The process of apoptosis is regulated at multiple levels through phosphorylation by several different protein kinases. The protein kinase C (PKC) family of isozymes have been shown to exert both inhibitory and stimulatory influences on apoptosis. During the apoptotic process phosphorylation events are known to occur also at the nuclear level. Evidence suggests that PKC isoforms play a key role in some steps that lead to nuclear disassembly during the execution phase of apoptosis. This review highlights the recent progress made in determining the roles played by individual PKC nuclear isoforms in the control of apoptosis.

Key words: nucleus, phosphorylation, proteins, nuclear matrix, nuclear lamins, gene expression.

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isoenzymes are either involved in the characteristic nuclear apoptotic changes, or may protect cells by apoptosis. We begin with brief overviews on the PKC family and nuclear apoptotic modifications.

**The PKC family**

At present 10 PKC isoforms have been cloned. They have been divided into three subfamilies that share a common requirement for phospholipids (phosphatidylserine, PS) for their activity, but differ in structure and dependencies on other activators. Conventional PKCs (cPKCs), $\alpha$, $\beta I$, $\beta II$, $\gamma$, meet the original definition of PKC as a Ca$^{2+}$- and phospholipid-dependent protein kinase. Indeed, they require PS, Ca$^{2+}$, and diacylglycerol (DAG); novel PKCs (nPKCs), $\delta$, $\epsilon$, $\eta$, $\theta$, require only DAG and PS; atypical PKCs (aPKCs), $\zeta$, $\eta\gamma$, are dependent solely on PS. (Hug and Sarre, 1993). The overall structure of PKC isoforms includes four conserved domains (C1-C4) that are interrupted by five variable regions (V1-V5). Each isoform contains a catalytic and a regulatory domain (Hug and Sarre, 1993). The catalytic domain can be active in the absence of cofactors after proteolytic removal of the regulatory domain by cleavage in the V1 (hinge) region. The regulatory domain, which is responsible for dependence on cofactors, contains an autoinhibitory pseudosubstrate region as well as sequences that mediate the interactions of PKC with phospholipids, DAG, and specific anchoring proteins. The Ca$^{2+}$ dependency is mediated by the C1 region (which is indeed absent in nPKCs), while DAG binding requires the presence of two cysteine-rich zinc-finger regions within the C2 domain. aPKCs lack one of the two cysteine-rich zinc-finger regions and therefore do not bind (and cannot be activated by) DAG. (Hug and Sarre, 1993).

**Nuclear apoptotic changes**

Apoptotic cell death was first described by Kerr and coworkers (1972) almost 30 years ago and was distinguished from necrosis exclusively on the basis of morphological criteria such as chromatin condensation and the formation of apoptotic bodies. Subsequently, it has become clear that apoptosis is also characterized by a variety of biochemical changes, which occur in several organelles, including the nucleus. The morphological changes typical of apoptosis are well known: they consist of a collapse of chromatin (which, in many cases adopts a striking crescent or half-moon shape) against the nuclear periphery, a progressive condensation of chromatin, a shrinkage of the entire nucleus into a single ball or, in other cases, chromatin budding outward into smaller balls resembling a cluster of grapes, with each grape being surrounded by nuclear envelope (Earnshaw, 1995). These balls are referred to as apoptotic bodies and some of them contain only DNA whereas others contain only RNA. The nuclear envelope remains morphologically intact even though the nuclear pores redistribute by sliding away from the surface of the condensed chromatin domains and accumulating between them (Earnshaw, 1995; Robertson et al., 2000; Martelli et al., 2001). The apoptotic nuclear changes are conceivably caused by both DNA fragmentation due to the activity of several distinct nucleases (Scovassi and Torriglia, 2003) and proteolysis of key nuclear polypeptides which include, among the others, topoisomerase II, NuMA, lamins, SATB1, SAF-A (Martelli et al., 2001). These proteins are the target of cysteine-dependent aspartate-directed proteases referred to as caspases (Shi, 2002). Many of nuclear proteins which are cleaved during apoptosis belong to the nuclear matrix, a mainly proteinaceous structure which is considered by several investigators to act as a sort of nuclear skeleton (Martelli et al., 2002; Berezney et al., 2003).

The cleavage of nuclear matrix proteins and the DNA fragmentation are interlinked processes that ultimately lead to the nuclear collapse seen during the execution phase of apoptosis (Liu et al., 2003).

**Pro-apoptotic nuclear PKC isoforms**

The first report hinting at a possible involvement of nuclear PKC in apoptosis showed a migration of the kinase (unknown isotype) to the nucleus in dexamethasone-treated thymocytes, a classic model of apoptotic cell death (Trubiani et al., 1994). However, the first direct link between nuclear PKC and apoptosis was provided by Shimizu et al. (1998). In HL60 cells induced to apoptosis by camptothecin, PKC-$\alpha$ phosphorylated lamin B one hour after the addition of the drug and this phenomenon preceded both lamin proteolytic degradation and DNA fragmentation. Several lines of evidence supported an important role for PKC-$\alpha$ in lamin B phosphorylation and subsequent degradation: a) camptothecin stimulated PKC-$\alpha$ activity in HL60 cells; b) both the PKC inhibitor UCN-01 and an anti-PKC antibody prevented lamin B phosphorylation; c) lamin B phosphorylation was restored...
by recombinant PKC-α; and d) if PKC-α was immunodepleted, phosphorylation of lamin B was reduced by about 90%. We have confirmed these data by showing that in camptothecin treatment of HL60 cells, DAG is generated within the nucleus through the action of a phospholipase D. This DAG is essential for the activation of nuclear PKC-α (Martelli et al., 1999). However, these results have been questioned by another group which showed that in HL60 cells exposed to the apoptotic inducer cytarabine, the –δ isozyme of PKC migrated to the nucleus where it co-localized with lamin B (Cross et al., 2000b). Moreover, proteolytic activation of PKC-δ by caspase-3 was concomitant with lamin B phosphorylation and proteolysis, while inhibition of PKC-δ by the selective inhibitor, rottlerin, delayed lamin proteolysis. Taking into account that PKC-δ was also capable of phosphorylating lamin B in vitro, it was concluded that this PKC isoform is an apoptotic lamin kinase and that efficient nuclear lamina disassembly at apoptosis requires lamin B phosphorylation followed by its caspase-6-mediated proteolytic degradation.

A key role played by nuclear PKC-δ during apoptosis has also been shown in C5 rat parotid salivary cells incubated with etoposide (DeVries et al., 2002). Indeed, both the caspase-3-generated, catalytic fragment of endogenous PKC-δ, and the full-length endogenous PKC-δ preferentially accumulated in the nucleus of C5 cells in response to etoposide treatment. Overexpression of PKC-δ catalytic fragment resulted in nuclear localization and apoptosis. The import of the catalytic fragment of PKC-δ was dependent on a novel nuclear localization signal (NLS) located in the carboxyl-terminus. Importantly, mutations within the NLS prevented nuclear accumulation of the catalytic fragment and apoptosis. In this context, it is important to recall that a catalytically active fragment of PKC-δ is generated by proteolysis in cells induced to undergo apoptosis in response to some, but not all, inducers of apoptotic cell death (Kikkawa et al., 2002; Brodie and Blumberg, 2003). The cleavage is presumably effectuated by caspase-3 between aspartic acid 327 and asparagine 328. This cleavage site of PKC-δ is located between two tyrosine residues (311 and 332) which can be phosphorylated. Phosphorylation of Tyr311 promotes degradation of PKC-δ presumably after ubiquitination (Kikkawa et al., 2002). PKC-phosphorylated at Tyr311 may be insensitive to proteolysis by caspase. The catalytic fragment and the tyrosine phosphorylated active form of PKC-δ seem to contribute to promotion of apoptosis independently (Kikkawa et al., 2002). Both the native form and the 40-kDa catalytic fragment of PKC-δ have been found associated with the nuclear matrix of apoptotic cells (Zemskov et al., 2003).

An important role for PKC-δ in apoptosis is also indicated by the fact that it translocates to the nucleus of insulin-secreting RIN1046-38 treated with free fatty acids. Apoptosis induced by this treatment was accompanied by lamin B disassembly that followed PKC-δ migration to the nucleus. Lamin B disassembly and apoptosis were decreased by cell transfection with a dominant negative mutant form of PKC-δ (Eitel et al., 2003). Nuclear translocation and activation of PKC-δ have also been reported for etoposide-treated C6 glioma cells (Blass et al., 2002) or Jurkat T cells exposed to ionizing radiation, even though in the latter case their relevance in the context of the apoptotic process has not been elucidated (Cataldi et al., 2002).

Taken together, the aforementioned findings strongly suggest that PKC-δ regulates a or some nuclear event(s) required for apoptotic cell death.

Is the role of nuclear PKC-δ in apoptosis restricted to the phosphorylation of lamin B? Most likely, the answer is that it is not. Indeed, results by DeVries et al. (2002) have suggested that, once in the nucleus, PKC-δ can regulate the cytosolic apoptotic machinery, conceivably by affecting the expression of key apoptotic molecules which act at, or upstream of, the mitochondria. Moreover, activated (cleaved) PKC-δ binds directly to the carboxyl-terminus of DNA-dependent protein kinase (DNA-PK), which plays an essential role in the repair of DNA double-strand breaks. Following binding, DNA-PK is phosphorylated and this results in its dissociation from DNA, the inhibition of DNA repair, and enhanced DNA fragmentation (Brodie and Blumberg, 2003).

However, the data which indicate PKC-δ as an important pro-apoptotic nuclear PKC isoform are in contrast to the findings reported by Pongracz et al. (1995) which demonstrated that in spontaneously apoptotic U937 cells PKC-δ expression was reduced at the nuclear level whereas PKC-δ amount was increased in this cell compartment.

Nevertheless, yet another PKC isoform, PKC-βII, has recently emerged as an apoptotic lamin B kinase in pyF111 rat fibroblasts treated with etoposide (Chiarini et al., 2002). In this case, it has been found...
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that large complexes, consisting of PKC-βII, lamin B1, and active caspase-3 and -6, could be immunoprecipitated from the nuclear envelope of etoposide-treated, but not of control, cells. PKC-βII moved from cytoplasm to the nuclear envelope in response to etoposide, while hispidin, a selective inhibitor of PKC-βs, partly reduced lamin B phosphorylation and completely blocked its proteolytic degradation, even in the presence of active caspase-6. In this experimental system, lamin B-bound PKC-δ was inactive, whereas PKC-α did not associate with lamin B. Therefore, we can infer that either different cells may have distinct PKC isozymes which act as lamin B apoptotic kinases or that different apoptosis inducers activate, in the same cell type, distinct PKC isotypes, all capable of cleaving lamin B (Figure 1).

Anti-apoptotic nuclear PKC Isoforms

Nuclear PKC isoforms may also be involved in the protection from apoptosis. For example, in an interleukin-3-dependent hematopoietic cell line (IC2.9), the v-Abl protein tyrosine kinase-mediated suppression of apoptosis was associated with nuclear translocation of PKC-βII (Evans et al., 1995). Interestingly, the PKC inhibitor calphostin inhibited PKC-βII migration to the nucleus and restored cell sensitivity to interleukin-3 deprivation. Similar results have been recently reported by the same group for the FDCP-Mix A4 multipotent hematopoietic cell line (Xenaki et al., 2004).

Another good candidate as an anti-apoptotic nuclear PKC isoform seems to be the ζ isotype (Mizukami et al., 1997; Cataldi et al., 2003; Marchisio et al., 2003; Polosukhina et al., 2003). It is unclear how PKC isoforms, once migrated to the nucleus, could protect cells from apoptotic stimuli. Since some of nuclear substrates for PKC are involved in mRNA transcription and metabolism, nuclear PKC isoforms might be involved in the control of genes that are important for cell survival. There are indeed some clues that PKC may regulate gene transcription in myocardial cells, although the molecular mechanisms of this regulation are still unclear (Ventura and Maioli, 2001). In addition, PKC-ζ phosphorylates 106-kDa C23/nucleolin (Zhou et al., 1997). Nucleolin is not a transcription factor but rather a multifunctional nucleolar phosphoprotein implicated in chromatin structure, rDNA transcription, rRNA processing, ribosome assembly and maturation, and nucleo-cytoplasmic transport (Ginisty et al., 1999).

Conclusions

The PKC family of isoforms is responsible for regulating a wide variety of cellular functions. The finding that some nuclear PKC isoforms are involved in apoptosis regulation introduces the possibility of targeting PKC for therapeutic treatment of diseases where there is a deregulation in the processes of cell survival and death such as cancer, inflammatory diseases, and neurodegenerative disorders (Carter, 2000; Battaini, 2001). Indeed, in the past few years a large number of PKC activators and inhibitors with potential as therapeutic agents have been developed and several of these compounds are already in Phase II clinical testing (Hofmann, 2001; Barry and Kazanietz, 2001).

Figure 1. Involvement of nuclear PKC isoforms in the apoptotic process. Several apoptotic stimuli induce translocation to the nucleus of different PKC isoforms. All of these isoforms seem to target lamin B which is phosphorylated on an unidentified residue. Lamin B phosphorylation facilitates its cleavage by caspase-3 or -6, which is then followed by lamin disassembly, a critical step in apoptotic nuclear collapse. Note that in the diagram we did not take into consideration cleavage of lamin A that, in some experimental models (MCF-7 cells for example, see Ruchaud et al., 2002), is performed by caspase-6 and is essential for the initiation and completion of the typical apoptotic nuclear changes. Very recent results point to the likelihood that also phosphorylation of lamin A/C is important in the context of apoptosis (Steen et al., 2003) but it is not known whether PKC is involved. Moreover, pro-apoptotic PKC isoforms may target other nuclear proteins important for the execution phase of apoptosis, such as DNA-PK.
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