Isoenzymes of protein kinase C: differential involvement in apoptosis and pathogenesis

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There has been an abundance of literature concerning apoptosis (programmed cell death) and its role in development and disease. Apoptotic cell death has characteristic morphological changes in that the cell shrinks and becomes denser, the nucleus breaks up, and the cell buds as a whole to produce membrane bound apoptotic bodies.^{1,2} Apoptotic cells are recognised and removed by "professional" phagocytes, or neighbouring cells, thus preventing loss of cell contents and initiation of inflammatory responses.³ Apoptosis occurs during embryonic development—for example, in the regression of the tadpole tail,⁴ and in the removal of interdigital webs during limb development in mammalian embryos.⁵ Apoptosis also occurs during biological processes in the adult—for example, in the deletion of autoreactive thymocytes⁶ and in the removal of aging neutrophils during resolution of the inflammatory response.⁷ Furthermore, the control of cell numbers is regulated by the rates of cell proliferation, differentiation, and apoptosis, and dysregulation of these processes will result in neoplasia.⁸ The induction of apoptosis therefore offers an alternative to cytostatic or differentiation therapies in the treatment of cancer, and it has become clear that several anticancer agents already in use are potent inducers of apoptosis.^{9,10} It is in this light that the role of apoptosis and its regulation in normal and disease states has come to the fore in recent years, as a potential treatment for cancer and other diseases which may involve a modulation of apoptosis in their pathogenesis.¹¹⁻¹⁶

The realisation that apoptosis is a fundamental cellular process that impacts the initiation and progression of disease states has led to the broadening of disease model systems under consideration for intervention at the level of the apoptotic programme. Many of these studies have focused on investigation of the signal transduction pathways involved in the regulation of cell proliferation and apoptosis, and more specifically how these pathways interact and how their dysregulation leads to disease. One signalling element implicated in the regulation of cell proliferation and apoptosis, and already a recognised target for therapeutic modulation, is the enzyme protein kinase C.¹⁷

Protein kinase C

Protein kinase C (PKC), a serine-threonine kinase, has been studied as a key element in signalling pathways regulating a wide range of cell functions.^{18,19} PKC was discovered in rat brain by Inoue and co-workers²⁰ who described it as a histone protein kinase that displayed

phospholipid and calcium dependency, and required diacylglycerol (DAG) for full enzyme activity. The range of cell functions known to be regulated through the PKC signalling pathway is extensive and includes secretion,²¹ cytoskeleton function,²²⁻²⁴ cell-cell contacts,²⁵ gene expression,²⁶⁻²⁸ and cell survival.²⁹⁻³¹ This pleiotropic involvement of PKC in cellular activity raised the question of how specificity of biological action could be maintained if a wide range of receptors were linked to the PKC signalling pathway. This paradox began to be resolved when the screening of rat cDNA libraries revealed the existence of several different, but closely related, isoenzymes of PKC.³²

PKC ISOENZYMES

The PKC isoenzyme family currently has 12 members that show a high degree of conservation across mammalian species,³³ suggesting that they may have specific functions within cells. This proposal is supported by the selective distribution of certain PKC isoenzymes. PKC α , δ , and ζ seem to be the most widely distributed.³⁴⁻³⁶ In contrast, PKC γ is expressed exclusively in the central nervous system,³⁷ PKC θ only in skeletal muscle and haemopoietic cells,³⁸ and PKC η expression is greatest in skin and lung tissue, with only low levels detected in the brain and spleen.³⁸ In addition to tissue specific distribution, the isoenzymes of PKC are differentially responsive to co-factors, allowing them to be regulated independently. Detailed descriptions of the biochemistry and regulation of PKC have been provided in several reviews in recent years^{18,19,33,39,40} and will consequently be given only brief consideration here.

While all PKC isoenzymes appear to require the presence of phospholipids for full activity, the PKC isoenzyme family can be divided into three main groups based on their additional activation requirements and ability to respond to phorbol esters. The latter bind to the regulatory domain of PKC, mimicking the physiological activation by diacylglycerol (DAG). *Classical PKC*— α , β I, β II, and γ are calcium dependent and activated by phorbol esters. *Novel PKC*— δ , ϵ , η , and θ are calcium independent, but can be activated by phorbol esters.

Atypical PKC— ζ , λ /I, and μ are calcium independent and do not bind phorbol esters.

Suggested physiological activators of these isoenzymes include ceramide^{41,42} and phosphatidylinositol 3,4,5-trisphosphate.⁴³

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PKC TRANSLOCATION

All PKC isoenzymes possess a catalytic and a regulatory domain separated by a hinge region V3 (fig 1) which is subject to proteolysis.⁴⁴ Depending on the class of PKC isoenzyme, the regulatory domain can include a calcium binding and a DAG-phorbol ester binding site. Activation of PKC isoenzymes requires their interaction with phospholipid, with acidic species such as phosphatidylserine being most effective.^{45 46} Inactive PKCs are reported to be located predominantly, though not exclusively, in the cytosol.¹⁹ Thus, activation of PKC is related to its redistribution and association with intracellular membranes. The direction of translocation and intracellular targeting of PKC isoenzymes is highly variable, being influenced by the nature of the initial signal, cell type, and developmental status. For example, in Swiss 3T3 cells treated with bombesin, PKC α translocates to the cell plasma membrane. In the same cells treated with IGF-1, PKC α is associated with the nuclear membrane.⁴⁷ PKC β translocates to the nucleus when K562 cells are treated with bryostatin-1 and to the plasma membrane following treatment with phorbol dibutyrate.⁴⁸ Clearly such differences will influence the actions of PKC isoenzymes, most notably regarding substrate availability,⁴⁹ and will dictate the role of PKC isoenzymes in cell function.

ACTIVATION BY LIPIDS

PKC isoenzymes show differential responsiveness to lipid species. For example, activation of classical PKCs is further enhanced by cis-unsaturated fatty acids and lysophosphatidylcholine; free fatty acids activate PKC ϵ and ζ , but inhibit PKC δ .⁵⁰⁻⁵² Ceramide is reported to activate PKC ζ ⁴³ but inhibits the activation of PKC α .⁵³ Thus the activation of specific PKC isoenzymes will vary dependent upon the lipid species generated as second messengers follow-

ing receptor ligation. Furthermore, analysis of total cellular DAG in Swiss 3T3 cells has revealed a complex mixture of 27 different molecular species. Stimulation of cells with bombesin induced an increase in only a few DAGs.⁵⁴ Although differential activation of PKC isoenzymes by DAG species has not been reported, this could clearly provide another route to selectivity in signalling through PKC.

PROTEOLYSIS AND DOWNREGULATION

The regulatory domain of PKC also includes a motif resembling the consensus site for PKC substrates, xRxxS/TxRx, in which a serine-threonine residue is changed to alanine. This motif represents a pseudosubstrate site which blocks the catalytic site in inactive PKCs. Binding of an activator such as DAG, induces a conformational change removing any inhibition by the pseudosubstrate site, and renders the hinge region open to proteolytic cleavage.⁴⁰ Proteolysis generates two distinct fragments, the regulatory domain and a catalytically active protein kinase domain known as PKM.^{55 56} In the case of classical PKCs, the hinge region contains cleavage sites for the calcium dependent neutral proteases calpains I and II.⁵⁷ Schaap *et al*⁵⁶ have also reported a cleavage site for trypsin in PKC ϵ . Persistent activation of PKC leads to the downregulation of PKC and interestingly the PKC isoenzymes vary in their susceptibility to such cleavage. PKC α has been shown to be relatively resistant to proteolysis mediated downregulation, whereas PKC β and PKC γ are more easily inactivated.⁵⁸ As will be discussed later, it has been shown recently that a novel PKC, PKC δ , can be cleaved by the ICE-like protease CPP32 to generate a catalytically active 40 kDa PKC.⁵⁹ This property appears to be unique to PKC δ . Whether PKC isoenzymes are targets for other proteases remains to be established, but clearly this mode

Figure 1 Structure of the three main groups of the protein kinase C isoenzyme family. c, classical; n, novel; a, atypical; V, variable region; C, constant region.

of activation could involve PKC in an even wider range of signalling pathways.

PKC SUBSTRATES

In determining a role for PKC in the regulation of a specific cell function, such as apoptosis, it is clearly important to establish whether a particular biological effect is correlated with activation or downregulation of a specific isoenzyme. In addition, significant progress will only be made if we can define precisely how PKC isoenzymes mediate their effects on cell regulation. In this respect it is the identification of PKC substrates in whole cell studies that will increase our understanding of PKC. PKC is known to phosphorylate a number of different substrates, which include other proteins involved in signal transduction (Raf-1),⁶⁰ proteins regulating DNA synthesis (transcription factors),^{27 28} DNA modifying enzymes,^{61 62} and proteins involved in cell cycle control (lamin B).⁶³ Identification of isoenzyme substrates will clearly define the role of PKC in cell regulation and clarify whether its modulation is a feasible therapeutic target. For example, PKC has been suggested to play a role in cell cycle control, though little is known of the molecular basis of this involvement. However, studies by Goss and co-workers have identified PKCβII as a mitotic lamin kinase,⁶³ and phosphorylation of lamin B by PKC contributes to disassembly of the nuclear lamina. More specifically, the phosphorylation of lamin B by PKCβII is required to maintain lamin B within the cytosol during mitosis.⁶⁴ In addition, cell cycle arrest at G1/S induced by tumour necrosis factor (TNF)α has been shown to involve dephosphorylation of Rb and to require the inactivation of PKCα, possibly mediated by ceramide activated protein phosphatase.⁵³ These examples illustrate the relevance of studies aiming to identify not only the PKC isoenzymes involved in regulation but also their physiologically relevant substrates.

It is apparent that PKC regulation is complex and there are many factors to be considered when looking at its role in apoptosis and disease. PKC involvement will be affected by the isoenzyme content of the tissue being studied, the second messengers generated following receptor ligation, degree of downregulation, direction of translocation, and substrate availability. To date the majority of studies that have considered the role of PKC in apoptosis have not taken these factors into account. However, it is becoming clear that PKC does regulate apoptosis and that specific isoenzymes are involved in signals that promote or delay apoptosis. The following is an attempt to review the current literature in this area, with emphasis on studies that have considered the role of individual PKC isoenzymes.

PKC and apoptosis

Understanding the role of PKC in apoptosis (table 1) has been complicated by conflicting reports of its action.³¹ This has resulted in part from studies that used broad range activators (12-0-tetradecanoylphorbol-13-acetate; TPA) or inhibitors (staurosporine) to investigate the

Table 1 Effects of TPA on apoptosis

Induced by TPA	Prevented by TPA
Hydrocortisone treated thymocytes ⁶⁵	IL-2 deprived T lymphocytes ¹²⁹
T cell hybridomas treated with anti-CD3 ¹³⁰	IL-3 derived promyeloid cells ²⁹
Human prostate cancer cells ⁶⁶	Germinal center B cells ⁷¹

involvement of PKC. Thus, TPA has been shown to induce apoptosis in isolated thymocytes⁶⁵ and human prostate cancer cells,⁶⁶ yet it inhibits apoptosis following growth factor withdrawal in interleukin (IL)-3 dependent myeloid cells.²⁹ The use of TPA can give apparently contradictory findings because it can activate several PKC isoenzymes, namely the classical and novel PKCs. In addition, unlike the physiological activator DAG, TPA is a stable compound which will induce a persistent activation of PKC leading to its downregulation. As stated earlier, the PKC isoenzymes are differentially sensitive to downregulation, introducing another variable to be considered when using TPA.^{67 68}

PROMOTION OF APOPTOSIS

Some of the earliest studies to investigate the precise involvement of PKC isoenzymes simply determined PKC isoenzyme expression or activation and correlated this with the onset or delay of apoptosis. PKCβ has been implicated in the regulation of apoptosis in myeloid cells using a variety of approaches. In promyeloid U937 cells, expression of PKCβ was shown to be increased in spontaneously apoptotic cells.⁶⁹ Promyeloid HL60 PET cells, which are deficient in PKCβ, are unable to undergo apoptosis in response to phorbol esters.⁷⁰ Induction of PKCβ expression in HL60 PET cells, by treatment with vitamin D₃, restores their ability to differentiate and apoptose when treated with phorbol esters. Furthermore, Knox *et al*⁷¹ reported that the level of bcl-2 expression was inversely related to the expression of PKCβ and α in tonsil epithelial cells. PKCβ actually exists as two isoforms, βI and βII, which differ at their 3' ends and are generated by alternate splicing.⁴⁵ Interestingly, the current literature suggests that the two PKCβ isoforms may have divergent effects on apoptosis, though none of the published studies have considered both PKCβ isoforms. The deoxyphorbol ester Doppa, a selective activator of PKCβI *in vitro*,⁷² is able to increase apoptosis in HL60 and U937 cell cultures.^{73 74} Our recent studies have shown that PKCβ expression was increased as neutrophils died by apoptosis, but that this effect was due to *de novo* expression of PKCβI, with no change in PKCβII (Pongracz *et al* 1996, unpublished observations). In contrast, studies to determine the molecular basis of suppression of apoptosis by the v-ABL protein tyrosine kinase in an IL-3 dependent cell line revealed that activation of v-ABL resulted in suppression of apoptosis induced by removal of IL-3. v-ABL activation was followed by the translocation of PKCβII to the nucleus. Inclusion of a PKC inhibitor, calphostin C, during v-ABL activation, inhibited the activation of

PKC β II and prevented the suppression of apoptosis by v-ABL.⁷⁵ The role of PKC β II as a suppressor of apoptosis is further supported by its confirmed role in proliferation.⁷⁶ As mentioned earlier, PKC β II is a mitotic lamin kinase.⁶³ The processes of proliferation and apoptosis are clearly very closely interrelated⁷⁷ and it is tempting to speculate that PKC β I and β II may be differentially involved in these mutually exclusive events. Whether the two PKC β isoforms can be regulated differentially is not known. However, the 50 amino acid difference in their composition at the C-terminus does affect their targeting within cells,⁴⁹ suggesting that they have distinct roles in the regulation of cell function.

It is unlikely that PKC β alone is involved in the regulation of apoptosis, PKC β is not present in all cells and several authors have reported an association of other isoenzymes with apoptosis.⁷⁸ We have used a cell free approach⁷⁹ to identify PKC isoenzymes that are required for the induction of apoptosis in human neutrophils. Nuclei isolated from healthy neutrophils were combined with the cytosol from apoptotic neutrophils and DNA fragmentation assessed as a measure of apoptosis. Removal of either PKC β or δ from the cytosol by immunoprecipitation significantly reduced the level of DNA fragmentation (Pon-gracz 1996, unpublished observations).

Much attention has been focused recently on the isoenzyme PKC δ . Like PKC β , this isoenzyme is expressed in a wide range of cell types.¹⁹ PKC δ is activated following treatment of U937 cells with ultraviolet irradiation, TNF α or anti-Fas antibody.^{80, 81} However, PKC δ activation in these studies was not mediated by lipid co-factors such as DAG, but as a result of proteolytic cleavage by the ICE protease CPP32.⁸¹ Datta *et al*⁸² stated that they had unpublished data showing that CPP32, but not other members of the ICE family, cleaved PKC δ in vitro. Transfection of cells with the protease generated catalytic fragment of PKC δ was sufficient to induce apoptosis. Moreover, transfection with a kinase defective PKC δ fragment did not induce apoptosis and proteolytic activation was blocked by bcl-2 and bcl-x, supporting an association of PKC δ with apoptosis. Proteolytic activation by CPP32 was unique to PKC δ and was not seen in PKC α , ϵ or ζ . Our own studies also support a role for PKC δ activation in the induction of apoptosis and suggest a possible mode of action for this isoenzyme. We have reported the characterisation of a marine compound, Bistratene A, an activator of PKC which appears to be selective for PKC δ .⁸³ We and others have shown that this agent induces growth arrest at G2/M and apoptosis in haemopoietic cell lines.⁸³⁻⁸⁵ Moreover, activation of PKC δ following Bistratene A treatment of HL60 cells, induces association of PKC δ with nuclear lamins and phosphorylation of lamin B.⁸⁵ We therefore propose that PKC δ may mediate disassembly of the nuclear lamina, a prerequisite for both mitosis and apoptosis. The G2/M arrest seen in cells which overexpress PKC δ ⁸⁶ may therefore be a consequence of disassembly of the nuclear lamina,

preventing further progress through the cell cycle. It is possible that persistent activation of PKC δ will maintain the lamina in a soluble form, which is then susceptible to proteolytic degradation. Lamin proteins are degraded before apoptosis^{87, 88} by the ICE-like protease Mch2 alpha,⁸⁹ thus preventing reformation of the lamina as occurs at the end of mitosis, and justifying the view of apoptosis as a "mitotic catastrophe".⁹⁰

While the majority of evidence currently available suggests that activation of PKC δ leads to apoptosis, other possibilities cannot be ruled out. Leszczynski *et al*⁹¹ used a PKC δ specific antisense oligonucleotide to downregulate PKC δ expression in rat vascular smooth muscle cells. Downregulation of PKC δ in this way resulted in apoptosis and Leszczynski has since suggested that downregulation of PKC, rather than activation, was responsible for cell death.⁷⁸ These data may represent an alternative involvement of PKC δ in apoptosis and at this stage all that can be concluded is that this isoenzyme appears to be an important regulator of cell death.

SUPPRESSION OF APOPTOSIS

A variety of reports in the literature have shown that inhibition of PKC results in apoptosis,⁹²⁻⁹⁴ therefore, a suppressive effect of certain PKC isoenzymes can be predicted. This has already been discussed for PKC β II but can be extended to other isoenzymes. The evidence appears to be most consistent and convincing in the case of PKC α and ζ . Several agents that induce apoptosis increase intracellular levels of ceramide, including TNF- α , via the activation of sphingomyelinase.⁹⁵ Ceramide has several potential targets, including stress-activated protein kinase (SAPK/JUNK),⁹⁶ ceramide-activated protein phosphatase (CAPP),⁹⁷ and PKC ζ . Increased intracellular levels of ceramide lead to an increase in apoptosis, which can be overcome by activation of PKC,^{92, 98} suggesting that inhibition of PKC is involved in ceramide induced apoptosis. Lee *et al*⁹³ have shown that ceramide inactivates PKC α in Molt-4 cells and that this is an indirect effect. Ceramide did not prevent translocation of PKC α in Molt-4 cells, but did prevent its activation by inhibiting phosphorylation of PKC α . All PKC isoenzymes are known to autophosphorylate as a requirement for enzyme activation,¹⁹ and Lee and co-workers suggest that ceramide is activating CAPP to prevent phosphorylation of PKC α .

We have previously reported that PKC ζ expression was markedly reduced in apoptotic U937 cells,⁶⁹ suggesting that this isoenzyme could also have a protective function in relation to apoptosis. This role was investigated further in a study on rat vascular smooth muscle using a PKC ζ specific antisense oligonucleotide. Loss of the ζ isoenzyme was found to induce apoptosis.⁹¹ More recently, Diazmeo *et al*⁹⁹ have shown that the product of par-4, a gene induced during apoptosis, interacts selectively with atypical PKCs, including PKC ζ . Interaction of par-4 with the regulatory domains of atypical PKCs dramatically inhibited their

Table 2 Diseases known to involve dysregulation of apoptosis and altered PKC function

Disease (reference)	Apoptosis	PKC	Activity/expression
Cancer			
Colorectal ^{122 131}	↓	↓	(↓ε, ↓β, ↓δ)
Breast ¹³²	↓	↑	(α, β)
Prostate ¹²¹	↓	↑	(ε)
Glioblastoma ^{105 125 133}	↓	↑↓	(↑α)
Immune function			
AIDS ^{104 134}	↑	↑	
Chronic granulomatous disease ^{101 111}	↓	↓	
Asthma ¹³⁵	↓	↑	
Other			
Vascular hypertension ^{103 136}	↑	↑	(↑α)
Alzheimer's ^{108 109 137}	↑	↓	(α, ζ)
Ataxia telangiectasia ^{113 114}	↑	—	
Psoriasis ¹³⁸⁻¹⁴⁰	↓	↓	(↑η, ↓α, ↓βII)

Letters in parentheses indicate changes detected in the expression or activation status of specific PKC isoenzymes; —, no difference detected between normal and disease tissue.

enzyme activity. These data suggest a role for PKCζ in cell survival and data from Berra *et al*⁶⁰ provide further support for this proposal. These authors showed that an active mutant of PKCζ could activate key elements of the mitogenic signalling cascade MAPK and MEK, a dominant negative mutant of PKCζ impaired activation of these kinases by growth factors.

Thus PKC isoenzymes are differentially involved in the regulation of apoptosis and while there are likely to be variations between cell types, the current literature suggests that PKCδ is involved in execution of the apoptotic programme. In contrast, PKCα and ζ are frequently associated with cell survival and suppression of apoptosis. The role of PKCβI and βII remains to be clarified, though both are intimately associated with signalling pathways that modulate cell proliferation and apoptosis.

PKC, apoptosis, and disease

PKC, together with other signalling elements that regulate cell function, has become of major interest in recent years as a target for therapeutic intervention in a range of different diseases. Several of these studies have centred on the role of PKC in the regulation of apoptosis. Removing a diseased cell by inducing it to undergo apoptosis has many advantages, not least that apoptotic cells are efficiently removed by phagocytic cells, preventing the release of potentially toxic cell contents.³ While the emphasis in the literature is given to apoptosis and cancer, other disease states clearly include dysregulation of apoptosis as a significant pathogenic factor (table 2): disorders of the immune system such as chronic allergic disease and chronic granulomatous disease, in which eosinophil¹⁰⁰ and neutrophil¹⁰¹ apoptosis, respectively, are reduced; neurodegenerative disorders, including Alzheimer's disease, which involves increased apoptosis of neuronal cells¹⁰²; vascular disease¹⁰³; AIDS¹⁰⁴ and ataxia telangiectasia,¹⁰⁵ which are both associated with accelerated lymphocyte apoptosis.

Although alterations in PKC expression or function have been established in several of these diseases (table 2), with the exception of cancer and ataxia telangiectasia the connecting factor does not appear to involve apoptosis. For example, in Alzheimer's disease decreased expression of PKCβ,¹⁰⁶ PKCε,¹⁰⁷ PKCα and

ζ¹⁰⁸⁻¹¹⁰ have been reported. Defective secretion of amyloid β protein has now been identified as the function directly affected by the alteration in PKCα.¹¹⁰ However, it is still likely that future studies that consider alterations in specific PKC isoenzymes in relation to disease states and apoptosis may reveal more positive connections. For example, chronic granulomatous disease is associated with defective neutrophil apoptosis and activation, with the latter associated with reduced PKC activity.¹¹¹ As Curnutte *et al*¹¹¹ did not consider individual PKC isoenzymes, further analysis is required to establish whether PKCs identified as involved in regulating neutrophil apoptosis¹¹² are altered in chronic granulomatous disease.

ATAXIA TELANGIECTASIA

Ataxia telangiectasia is an inherited autosomal recessive disorder, which includes a characteristic hypersensitivity to ionising radiation. The ataxia telangiectasia group D complementing gene (ATGD) can restore normal radiosensitivity and the ability of the ATGD gene product to modulate radiosensitivity is regulated by phosphorylation. PKC has been identified recently as the kinase responsible for this phosphorylation.¹¹³ To date the isoenzyme involved has not been identified and altered PKC expression or activation has not been associated with ataxia telangiectasia.¹¹⁴

NEOPLASIA

It has been known for some time that PKC plays a role in neoplasia, with the expression and activation status of individual isoenzymes varying in a number of neoplastic tissues.¹¹⁵ As the literature in this area is vast, we have highlighted only a few of the studies that have considered the involvement of PKC isoenzymes in oncogenesis. Although PKCε is the only isoenzyme with full oncogenic potential,¹¹⁶ there are very few reports of its involvement in apoptosis or neoplasia. However, recent preliminary studies have suggested that PKCε may be an "early response" protein, involved in entry of quiescent cells into cell cycle and DNA replication.¹¹⁷ Li *et al*¹¹⁸ have also shown that PKCε is required for induction of c-myc and DNA replication by erythropoietin in erythroleukaemia cells. With regard to cancer, the literature is conflicting, not only with respect to PKCε but also the other PKC isoenzymes. PKCε is expressed at high levels in tumorigenic rat colonic epithelial cells,¹¹⁹ MCF-7 breast cancer cells,¹²⁰ and neoplastic rat prostate cancer cells,¹²¹ supporting the suggestion of an anti-apoptotic, mitogenic role for this isoenzyme. However, our own studies analysing PKC isoenzyme levels in human colon cancer tissue found a significant decrease in PKCε.¹²² Kiss and Anderson¹²³ showed that carcinogens reduced expression of PKCε in mouse embryo fibroblasts and inhibition of apoptosis in prostatic carcinoma cells was mediated by a activation and downregulation of PKCε.¹²⁴ Thus, although several cell line studies suggest an oncogenic role for PKCε, this remains to be confirmed in analyses of human tumour biopsies.

The involvement of PKC α in neoplasia appears less controversial and, encouragingly, antisense oligonucleotides to PKC α have been used recently to reduce elevated levels of this PKC in glioblastoma cells and inhibit tumour growth.¹²⁵ Whether this reduction in tumour growth was mediated via alterations in proliferation or apoptosis was not determined. The role of PKC α has also been investigated in breast carcinoma, using MCF-7 cells stably transfected to overexpress PKC α .¹²⁶ Cells overexpressing PKC α displayed a more transformed aggressive phenotype as shown by an increase in proliferative rate, loss of epithelioid appearance, downregulation of oestrogen receptor expression, and enhanced tumorigenicity with metastatic potential in nude mice. However, caution is required in interpreting these data, as the transfected cells also showed alterations in the expression of other PKC isoenzymes, most notably a decrease in PKC δ . Thus enhanced tumorigenicity could be a consequence of the increased expression of an isoenzyme involved in suppression of apoptosis and the reduction of a pro-apoptotic PKC.

Because of space limitations it has been possible to review only the major findings in the literature concerning PKC and its involvement in apoptosis and pathogenesis. What has become apparent is that the true role of PKC in apoptosis and disease will only be realised when individual family members are considered. Access to specific inhibitors and activators of PKC isoenzymes will also improve our understanding of PKC isoenzyme function and may uncover therapeutically useful compounds.¹⁷⁻⁸³ In addition, the increased use of specific antisense oligonucleotides and constitutively active PKC isoenzyme mutants, mentioned briefly in this review, has already begun to increase understanding of PKC isoenzyme function.¹²⁷⁻¹²⁸ Precise studies of PKC function such as these will allow informed decisions to be made regarding the suitability of PKC as a target for drug therapy in the treatment of a multitude of diseases.

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