensus over the roles of PKC isoforms as regulators of apoptosis. Thus, conventional and atypical PKCs are generally considered to be predominantly anti-apoptotic, being principally involved in promoting cell survival and proliferation. The novel PKCs, however, generally have a tumour suppressor function and are regarded as pro-apoptotic proteins. PKCα and PKCδ are two of the PKCs better characterised with respect to their important functions in preventing and promoting apoptosis, respectively. It is interesting that these two protein kinases have been demonstrated as mediating key roles in apoptosis, as these isoforms are present in the majority of cells [19].

Anti-apoptotic PKC isoforms

PKCα has emerged as an important isoform in promoting cell survival. In several cell lines, including endothelial cells [20] and glioma cells [21, 22], apoptosis was induced as a result of cellular PKCα depletion using antisense oligonucleotides or phorbol ester-mediated downregulation. This was consistent with the apoptotic response that was induced by expressing a dominant-negative form of PKCα in both COS1 cells [23] and salivary gland epithelial cells [24], and with the increased PKCα expression that is observed in many cancer cell types. Although the majority of published work suggests a suppressive role for PKCα in apoptosis, conflicting data indicating a pro-

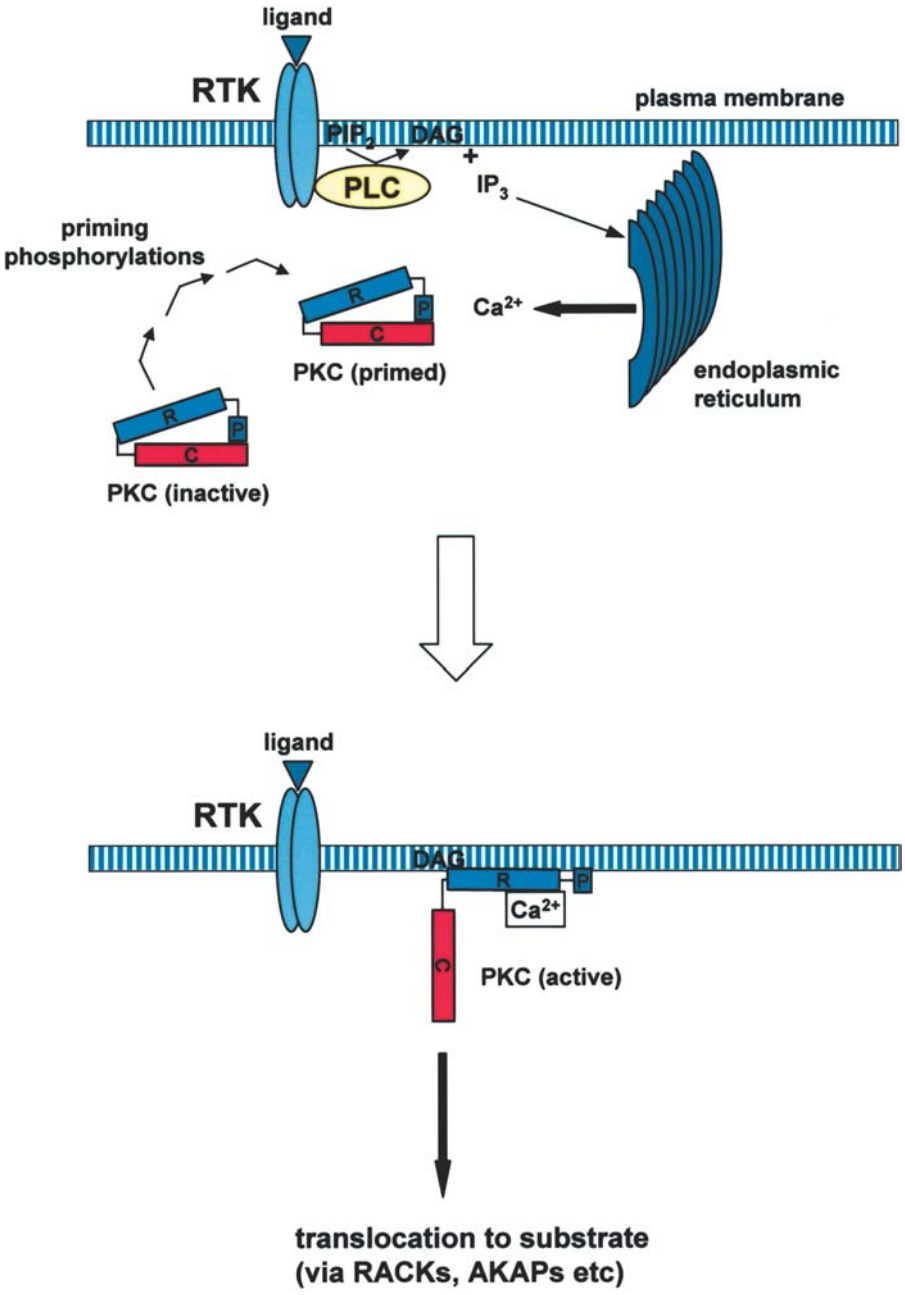

Figure 3. PKC activation. Following its initial synthesis, PKC undergoes a series of phosphorylations which do not activate the enzyme, but prime it for activation by second messengers. Ligation of receptor tyrosine kinases (RTKs) or other cell surface receptors leads to activation of phosphoinositide-specific phospholipase C (PLC). PLC cleaves phosphotidylinositol-4,5-bisphosphate (PIP_2) to form diacylglycerol (DAG) and inositol trisphosphate (IP_3). IP_3 binds to receptors on the endoplasmic reticulum and promotes release of Ca^{2+}. DAG (and Ca^{2+} in the case of cPKCs) induce conformational changes in PKC at the plasma membrane, releasing it from its inhibitory conformation and increasing catalytic activity. Following its activation, PKC translocates to other subcellular locations and binds to specific anchoring proteins, RACKs and AKAPs.

apoptotic function have still been observed. In human prostate cancer cell lines, the presence of PKCα in the mitochondrial membrane was associated with apoptosis, while its absence corresponded to resistance [25]. This pro-apoptotic role was in agreement with results from epithelial cell lines of the tonsil: the cytoplasm normally expresses PKC α, β, δ, ε and ζ isoforms, but during apoptosis, the levels of PKCα and PKCβ were increased [26]. More recently, PKCα was shown to mediate activation of caspase-3 downstream of cytochrome c release in renal proximal tubule cells treated with the DNA-damaging agent cisplatin [27].

The mechanism by which PKCα prevents apoptosis is only partly known. One target that has been identified is

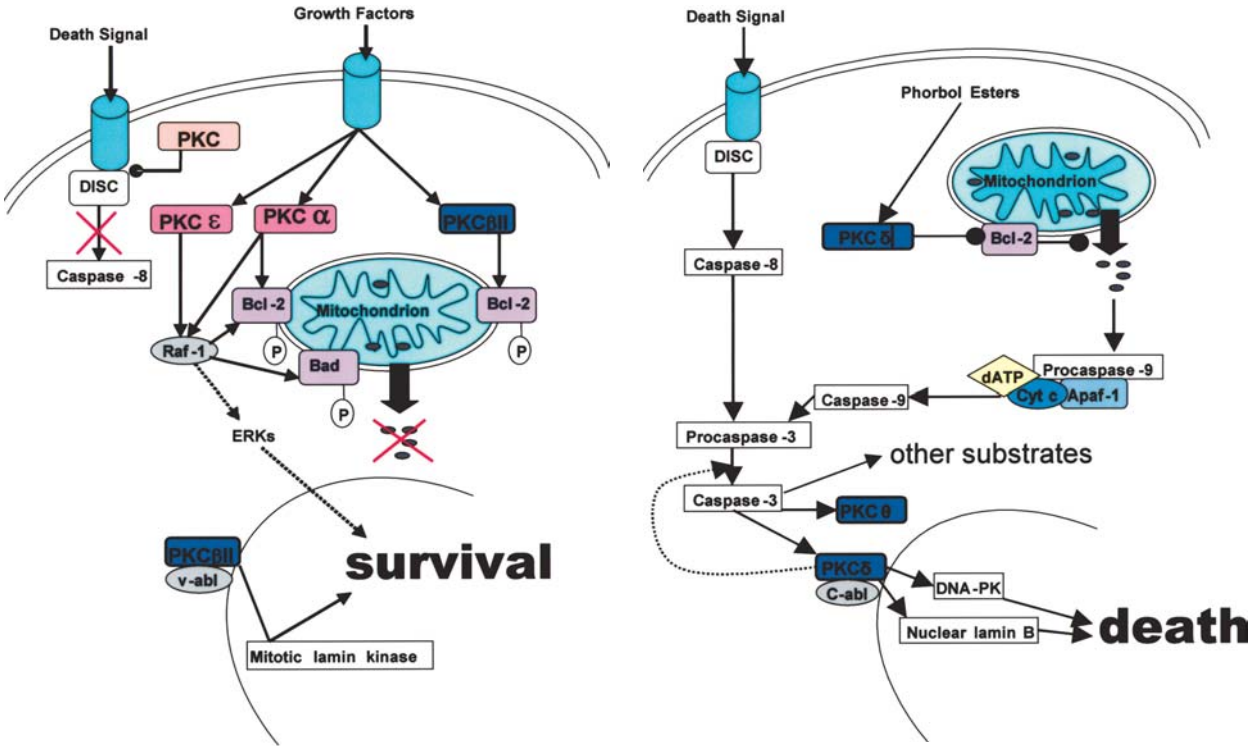

Figure 4. Regulation of apoptosis by PKC isoforms. Targets for isoforms of PKC having an established role in preventing (*left picture*) or promoting (*right picture*) apoptosis. For clarity only major pathways are shown. Full and further details are provided in the text.

the anti-apoptotic Bcl-2 protein. In HL-60 cells, PKCα co-localised with Bcl-2 in the mitochondria [28], while experiments with murine growth factor-dependent cell lines demonstrated that PKCα phosphorylated Bcl-2 on ser70 [29]. Phosphorylation of this site had the effect of stabilising Bcl-2 and enhancing its ability to prevent apoptosis. Another possible target for PKCα is the serine/threonine protein kinase Raf-1. Raf-1 has been shown to mediate the anti-apoptotic function of PKB/Akt in haematopoeitic cells, through a mechanism that is PKC dependent [30]. The activation of Raf-1 in this way targets it to the mitochondrial membrane through an interaction with Bcl-2. Once in the mitochondrial membrane Raf-1 is believed to phosphorylate and inactivate the pro-apoptotic protein BAD. Although Raf-1 is activated by PKCα in vitro, it can similarly be activated by PKCs δ, ε and ζ [31]. Moreover, selective downregulation of cellular PKCα by phorbol esters did not concomitantly inhibit Raf-1. Other studies have shown that nPKCε is primarily responsible for phosphorylating and activating Raf-1 [32]. Thus, the PKC isoforms responsible for Raf-1 activation in the context of mitochondrial targeting are still unclear. It is also worth mentioning that as Raf-1 is a target for PKC, it is likely, at least in some cell contexts, that PKC promotes cell survival by activating the extracellular signal-regulated kinase (ERK) pathway [33].

PKCs may also prevent apoptosis by modulating the function of death receptor components. For example, activation of PKC inhibited FADD recruitment and consequent DISC formation, thus preventing apoptosis, in Jurkat T-cells. This effect was prevented by an inhibitor of cPKC [34].

Initial studies demonstrated a role for PKCβ in the promotion of apoptosis [35]. Two PKCβ isoforms exist, PKCβ_{I} and PKCβ_{II}, which are generated from PKCβ by alternative splicing. PKCβ_{II} is thought to be involved in the prevention of apoptosis, although the evidence is somewhat contradictory. The tyrosine kinase c-abl is known to promote apoptosis by inducing the translocation of PKCδ from the cytosol to the nucleus [36]. However, expression of the oncogene v-*abl* causes translocation of PKCβ_{II} to the nucleus, thereby preventing apoptosis and implying that PKCβ_{II} is anti-apoptotic [37]. A mitotic lamin kinase has been identified as a target for PKCβ_{II}, and its interaction with this substrate promotes cell survival and proliferation [38, 39]. It seems likely, though, that in some pathways PKCβ_{II} also functions in a similar manner to PKCα because in ara-C-induced apoptosis of HL-60 cells, PKCβ_{II} had a protective role as a result of increasing the levels of Bcl-2 present in the mitochondrial membrane [40]. A recent study with PKCβ knockout transgenic mice demonstrated an essential role for this isoform in the activation of NFκB and NFκB-de-

pendent survival events in B lymphocytes [41]. Possible pro-apoptotic activity of PKCβ has also been reported. Activation of PKCβ_I by Doppa, which is a selective activator of this isoform in vitro, induced apoptosis in HL60 cells [42], indicating that PKCβ_I and PKCβ_{II} might have opposite roles in the regulation of apoptosis. Recently, PKCβ was demonstrated as being necessary in the targeting of stress-activated protein kinase (SAPK) to the mitochondria and the induction of apoptosis induced by phorbol esters in monocytes [43]. SAPK was shown to interact with and phosphorylate the anti-apoptotic Bcl-2 family member Bcl-x(L) in the mitochondria and thereby promote the release of cytochrome c. Therefore, a pro-apoptotic role for PKCβ, although not specified with regards to PKCβ_I or PKCβ_{II}, also seems to be a possibility in certain cell types.

PKCε is widely regarded as having anti-apoptotic properties. In addition, it is the one PKC isoform consistently demonstrated to be oncogenic (see [6]). The mechanisms responsible for its anti-apoptotic function are unclear, and very little is known about the physiologically relevant substrates of this isoform. In T lymphocytes PKCε was demonstrated to participate in a survival signalling pathway that culminated in the phosphorylation and inactivation of BAD [44]. More recently, PKCε was shown to prevent apoptosis by blocking mitochondrial-dependent caspase activation in lung cancer cells [45].

The atypical PKC isoenzymes are principally anti-apoptotic molecules, although very little is known about their mechanisms of action and physiological targets. PKCι overexpression prevented drug-induced apoptosis in K562 cells, although overexpression of PKCζ had no effect [46]. PKCλ/ι appears to be a downstream target of bcr-abl-mediated resistance to drug-induced apoptosis in these cells [47]. PKCλ/ι also promoted resistance to apoptosis in the absence of the correct extracellular matrix by increasing phosphorylation of p53, thereby enhancing its stability, while a reduction in its expression occurs during apoptosis of U937 cells [35]. In addition, the expression of the par-4 gene product, which is associated with apoptosis, can interact with and inhibit the PKCζ isoform [48]. Caspases cleave PKCζ, causing its activation and subsequent degradation via the ubiquitin-proteasome pathway [49]. Overexpression of PKCζ reduced topoisomerase II catalytic activity, cleavable complex formation and drug-induced cytotoxicity in monocytic U937 leukemia cells [50]. Recent evidence from PKCζ transgenic knockout animals indicates that the anti-apoptotic action of this isoform may be mediated through its regulation of ERK activation and NF-κB transcription [51, 52]. Although most studies indicate that the aPKCs are anti-apoptotic and important for cell growth and survival, there are reports suggesting that PKCζ may inhibit cell survival by inhibiting the pro-survival kinase PKB/Akt [53, 54].

Pro-apoptotic PKC isoforms

PKC isoforms most predominantly associated with apoptosis promotion are the novel PKCs δ and θ, in particular the ubiquitous PKCδ isotype. The activation of PKCδ occurs in response to a variety of stimuli, such as signals initiated by the death receptor [55], ultraviolet (UV) radiation [56] and etoposide, all of which induce apoptosis [57]. In addition, it has been demonstrated that a loss of PKCδ expression is associated with tumour growth [58]. PKCδ is also a substrate for the effector caspase, caspase-3, which proteolytically cleaves PKCδ at a site within the V3 domain to release the catalytically active 40-kDa fragment from the regulatory domain [59]. The kinase active catalytic domain is thought to be essential for apoptosis, as its overexpression in HeLa cells causes apoptosis, whereas no effect is produced when these cells are transfected with a kinase dead fragment [60]. The key targets for PKCδ are located in the nucleus, and it has been shown in HL60 cells [61] and T cells [62] that PKCδ translocates to the nucleus prior to cleavage by caspase-3, and is therefore in its full-length form. Nuclear proteins that are targets for PKCδ include the nuclear structural protein lamin B [61, 63], the catalytic fragment of DNA-dependent protein kinase (DNA-PK) [64], an enzyme that is essential for the repair of DNA double-strand breaks, and the p53 homologue p73 [65]. PKCδ phosphorylates DNA-PK upon activation, thus inactivating it and enhancing DNA damage-induced apoptosis [66]. Other intermediate filaments also constitute substrates for PKCδ [67]. In addition to nuclear targets, it appears that caspase-3 is itself a target for cleaved PKCδ [68], which creates a positive feedback loop with the effect of amplifying downstream events. Recent evidence indicates that proteolytically cleaved, activated forms of PKCε also regulate apoptosis. Their precise role may be context dependent, since one study with rat pituitary adenoma cells showed that it functions in a manner similar to PKCδ, undergoing caspase-3-mediated activation and participating in a positive feedback loop on caspase-3 activation [69], whereas another recent study indicated that cleaved PKCε promotes survival in MCF-7 breast carcinoma cells [70].

There is increasing evidence that PKCδ is involved in a pathway upstream of caspase activation. PKCδ is activated to promote apoptosis in response to phorbol ester, but in LNCaP prostate cancer cells, it was observed that PKCδ activation occurred without prior cleavage by caspase-3 [71]. This implied that an upstream noncleaved PKCδ, as well as a cleaved, activated PKCδ, has a role in promoting apoptosis. Previous studies have shown that on exposure of cells to TPA, PKCδ translocates from the cytosol to nuclear and plasma membranes [72]. Recent evidence demonstrates that activation of PKCδ in keratinocytes and human U937 myeloid leukaemia cells induces its translocation to the mitochondrial membrane,

where it promotes the release of cytochrome c via a change in the mitochondrial membrane potential [73, 74]. This function may occur through an allosteric activation of the PKC isozyme mediated by the PKC activator [75]. The demonstration that full-length PKCδ mediates mitochondrial-dependent apoptosis in salivary epithelial cells, although it is not known whether this is through a direct mechanism of PKCδ on the mitochondria, is consistent with these results [24]. Therefore, full-length PKCδ may regulate early events of apoptosis, while cleaved PKCδ serves in a later amplification stage of apoptosis. This is in agreement with the observation that phorbol ester-induced apoptosis is weaker than that produced by agents that cause the cleavage of PKCδ [57]. Activation and translocation of the full-length isoform or the cleaved isoform may depend on the anchoring protein with which PKCδ is associated. It has been reported that c-abl, a protein tyrosine kinase activated by DNA-damaging agents, is constitutively associated with PKCδ and induces its translocation to the nucleus upon activation [36]. Cellular conditions are also important, as PKCδ binds more weakly to phospholipids than PKCα under conditions of calcium sufficiency [75]. It is worth noting that many studies that have implicated PKCδ in promoting apoptosis have employed the selective chemical inhibitor rottlerin [66]. It has emerged more recently that rottlerin is rather nonspecific in its action and probably inhibits many other protein kinases [76]. Indeed, recent studies indicate that rottlerin may promote apoptosis by effects on mitochondrial function that indirectly inhibit PKCδ [77, 78]. Despite this caveat, available evidence indicates that PKCδ is predominantly pro-apoptotic, functioning at the level of the mitochondria and/or downstream of the caspase cascade. Additionally, the recent identification of PKCδII, a novel PKCδ isoform that is generated by alternative splicing and is insensitive to caspase-3 [79], will add to the complexity of PKCδ function in apoptosis.

PKCθ has also been shown to promote apoptosis and to serve as a substrate for caspase-3. On the other hand, there is evidence that PKCθ can mediate a survival signal that is selective for T cells, occurring through the phosphorylation of BAD via RSK2 [44, 80]. Overall, however, there is limited data concerning the role this isoform plays in apoptosis, as it has a restricted tissue distribution, being confined mostly to skeletal muscle and haemopoietic cells [81].

Conclusions

The PKC family is responsible for regulating a variety of cellular functions in an isoform-specific manner. The finding that PKC is involved in apoptosis introduces the possibility of targeting PKC during the therapeutic intervention of diseases where there is deregulation in the processes of cell survival and cell death such as cancer, inflammatory disease and neurodegenerative disorders. Elevated levels of PKC have been demonstrated in many cancer cells, for example in breast cancers [82] and thyroid cancers [83]. In addition, decreasing PKCα activity, using inhibitors or antisense oligonucleotides, caused inhibition of tumour growth by preventing proliferation or inducing apoptosis. In this regard PKC-α-directed antisense oligonucleotides and PKC-selective inhibitors such as 7-hydroxystaurosporine are already undergoing clinical evaluation as novel anticancer agents [84–86]. However, as discussed in this review, the literature concerning PKC involvement in apoptosis is still contradictory, and the activities of individual isoforms remain to be fully characterised. Such information should lead to the identification of particular isoforms that might be targeted therapeutically. When knockout mice were generated for isoforms β, δ, θ, ε or γ, the consequences were fairly mild and cell/tissue specific, suggesting there could be a degree of functional redundancy among the PKC family. Because of its ubiquitous distribution and important anti-apoptotic role, the phenotype of animals with PKCα deletion may be more informative, as would the targeted deletion of isoforms within particular tissues.

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