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. Apoptotic cells are recognised and removed by "professional" phagocytes, or neighbouring cells, thus preventing loss of cell contents and initiation of inflammatory responses. Apoptosis occurs during embryonic development—for example, in the regression of the tadpole tail, and in the removal of interdigital webs during limb development in mammalian embryos. Apoptosis also occurs during biological processes in the adult—for example, in the deletion of autoreactive thymocytes and in the removal of aging neutrophils during resolution of the inflammatory response. Furthermore, the control of cell numbers is regulated by the rates of cell proliferation, differentiation, and apoptosis, and disregulation of these processes will result in neoplasia. 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. 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.

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 disregulation 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.

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. PKC was discovered in rat brain by Inoue and co-workers 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, cytoskeleton function, cell–cell contacts, gene expression, and cell survival. 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.

PKC ISOENZYMES

The PKC isoenzyme family currently has 12 members that show a high degree of conserva-
tion across mammalian species, suggesting that they may have specific functions within cells. This proposal is supported by the selective distribution of certain PKC iso-
enzymes. PKCδ and ζ seem to be the most widely distributed. In contrast, PKCγ is expressed exclusively in the central nervous system, PKCθ only in skeletal muscle and haemopoetic cells, and PKCγ expression is greatest in skin and lung tissue, with only low levels detected in the brain and spleen. 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 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 physio-
logological activation by diacylglycerol (DAG). Classical PKC—δ1, δ2, δ3, ε, η, and θ are calcium dependent and activated by phorbol esters. Novel PKC—ζ, λ, μ, and ν are calcium independent, but can be activated by phorbol esters.

Atypical PKC—ζ, λ, μ, and ν are calcium independent and do not bind phorbol esters. Suggested physiological activators of these isoenzymes include ceramide and phosphatidylinositol 3,4,5-trisphosphate.
PKC TRANSLLOCATION
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, PKCa translocates to the cell plasma membrane. In the same cells treated with IGF-1, PKCu is associated with the nuclear membrane.47 PKCb 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,19 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 cis unsaturated fatty acids and lysophosphatidylcholine; free fatty acids activate PKCc and Cζ, but inhibit PKCδ.49-50 Ceramide is reported to activate PKCy5 but inhibits the activation of PKCu.51 Thus the activation of specific PKC isoenzymes will vary dependent upon the lipid species generated as second messengers following 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.52 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 DOWNNREGULATION
The regulatory domain of PKC also includes a motif resembling the consensus site for PKC substrates, xRxSxTxRx, 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.53-54 In the case of classical PKCs, the hinge region contains cleavage sites for the calcium dependent neutral proteases calpains I and II.55 Schaap et al56 have also reported a cleavage site for trypsin in PKCe. Persistent activation of PKC leads to the downregulation of PKC and interestingly the PKC isoenzymes vary in their susceptibility to such cleavage. PKCa has been shown to be relatively resistant to proteolysis mediated downregulation, whereas PKCb and PKCγ are more easily inactivated.57 As will be discussed later, it has been shown recently that a novel PKC, PKCy, can be cleaved by the ICE-like protease CPP32 to generate a catalytically active 40 kDa PKC.58 This property appears to be unique to PKCy. Whether PKC isoenzymes are targets for other proteases remains to be established, but clearly this mode

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**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), proteins regulating DNA synthesis (transcription factors), DNA modifying enzymes, and proteins involved in cell cycle control (lamin B). 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, 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. 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 PKCoα, possibly mediated by ceramide activated protein phosphatase. 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. This has resulted in part from studies that used broad range activators (12-0-tetradecanoylphorbol-13-acetate; TPA) or inhibitors (staurosporine) to investigate the involvement of PKC. Thus, TPA has been shown to induce apoptosis in isolated thymocytes and human prostate cancer cells, yet it inhibits apoptosis following growth factor withdrawal in interleukin (IL)-3 dependent myeloid cells. 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.

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**Table 1: Effects of TPA on apoptosis**

<table>
<thead>
<tr>
<th>Induced by TPA</th>
<th>Prevented by TPA</th>
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<tbody>
<tr>
<td>Hydrocortisone treated thymocytes</td>
<td>IL-2 deprived T lymphocytes</td>
</tr>
<tr>
<td>T cell hybridomas treated with anti-CD3</td>
<td>IL-3 derived promyeloid cells</td>
</tr>
<tr>
<td>Human prostate cancer cells</td>
<td>Germinal center B cells</td>
</tr>
</tbody>
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**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 increase in spontaneously apoptotic cells. In promyeloid HL60 PET cells, which are deficient in PKCβ, are unable to undergo apoptosis in response to phorbol esters. Induction of PKCβ expression in HL60 PET cells, by treatment with vitamin D₃, restores their ability to differentiate and apoptosis when treated with phorbol esters. Furthermore, Knox et al 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. Interestingly, the current literature suggests that the two PKCβ isoforms may have divergent effects on apoptosis, though none of the published studies has considered both PKCβ isoforms. The deoxyphorbol ester Doppa, a selective activator of PKCβII in vitro, is able to increase apoptosis in HL60 and U937 cell cultures. 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 PKCβII, with no change in PKCβI (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 PKCβIII to the nucleus. Inclusion of a PKC inhibitor, calphostin C, during v-ABL activation, inhibited the activation of
PKCβII and prevented the suppression of apoptosis by v-ABL. The role of PKCβII as a suppressor of apoptosis is further supported by its confirmed role in proliferation. As mentioned earlier, PKCβII is a mitotic lamina kinase. The processes of proliferation and apoptosis are clearly very closely interrelated and it is tempting to speculate that PKCβ and βII may be differentially involved in these mutually exclusive events. Whether the two PKCβ 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, suggesting that they have distinct roles in the regulation of cell function.

It is unlikely that PKCβ alone is involved in the regulation of apoptosis, PKCβ is not present in all cells and several authors have reported an association of other isoenzymes with apoptosis. We have used a cell free approach 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 PKCβ or δ from the cytosol by immunoprecipitation significantly reduced the level of DNA fragmentation (Pongracz 1996, unpublished observations).

Much attention has been focused recently on the isoenzyme PKCδ. Like PKCβ, this isoenzyme is expressed in a wide range of cell types. PKCδ is activated following treatment of U937 cells with ultraviolet irradiation, TNFα or anti-Fas antibody. However, PKCδ 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. Datta et al. stated that they had unpublished data showing that CPP32, but not other members of the ICE family, cleaved PKCδ in vitro. Transfection of cells with the protease generated catalytic fragment of PKCδ was sufficient to induce apoptosis. Moreover, transfection with a kinase defective PKCδ fragment did not induce apoptosis and proteolytic activation was blocked by bcl-2 and bcl-x, supporting an association of PKCδ with apoptosis. Proteolytic activation by CPP32 was unique to PKCδ and was not seen in PKCα, ε or ζ. Our own studies also support a role for PKCδ activation in the induction of apoptosis and suggest a possible role for this isoenzyme. We have reported the characterisation of a marine compound, Bistratene A, an activator of PKCδ which appears to be selective for PKCδ. We and others have shown that this agent induces growth arrest at G2/M and apoptosis in haemopoietic cell lines. Moreover, activation of PKCδ following Bistratene A treatment of HL60 cells, induces association of PKCδ with nuclear lamins and phosphorylation of lamin B. We therefore propose that PKCδ may mediate disassembly of the nuclear lamina, a prerequisite for both mitosis and apoptosis. The G2/M arrest seen in cells which overexpress PKCδ 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 PKCδ may maintain the lamina in a soluble form, which is then susceptible to proteolytic degradation. Lamin proteins are degraded before apoptosis by the ICE-like protease Mch2 alpha, thus preventing reformation of the lamina as occurs at the end of mitosis, and justifying the view of apoptosis as a "mitotic catastrophe." While the majority of evidence currently available suggests that activation of PKCδ leads to apoptosis, other possibilities cannot be ruled out. Leszczynski et al. used a PKCδ specific antisense oligonucleotide to downregulate PKCδ expression in rat vascular smooth muscle cells. Downregulation of PKCδ in this way resulted in apoptosis and Leszczynsk has since suggested that downregulation of PKC, rather than activation, was responsible for cell death. These data may represent an alternative involvement of PKCδ 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. Therefore, a suppressive effect of certain PKC isoenzymes can be predicted. This has already been discussed for PKCβII but can be extended to other isoenzymes. The evidence appears to be most consistent and convincing in the case of PKCa and ζ. Several agents that induce apoptosis increase intracellular levels of ceramide, including TNF-α, via the activation of sphingomyelinase. Ceramide has several potential targets, including stress-activated protein kinase (SAPK/JNK), ceramide-activated protein phosphatase (CAPP), and PKCζ. Increased intracellular levels of ceramide lead to an increase in apoptosis, which can be overcome by activation of PKCζ, suggesting that inhibition of PKCζ is involved in ceramide induced apoptosis. Lee et al. have shown that ceramide inactivates PKCa in Molt-4 cells and that this is an indirect effect. Ceramide did not prevent translocation of PKCa in Molt-4 cells, but did prevent its activation by inhibiting phosphorylation of PKCa. All PKC isoenzymes are known to autophosphorylate as a requirement for enzyme activation, and Lee and co-workers suggest that ceramide is activating CAPP to prevent phosphorylation of PKCa.

We have previously reported that PKCζ expression was markedly reduced in apoptotic U937 cells, 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 PKCζ specific antisense oligonucleotide. Loss of the ζ isoenzyme was found to induce apoptosis. More recently, Diazmeco et al. have shown that the product of par-4, a gene induced during apoptosis, interacts selectively with atypical PKCs, thus preventing further apoptosis. Interaction of par-4 with the regulatory domains of atypical PKCs dramatically inhibited their
Table 2  Diseases known to involve disregulation of apoptosis and altered PKC function

<table>
<thead>
<tr>
<th>Disease (reference)</th>
<th>Apoptosis</th>
<th>PKC</th>
<th>Activity/expression</th>
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<tbody>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal112, 114</td>
<td></td>
<td></td>
<td>(δE, ζ, δβ, θη)</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td>(α, δβ)</td>
</tr>
<tr>
<td>Prostate</td>
<td></td>
<td></td>
<td>(δ, ζ)</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td></td>
<td></td>
<td>(γ, δ, θα)</td>
</tr>
<tr>
<td>Immune function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS104, 114</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Chronic granulomatous disease110, 111</td>
<td>θα</td>
<td></td>
<td>(θα)</td>
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<tr>
<td>Asthma110</td>
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<td></td>
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<tr>
<td>Other</td>
<td></td>
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<tr>
<td>Vascular hypertension112, 113</td>
<td>θη</td>
<td></td>
<td>(θη, θδ, δθ)</td>
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<tr>
<td>Alzheimer106, 109, 117</td>
<td>θη</td>
<td></td>
<td>(θη, θζ)</td>
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<tr>
<td>Ataxia telangietasia113, 114</td>
<td>θη</td>
<td></td>
<td>(θη, θ, δθ)</td>
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<tr>
<td>Psoriasis110, 114</td>
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</tbody>
</table>

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

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 apopotic cells are efficiently removed by phagocytic cells, preventing the release of potentially toxic cell contents. While the emphasis in the literature is given to apoptosis and cancer, other disease states clearly include disregulation 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 eosinophil106 and neutrophil107 apoptosis, respectively, are reduced; neurodegenerative disorders, including Alzheimer's disease, which involves increased apoptosis of neuronal cells108; vascular disease109; AIDS100 and ataxia telangietasia,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 telangietasia the connecting factor does not appear to involve apoptosis. For example, in Alzheimer's disease decreased expression of PKCB106 PKCE,107 PKCa and enzyme activity. These data suggest a role for PKCζ in cell survival and data from Berra et al.100 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 PKCζ is involved in execution of the apoptotic programme. In contrast, PKCa and ζ are frequently associated with cell survival and suppression of apoptosis. The role of PKCBβ and ζ remains to be clarified, though both are intimately associated with signalling pathways that modulate cell proliferation and apoptosis.  

ATAXIA TELANGIECTASIA

Ataxia telangietasia is an inherited autosomal recessive disorder, which includes a characteristic hypersensitivity to ionising radiation. The ataxia telangietasia 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 telangietasia.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 PKCe 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 PKCe 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 PKCe 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 PKCe but also the other PKC isoenzymes. PKCe is expressed at high levels in tumorigenic rat colon 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 PKCe.122 Kiss and Anderson123 showed that carcinogens reduced expression of PKCe in mouse embryo fibroblasts and inhibition of apoptosis in pros-tatic carcinoma cells was mediated by a activation and downregulation of PKCe.124 Thus, although several cell line studies suggest an oncogenic role for PKCe, this remains to be confirmed in analyses of human tumour biopsies.

<sup>106-110</sup> have been reported. Defective secretion of amyloid β protein has now been identified as the function directly affected by the alteration in PKCa.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 Curtum et al.112 did not consider individual PKC isoenzymes, further analysis is required to establish whether PKCs identified as involved in regulating neutrophil apoptosis113 are altered in chronic granulomatous disease.
The involvement of PKCa in neoplasia appears less controversial and, encouragingly, antisense oligonucleotides to PKCa have been used recently to decrease elevated levels of this PKC in glioblastoma cells and inhibit tumour growth. Whether this reduction in tumour growth was mediated via alterations in proliferation or apoptosis was not determined. The role of PKCa has also been investigated in breast carcinoma, using MCF-7 cells stably transfected to overexpress PKCa. Cells overexpressing PKCa 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 PKCβ.

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.

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. 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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