

therapy (Cory and Adams, 1998; Reed, 1999; Adams and Cory, 2001; Green and Evan, 2002). Although therapeutic approaches that block survival signals may tip the balance toward cell death, blocking such survival signals does not necessarily mean that cancer cells will automatically undergo apoptosis. In contrast, induction of apoptosis through modulation of key factors in the apoptotic pathway would have a direct and dominant effect on cells. Thus direct activation of the cell death response may be a better approach for cancer therapies. In this review, we focus on the PKC isoform best characterized in triggering apoptosis, PKC- (Brodie and Blumberg, 2003). Compared with PKC- , which provides a survival and proliferation signal, PKC- provides a more direct target to enhance apoptosis (Mandil et al, 2001).

Involvement of PKC- in apoptosis was first demonstrated by activation of PKC- in cells treated with a variety of apoptotic stimuli, including H₂O₂ (Konishi et al, 2001; Majumder et al, 2001), TNF- (Emoto et al, 1995), the Fas ligand (Scheel-Toellner et al, 1999), UV and irradiation (Denning et al, 1998; Yuan et al, 1998), and etoposide treatment (Reyland et al, 1999; Blass et al, 2002). Inhibition of PKC- activity by a PKC- -specific inhibitor, rottlerin, or by a dominant negative mutant resulted in suppression of the apoptotic response (Li et al, 1999; Majumder et al, 2000). Another important clue was shown in PKC- -deficient mice, which had an increased B cell population and formed numerous germinal centers in the absence of stimulation (Mecklenbrauker et al, 2002; Miyamoto et al, 2002). The observed abnormal B cell proliferation was associated with enhanced autoimmunity due to the persistence of self antigen-recognizing B cells that failed to undergo apoptosis during positive selection. Analysis of these knockout mice thus established a role for PKC- in controlling B-cell apoptosis in the regulation of B cell tolerance.

IV. Regulation of the activity of PKC-

Regulation of PKC- activity is mediated by at least three mechanisms. The regulatory C1 domain has an inhibitory effect on the catalytic domain found at the carboxyl terminus (Ohno, 1997; Parker, 1997). One way to release inhibition is by interaction of diacylglycerol or phorbol esters with the C1 domain, which triggers a conformational change. A second mechanism is mediated by cleavage of the catalytic domain from the C1 regulatory domain, which is achieved during apoptosis by activated caspase 3 (Ghayur et al, 1996; Denning et al, 2002). Tyrosine phosphorylation of PKC- at Tyr64 and 187 is essential for the cleavage and the apoptotic effect of PKC- (Blass et al, 2002). Tyr311 phosphorylation by Lck kinase after H₂O₂ treatment enhances basal PKC- activity and elevates its maximal activity in the presence of diacylglycerol (Konishi et al, 2001). Finally, activated PKC- undergoes ubiquitination and degradation through the proteasome pathway, which prevents a persistent effect of PKC- (Lu et al, 1998).

V. Translocation of PKC- during apoptotic responses

Depending on the cell types and the apoptotic stimuli, PKC- has been reported to translocate to nearly all subcellular organelles, including nuclei, mitochondria, the Golgi complex, endoplasmic reticulum (ER) and the plasma membrane (Brodie and Blumberg, 2003; Roychowdhury and Lahn, 2003). At each subcellular organelle, PKC- phosphorylates different substrates, inducing various responses that eventually lead to cell death. Identification of the substrates is critical to understanding the mechanism of PKC- , but it has been very challenging to identify physiologic substrates in each organelle. We define three criteria that must be met to convincingly claim any protein as a physiologic substrate of PKC- ; (1) there must be evidence that PKC- phosphorylates the protein, (2) there should be evidence for their interaction, and (3) most importantly, deletion or inactivation of the substrate must lead to at least a partial loss of PKC- -induced response. Here we review several known substrates of PKC- with a goal of connecting these substrates to the apoptotic pathway so that we can understand how PKC- induces apoptosis.

VI. PKC- substrates in the nucleus

Translocation of PKC- to the nucleus has been established in T cells and C6 glioma cells (Scheel-Toellner et al, 1999; Blass et al, 2002). A putative nuclear localization signal has been identified at the carboxyl terminus of the catalytic domain of PKC- (DeVries et al, 2002). Previously, nucleolin, which is required for nerve-growth factor (NGF)-induced differentiation of pheochromocytoma cells PC12, was identified as a substrate of PKC- (Zhou et al, 1997). However, neither PKC- nor PKC- can phosphorylate nucleolin, and nucleolin is not involved in the apoptotic response.

Recently, Yoshida et al, (2003) reported that PKC- is responsible for constitutive and DNA damage-induced phosphorylation of Rad9, a key factor involved in checkpoint regulation of the DNA damage response (al-Khodairy et al, 1994). The authors also demonstrated an interaction between PKC- and Rad9, and showed that PKC- phosphorylated Rad9 both *in vitro* and in cells treated with Cytarabine (ara-C) or irradiation (Yoshida et al, 2003). Nuclear Rad9 forms a critical heterotrimeric complex with Hus1 and Rad1, the 9-1-1 complex that is involved in DNA damage checkpoint control (Volkmer and Karnitz, 1999). PKC- , which translocated to the nucleus during apoptosis, enhanced the phosphorylation of Rad9 and the formation of the Rad9-Hus1-Rad1 complex (Yoshida et al, 2003). Interestingly, Rad9 is also phosphorylated by ATM (Chen et al, 2001) and c-Abl (Yoshida et al, 2002). The latter kinase also interacts with PKC- (Sun et al, 2000a, b). Using ATM siRNA to down-regulate the level of ATM, a diminished nuclear targeting of PKC- was observed, suggesting that ATM is required for nuclear targeting of PKC- and is functionally upstream of PKC- (Yoshida et al, 2003). These studies provide a direct linkage between PKC- and DNA

damage-induced checkpoint regulation, and form the basis for future studies of the mechanism of PKC- δ -induced apoptosis.

Another downstream effector of PKC- δ in DNA damage response in cells treated with ara-C is stress-activator protein kinase (SAPK/JNK) (Yoshida et al, 2002) (**Figure 1**). DNA damage-induced SAPK/JNK activation was attenuated by rottlerin, a dominant negative mutant of PKC- δ , and PKC- δ siRNA. PKC- δ did not directly phosphorylate SAPK/JNK, rather SAPK/JNK was indirectly phosphorylated through the mitogen-activated protein kinase (MAPK) pathway, PKC- δ → MEKK1 → MKK7 → SAPK/JNK (Yoshida et al, 2002). The finding that SAPK/JNK is a downstream effector of PKC- δ provides another mechanism of PKC- δ -induced apoptosis. Interestingly, SAPK/JNK was shown to be the substrate of PKC- δ and to translocate to mitochondria after phosphorylation to induce cytochrome c release (Ito et al, 2001a).

VII. PKC- δ substrates in the mitochondria

Translocation of PKC- δ to mitochondria was shown in U937 myeloid leukemia cells and keratinocytes (Li et al, 1999; Majumder et al, 2000). The translocation can be induced by phorbol ester (Denning et al, 1998) and the oxidative stress (Majumder et al, 2001). With UV irradiation, mitochondrial targeted PKC- δ was cleaved by caspase 3 to generate the active catalytic fragment of

PKC- δ (Denning et al, 2002). One known substrate of PKC- δ is c-Abl kinase (Sun et al, 2000a). It has been demonstrated that PKC- δ interacts with c-Abl, and that the phosphorylation of c-Abl results in activation of c-Abl kinase. Cells treated with H₂O₂ had an increase in c-Abl activity, which was attenuated by the PKC- δ inhibitor, rottlerin, and by overexpression of the regulatory domain of PKC- δ (Sun et al, 2000a). In the unstimulated condition, c-Abl localized to the nucleus, ER and cytoplasm. On ER stress caused by calcium ionophore A23187, brefeldin A or tunicamycin treatment, c-Abl translocated to mitochondria (Ito et al, 2001b).

The second mitochondrial target of PKC- δ is the phospholipid scramblase 3 (PLS3) (Liu et al, 2003a), a member of the scramblase family that is responsible for bidirectional movement of phospholipids in the lipid bilayer. Unlike PLS1, which is localized in the plasma membrane (Zhou et al, 1997; Sims and Wiedmer, 2001), PLS3 is found exclusively in the mitochondria (Liu et al, 2003a, b). The function of PLS3 is currently unclear, but is likely involved in translocation of cardiolipin from the mitochondrial inner membrane to the outer membrane during apoptosis. Mitochondria with expression of an inactive mutant of PLS3 have a low level of cardiolipin and poor respiration (Liu et al, 2003b). They also display a unique morphology, being larger in size, fewer in number, and with tightly packed cristae, consistent with the notion that PLS3 moves phospholipids from the inner membrane to the outer membrane (Liu et al, 2003b).

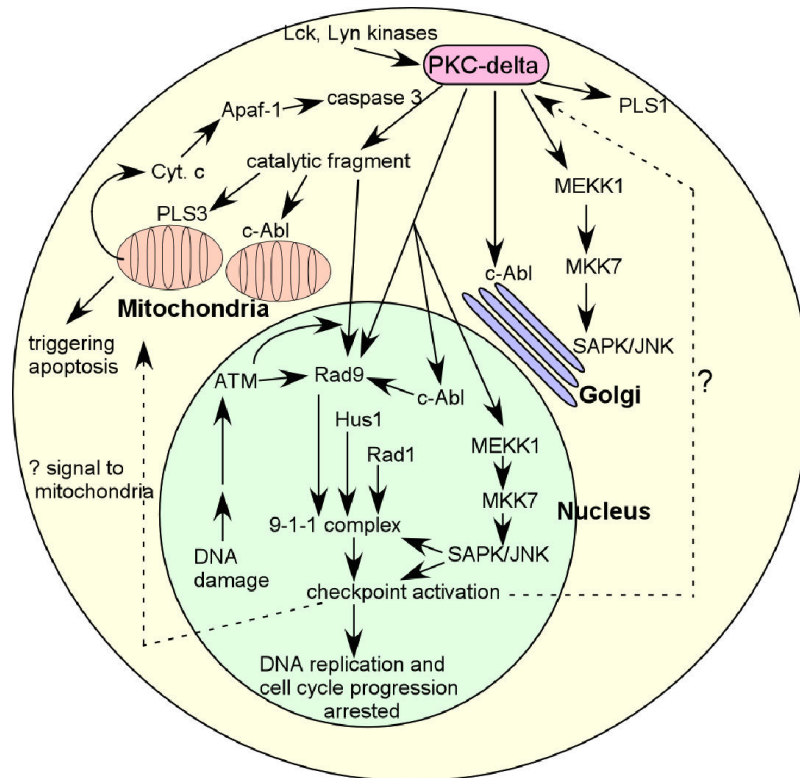


Figure 1. Diagram of PKC- δ and its downstream effectors in DNA damage-induced apoptosis. PKC- δ is localized in the cytoplasm before the induction of apoptosis. When DNA is damaged by apoptotic stimuli, PKC- δ translocates to the nucleus, where it phosphorylates Rad9 and c-Abl and activates MEKK1. PKC- δ also translocates to mitochondria, where it phosphorylates PLS3 and c-Abl, and to the Golgi, where it phosphorylates c-Abl and SAPK/JNK. In the plasma membrane, PKC- δ phosphorylates PLS1. The pathway from DNA damage to checkpoint activation and apoptosis is outlined.

Cardiolipin translocation is directly tied to the sensitivity of Cardiolipin translocation is directly tied to the sensitivity of mitochondria to tBid-induced cytochrome c release. Because tBid targeting to the mitochondria is mediated by cardiolipin (Lutter et al, 2000), the translocation of cardiolipin from the inner membrane to outer membrane facilitates the recruitment of tBid (Liu et al, 2003b). This idea was confirmed by the finding that mitochondria overexpressing PLS3 were more sensitive to tBid-induced cytochrome c release, whereas those expressing inactive mutant PLS3 were more resistant (Liu et al, 2003b).

PLS3 fulfills the three criteria we defined for a physiological substrate of PKC- . PLS3 can interact with PKC- and be phosphorylated by PKC- *in vitro* (Liu et al, 2003a). HeLa cells expressing PLS3 become more positive in TUNEL studies when they were treated with the phorbol ester, PMA. Expression of mitochondria-targeted PKC- in cells resulted in apoptosis, and overexpression of PLS3 enhanced this effect. In contrast, overexpression of the inactive PLS3 mutant did not generate this response (Liu et al, 2003a). These data support the view that PLS3 is a mitochondrial target of PKC- -induced apoptosis.

VIII. PKC- substrates in the plasma

Another member of PLS family, PLS1, has been shown to be a target of PKC- in the plasma membrane (Frasch et al, 2000). PLS1 is phosphorylated by PKC- at a PKC phosphorylation consensus site, Thr161. Co-expression of PKC- and PLS1 significantly increased the activity of scramblase following PMA treatment (Frasch et al, 2000). In contrast, co-expression of PKC- and a T161A mutant of PLS1 showed no increase in scramblase activity, indicating that phosphorylation of Thr161 by PKC- is important for scramblase function (Frasch et al, 2000). In addition, PLS1 can be phosphorylated by c-Abl, a kinase known to interact with PKC- in other organelles (Sun et al, 2001).

Although there is solid evidence that PLS1 is activated during apoptosis (Zhao et al, 1998 ; Frasch et al, 2000), a direct link between PLS1 and apoptosis has not been fully established. During apoptosis, phosphatidylserine (PS) translocates from the inner leaflet to the outer leaflet of the plasma membrane. The regulation of transbilayer movement of phospholipids is controlled by at least three enzymes. One is aminophospholipid translocase, or flippase, which moves phospholipids inwards. One is the phospholipid scramblase (PLS1) that moves phospholipids bidirectionally. The third is a less well-characterized outward-directed floppase (Bevers et al, 1999). Probably due to the complexity of the regulation of phospholipid topology in lipid bilayers, cells from mice with homozygous for a deletion of PLS1 still maintain their ability to translocate PS to the surface (Zhou et al, 2002). This could presumably be due to compensation by aminophospholipid translocase activity. Therefore the mechanism of surface translocation of PS remains unclear. It has been hypothesized that apoptosis is associated with

inactivation of aminophospholipid translocase and activation of the scramblase (Bevers et al, 1998; 1999), but definitive proof of this hypothesis has not yet been materialized.

A second plasma membrane target of PKC- is Fyn kinase, found in the plasma membrane of platelets (Crosby and Poole, 2003). In platelets, activation of PKC by phorbol ester induces platelet degranulation and activation of the integrin IIb III . Crosby *et al.* (2003) showed that activation of the platelet adhesion complex is associated with interaction of Fyn kinase and PKC- , but not other members of the PKC family. Fyn kinase is also phosphorylated at a serine residue that is found within a PKC consensus sequence. Whether this finding is tied to apoptosis is unknown.

IX. PKC- is a survival factor in several cancer cells

In addition to promoting apoptosis, PKC- also enhances survival in several types of cancer cells. For example, one study using non-small cell lung cancer (NSCLC) cells, most of the PKC isoforms had enhanced phosphorylation compared to primary human lung epithelial cells (Clark et al, 2003). These authors also showed that blocking PKC- with rottlerin was highly effective in potentiating chemotherapy-induced apoptosis, and that transfection of cells with a kinase-dead mutant of PKC- increased apoptosis (Clark et al, 2003). McCracken et al, (2003) used antisense oligonucleotides to down-regulate various PKC isoforms in MCF-7 breast cancer cells, and found that down-regulation of PKC- impaired survival in response to -irradiation. Similar findings were achieved with rottlerin and a dominant-negative mutant of PKC- . However, neither of these two studies addressed the translocation of PKC- . Therefore, it is unclear whether the observed opposite effects of PKC- are due to differences in translocation or to targeting of different substrates in each cell line.

X. Unanswered questions

When DNA is damaged by chemotherapeutic agents or irradiation, one would imagine that an early event would be activation of a checkpoint to stop DNA replication or cell cycle progression. This step might be followed by DNA repair or by apoptosis if the damage is too extensive to repair. PKC- clearly plays an important role in both of these steps. However, it remains an unanswered question how signals are transmitted from checkpoint activation to PKC- or to mitochondria to activate the cell death cascade (see diagram). This is perhaps the most important question in current studies of apoptosis.

XI. Using PKC- as a therapeutic target

Based on the ample evidence that PKC- enhances apoptotic responses in certain systems, attempts have been

made to target PKC- in cancers in which PKC- is known to play a pro-apoptotic, but not pro-survival, role. This has been a very challenging task because none of the PKC activators is specific enough to activate solely PKC- without any stimulation of the other classic PKCs. One promising drug is a derivative of adriamycin, N-benzyladriamycin-14-valerate (AD198), which does not inhibit topoisomerase II and binds DNA weakly in contrast to its parental drug adriamycin (Barrett et al, 2002, Roaten et al, 2002). AD198 has a potent anti-tumor effect through activation of PKC- by interacting with the regulatory domain of PKC- like the phorbol ester. More importantly, AD198 can override the anti-apoptotic effect of Bcl-2, a common problem in many malignancies (Barrett et al, 2002). It will be interesting to see how effective AD198 is in future clinical studies.

Another potential approach is gene therapy to introduce PKC- by adenoviral vectors, which have been achieved in cell lines (Li et al, 1999). Other approaches include blocking PKC- degradation through inhibition of the ubiquitin-proteasome pathway (Lu et al, 1998), for which bortezomib (PS-341) is available. We have observed that bortezomib induces activation and accumulation of PKC- in mitochondria and that the PKC-inhibitor rottlerin compromises the apoptotic effect of bortezomib (Durrant and Lee, manuscript in preparation). Finally, modulation of tyrosine phosphorylation of PKC- through blocking of Src kinase family and activation of PKC- is also possible (Joseloff et al, 2002), as there are Src kinase inhibitors in the preclinical developmental stage.

XII. Using downstream effectors of PKC- as therapeutic targets

Given the dual roles of PKC- in promoting survival and apoptosis, it might be better to utilize downstream effectors of PKC- as potential targets for induction of apoptosis in cancer therapy. One potential target in both the nucleus and mitochondria is c-Abl, since a drug is available, STI-571 (Gleevec), that blocks the tyrosine kinase activity of the fusion protein Bcr-Abl, and effectively controls the proliferation and induces apoptosis in chronic myelocytic leukemia (CML) cells with minimal side effects. STI-571 is widely used to treat CML (Druker et al, 2001; O'Dwyer et al, 2003). However, the *in vivo* situation may be more complicated with the kinase activity of c-Abl acting as a double-edged sword, like PKC-. With DNA damage-induced apoptosis, the kinase activity of c-Abl is activated by PKC--induced phosphorylation, and is required for the apoptotic response. Given this fact, we predict that blocking the kinase activity of c-Abl will inhibit apoptosis, an opposite effect to that observed in CML cells. This concern was confirmed by Kumar *et al.*, who demonstrated that STI-571 abrogated the cell death response to H₂O₂ in U937 myeloid leukemia cells (Kumar et al, 2003). This anti-apoptotic effect of STI-571 may explain why STI-571 is only effective in very limited numbers of cancers.

What about the possibility of using Rad9 as a target for the induction of apoptosis? Indeed, overexpression of Rad9 in cells was shown to be pro-apoptotic; whereas down-regulation of Rad9 by an antisense vector suppressed apoptosis (Komatsu et al, 2000). The mechanism of Rad9-induced apoptosis is unclear, but it has been shown that Rad9 contains a BH3 domain and can interact with Bcl-2 and Bcl-xL. Hence Rad9-induced apoptosis could be mediated by inhibition of Bcl-2 through its BH3 domain, or indirectly through the general DNA damage-induced apoptotic pathway. Until the mechanism is sorted out, it may not be practical to use Rad9 as a therapeutic target.

Another potential target is PLS3, a newly recognized member of the scramblase family that is present in the mitochondria. There is limited information regarding the function of this enzyme, though its function is apparently very critical for mitochondria. It has been reported that overexpression of wild-type PLS3 enhanced UV-induced apoptosis; whereas expression of an inactive PLS3 mutant suppressed apoptosis. Using ³²P labeling and TLC analysis of the phospholipids in mitochondrial inner and outer membranes, Liu *et al.* (2003b) found that PLS3 may be responsible for moving cardiolipin from the mitochondrial inner membrane to the outer membrane during apoptosis. The translocation of cardiolipin to the mitochondrial outer membrane enhanced sensitivity to tBid-induced cytochrome c release. To target PLS3 to induce apoptosis, we need to have a better understanding of PLS3, and to identify compounds that activate PLS3 to induce mitochondrial apoptosis. A caveat to this approach is that PLS3 has transmembrane domains and thus it is technically difficult to obtain large quantities of recombinant protein for high-throughput screening to identify such activators.

Could the MAPK pathway be targeted based on the observation that PKC- activates SAPK/JNK? The activation of the DNA-damage checkpoint is a protective mechanism to prevent cell cycle progression in the presence of DNA damage. When DNA damage becomes so severe that it is beyond the capacity of repair, the apoptotic pathway is activated. It is currently unknown how DNA damage signals are transmitted to mitochondria. PKC- and SAPK/JNK are candidates, but the exact mechanism is far from clear. Inhibitors of various MAPK pathways are currently available and may proceed to clinical trials in the near future. Potential advantages include the fact that they block survival signals and down-regulate checkpoint regulation to facilitate the activation of apoptosis.

In conclusion, PKC- is clearly an important mediator of the apoptotic pathway with diverse downstream effectors in cells. These facts make it a good target for the development of therapeutic interventions, with the potential benefit of avoiding the development of drug resistance. We anticipate the future development of PKC--specific activators, such as AD198, and further understanding of its downstream effectors such as Rad9 and PLS3, so that novel cancer therapeutic approaches can be developed utilizing these targets. Moreover, the novel PKC--based therapy may well be used in combination to

create synergism with other agents and help to prevent the development of drug resistance.

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References

- Adams, J.M. and S. Cory (2001) Life-or-death decisions by the Bcl-2 protein family. **Trends Biochem Sci** 26, 61-6.
- al-Khodairy, F., E. Fotou, K.S. Sheldrick, D.J. Griffiths, A.R. Lehmann, and A.M. Carr (1994) Identification and characterization of new elements involved in checkpoint and feedback controls in fission yeast. **Mol Biol Cell** 5, 147-60
- Barrett, C. M., F. L. Lewis, et al. (2002) Novel extranuclear-targeted anthracyclines override the antiapoptotic functions of Bcl-2 and target protein kinase C pathways to induce apoptosis. **Mol Cancer Ther** 1: 469-81.
- Bevers, E.M., P. Comfurius, D.W. Dekkers, and R.F. Zwaal (1999) Lipid translocation across the plasma membrane of mammalian cells. **Biochim Biophys Acta** 1439, 317-30.
- Bevers, E.M., P. Comfurius, D.W. Dekkers, M. Harmsma, and R.F. Zwaal (1998) Transmembrane phospholipid distribution in blood cells: control mechanisms and pathophysiological significance. **Biol Chem** 379, 973-86.
- Blass, M., I. Kronfeld, G. Kazimirsky, P.M. Blumberg, and C. Brodie (2002) Tyrosine phosphorylation of protein kinase Cdelta is essential for its apoptotic effect in response to etoposide. **Mol Cell Biol** 22, 182-95.
- Brodie, C. and P.M. Blumberg (2003) Regulation of cell apoptosis by protein kinase c delta. **Apoptosis** 8, 19-27.
- Chen, M.J., Y.T. Lin, H.B. Lieberman, G. Chen, and E.Y. Lee (2001) ATM-dependent phosphorylation of human Rad9 is required for ionizing radiation-induced checkpoint activation. **J Biol Chem** 276, 16580-6.
- Clark, A.S., K.A. West, P.M. Blumberg, and P.A. Dennis (2003) Altered protein kinase C (PKC) isoforms in non-small cell lung cancer cells: PKCdelta promotes cellular survival and chemotherapeutic resistance. **Cancer Res** 63, 780-6.
- Cory, S. and J.M. Adams (1998) Matters of life and death: programmed cell death at Cold Spring Harbor. **Biochim Biophys Acta** 1377, R25-44.
- Crosby, D. and A.W. Poole (2003) Physical and functional interaction between protein kinase C delta and Fyn tyrosine kinase in human platelets. **J Biol Chem** 278, 24533-41.
- Denning, M.F., Y. Wang, B.J. Nickoloff, and T. Wrone-Smith (1998) Protein kinase Cdelta is activated by caspase-dependent proteolysis during ultraviolet radiation-induced apoptosis of human keratinocytes. **J Biol Chem** 273, 29995-30002.
- Denning, M.F., Y. Wang, S. Tibudan, S. Alkan, B.J. Nickoloff, and J.Z. Qin (2002) Caspase activation and disruption of mitochondrial membrane potential during UV radiation-induced apoptosis of human keratinocytes requires activation of protein kinase C. **Cell Death Differ** 9, 40-52.
- DeVries, T.A., M.C. Neville, and M.E. Reyland (2002) Nuclear import of PKCdelta is required for apoptosis: identification of a novel nuclear import sequence. **EMBO J** 21, 6050-60.
- Druker, B.J., M. Talpaz, D.J. Resta, B. Peng, E. Buchdunger, J.M. Ford, N.B. Lydon, H. Kantarjian, R. Capdeville, S. Ohno-Jones, and C.L. Sawyers (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. **N Engl J Med** 344, 1031-7.
- Emoto, Y., Y. Manome, G. Meinhardt, H. Kasaki, S. Kharbanda, M. Robertson, T. Ghayur, W.W. Wong, R. Kamen, R. Weichselbaum, and et al. (1995) Proteolytic activation of protein kinase C delta by an ICE-like protease in apoptotic cells. **EMBO J** 14, 6148-56.
- Frasch, S.C., P.M. Henson, J.M. Kailey, D.A. Richter, M.S. Janes, V.A. Fadok, and D.L. Bratton (2000) Regulation of phospholipid scramblase activity during apoptosis and cell activation by protein kinase Cdelta. **J Biol Chem** 275, 23065-73.
- Ghayur, T., M. Hugunin, R.V. Talanian, S. Ratnofsky, C. Quinlan, Y. Emoto, P. Pandey, R. Datta, Y. Huang, S. Kharbanda, H. Allen, R. Kamen, W. Wong, and D. Kufe (1996) Proteolytic activation of protein kinase C delta by an ICE/CED 3-like protease induces characteristics of apoptosis. **J Exp Med** 184, 2399-404.
- Green, D.R. and G.I. Evan (2002) A matter of life and death. **Cancer Cell** 1, 19-30.
- Hofmann, J. (2001) Modulation of protein kinase C in antitumor treatment. **Rev Physiol Biochem Pharmacol** 142, 1-96.
- Ito, Y., N.C. Mishra, K. Yoshida, S. Kharbanda, S. Saxena, and D. Kufe (2001) Mitochondrial targeting of JNK/SAPK in the phorbol ester response of myeloid leukemia cells. **Cell Death Differ** 8, 794-800.
- Ito, Y., P. Pandey, N. Mishra, S. Kumar, N. Narula, S. Kharbanda, S. Saxena, and D. Kufe (2001) Targeting of the c-Abl Tyrosine Kinase to Mitochondria in Endoplasmic Reticulum Stress-Induced Apoptosis. **Mol Cell Biol** 21, 6233-42.
- Joseloff, E., C. Cataisson, H. Aamodt, H. Ocheni, P. Blumberg, A.J. Kraker, S.H. Yuspa (2002) Src family kinases phosphorylate protein kinase C delta on tyrosine residues and modify the neoplastic phenotype of skin keratinocytes. **J Biol Chem** 277: 12318-23.
- Komatsu, K., T. Miyashita, H. Hang, K.M. Hopkins, W. Zheng, S. Cuddeback, M. Yamada, H.B. Lieberman, and H.G. Wang (2000) Human homologue of S. pombe Rad9 interacts with BCL-2/BCL-xL and promotes apoptosis. **Nat Cell Biol** 2, 1-6.
- Konishi, H., E. Yamauchi, H. Taniguchi, T. Yamamoto, H. Matsuzaki, Y. Takemura, K. Ohmae, U. Kikkawa, and Y. Nishizuka (2001) Phosphorylation sites of protein kinase C delta in H2O2-treated cells and its activation by tyrosine kinase in vitro. **Proc Natl Acad Sci U S A** 98, 6587-92.
- Kumar, S., N. Mishra, D. Raina, S. Saxena, and D. Kufe (2003) Abrogation of the cell death response to oxidative stress by the c-Abl tyrosine kinase inhibitor STI571. **Mol Pharmacol** 63, 276-82.
- Li, L., P.S. Lorenzo, K. Bogi, P.M. Blumberg, and S.H. Yuspa (1999) Protein kinase Cdelta targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector. **Mol Cell Biol** 19, 8547-58.
- Liu, J., J. Chen, Q. Dai, and R.M. Lee (2003) Phospholipid Scramblase 3 Is the Mitochondrial Target of Protein Kinase C delta-induced Apoptosis. **Cancer Res** 63, 1153-6.
- Liu, j., Q. Dai, J. Chen, D. Durrant, A. Freeman, T. Liu, D. Grossman, and R.M. Lee (2003) Phospholipid scramblase 3 controls mitochondrial structure, function and apoptotic response. **Molecular Cancer Research** 1, 892-902.
- Lu, Z., D. Liu, A. Hornia, W. Devonish, M. Pagano, and D.A. Foster (1998) Activation of protein kinase C triggers its ubiquitination and degradation. **Mol Cell Biol** 18, 839-45.
- Lutter, M., M. Fang, X. Luo, M. Nishijima, X. Xie, and X. Wang (2000) Cardiolipin provides specificity for targeting of tBid to mitochondria. **Nat Cell Biol** 2, 754-761.
- Majumder, P.K., N.C. Mishra, X. Sun, A. Bharti, S. Kharbanda, S. Saxena, and D. Kufe (2001) Targeting of protein kinase C delta to mitochondria in the oxidative stress response. **Cell Growth Differ** 12, 465-70.

- Majumder, P.K., P. Pandey, X. Sun, K. Cheng, R. Datta, S. Saxena, S. Kharbanda, and D. Kufe (2000) Mitochondrial translocation of protein kinase C delta in phorbol ester-induced cytochrome c release and apoptosis. **J Biol Chem** 275, 21793-6.
- Mandil, R., E. Ashkenazi, M. Blass, I. Kronfeld, G. Kazimirsky, G. Rosenthal, F. Umansky, P.S. Lorenzo, P.M. Blumberg, and C. Brodie (2001) Protein kinase Calpha and protein kinase Cdelta play opposite roles in the proliferation and apoptosis of glioma cells. **Cancer Res** 61, 4612-9.
- Marshall, J.L., N. Bangalore, D. El-Ashry, Y. Fuxman, M. Johnson, B. Norris, M. Oberst, E. Ness, S. Wojtowicz-Praga, P. Bhargava, N. Rizvi, S. Baidas, and M.J. Hawkins (2002) Phase I study of prolonged infusion Bryostatin-1 in patients with advanced malignancies. **Cancer Biol Ther** 1, 409-16.
- McCracken, M.A., L.J. Miraglia, R.A. McKay, and J.S. Strobl (2003) Protein kinase C delta is a prosurvival factor in human breast tumor cell lines. **Mol Cancer Ther** 2, 273-81.
- Mecklenbrauker, I., K. Saijo, N.Y. Zheng, M. Leitges, and A. Tarakhovsky (2002) Protein kinase Cdelta controls self-antigen-induced B-cell tolerance. **Nature** 416, 860-5.
- Miyamoto, A., K. Nakayama, H. Imaki, S. Hirose, Y. Jiang, M. Abe, T. Tsukiyama, H. Nagahama, S. Ohno, S. Hatakeyama, and K.I. Nakayama (2002) Increased proliferation of B cells and auto-immunity in mice lacking protein kinase Cdelta. **Nature** 416, 865-9.
- O'Dwyer, M.E., M.J. Mauro, and B.J. Druker (2003) STI571 as a targeted therapy for CML. **Cancer Invest** 21, 429-38.
- Ohno, S., The distinct Biological Potential of PKC Isozymes, in Protein kinase C, P. Parker and L.V. Dekker Editors. 1997, R.G. Landes Company.
- Parker, P.J. and Dekker, L.V., Protein kinase C. 1997: R. G. Landes Company.
- Reed, J.C. (1999) Dysregulation of apoptosis in cancer. **J Clin Oncol** 17, 2941-53.
- Reyland, M.E., S.M. Anderson, A.A. Matassa, K.A. Barzen, and D.O. Quissell (1999) Protein kinase C delta is essential for etoposide-induced apoptosis in salivary gland acinar cells. **J Biol Chem** 274, 19115-23.
- Roaten, J. B., M. G. Kazanietz, M.J. Caloca, P.J. Bertics, L. Lothstein, A.L. Parrill, M. Israel, T.W. Sweatman (2002) Interaction of the novel anthracycline antitumor agent N-benzyladriamycin-14-valerate with the C1-regulatory domain of protein kinase C: structural requirements, isoform specificity, and correlation with drug cytotoxicity. **Mol Cancer Ther** 1: 483-92.
- Roychowdhury, D. and M. Lahn (2003) Antisense therapy directed to protein kinase C-alpha (Affinitak, LY900003/ISIS 3521): potential role in breast cancer. **Semin Oncol** 30, 30-3.
- Scheel-Toellner, D., D. Pilling, A.N. Akbar, D. Hardie, G. Lombardi, M. Salmon, and J.M. Lord (1999) Inhibition of T cell apoptosis by IFN-beta rapidly reverses nuclear translocation of protein kinase C-delta. **Eur J Immunol** 29, 2603-12.
- Sims, P.J. and T. Wiedmer (2001) Unraveling the mysteries of phospholipid scrambling. **Thromb Haemost** 86, 266-75.
- Sun, J., J. Zhao, M.A. Schwartz, J.Y. Wang, T. Wiedmer, and P.J. Sims (2001) c-Abl tyrosine kinase binds and phosphorylates phospholipid scramblase 1. **J Biol Chem** 276, 28984-90.
- Sun, X., F. Wu, R. Datta, S. Kharbanda, and D. Kufe (2000) Interaction between protein kinase C delta and the c-Abl tyrosine kinase in the cellular response to oxidative stress. **J Biol Chem** 275, 7470-3.
- Sun, X., P. Majumder, H. Shioya, F. Wu, S. Kumar, R. Weichselbaum, S. Kharbanda, and D. Kufe (2000) Activation of the cytoplasmic c-Abl tyrosine kinase by reactive oxygen species. **J Biol Chem** 275, 17237-40.
- Swannie, H.C. and S.B. Kaye (2002) Protein kinase C inhibitors. **Curr Oncol Rep** 4, 37-46.
- Volkmer, E. and L.M. Karnitz (1999) Human homologs of Schizosaccharomyces pombe rad1, hus1, and rad9 form a DNA damage-responsive protein complex. **J Biol Chem** 274, 567-70.
- Wang, X.Y., E. Repasky, and H.T. Liu (1999) Antisense inhibition of protein kinase Calpha reverses the transformed phenotype in human lung carcinoma cells. **Exp Cell Res** 250, 253-63.
- Yoshida, K., H.G. Wang, Y. Miki, and D. Kufe (2003) Protein kinase Cdelta is responsible for constitutive and DNA damage-induced phosphorylation of Rad9. **Embo J** 22, 1431-41.
- Yoshida, K., K. Komatsu, H.G. Wang, and D. Kufe (2002) c-Abl tyrosine kinase regulates the human Rad9 checkpoint protein in response to DNA damage. **Mol Cell Biol** 22, 3292-300.
- Yoshida, K., Y. Miki, and D. Kufe (2002) Activation of SAPK/JNK signaling by protein kinase Cdelta in response to DNA damage. **J Biol Chem** 277, 48372-8.
- Yuan, Z.M., T. Utsugisawa, T. Ishiko, S. Nakada, Y. Huang, S. Kharbanda, R. Weichselbaum, and D. Kufe (1998) Activation of protein kinase C delta by the c-Abl tyrosine kinase in response to ionizing radiation. **Oncogene** 16, 1643-8.
- Zhao, J., Q. Zhou, T. Wiedmer, and P.J. Sims (1998) Level of expression of phospholipid scramblase regulates induced movement of phosphatidylserine to the cell surface. **J Biol Chem** 273, 6603-6.
- Zhou, G., M.L. Seibenhener, and M.W. Wooten (1997) Nucleolin is a protein kinase C-zeta substrate. Connection between cell surface signaling and nucleus in PC12 cells. **J Biol Chem** 272, 31130-7.
- Zhou, Q., J. Zhao, J.G. Stout, R.A. Luhm, T. Wiedmer, and P.J. Sims (1997) Molecular cloning of human plasma membrane phospholipid scramblase. A protein mediating transbilayer movement of plasma membrane phospholipids. **J Biol Chem** 272, 18240-4.
- Zhou, Q., J. Zhao, T. Wiedmer, and P.J. Sims (2002) Normal hemostasis but defective hematopoietic response to growth factors in mice deficient in phospholipid scramblase 1. **Blood** 99, 4030-8.



Dr. Ray M. Lee

