We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

8

Pathophysiology of Protein Kinase C Isozymes in Chronic Lymphocytic Leukaemia

John C. Allen and Joseph R. Slupsky
Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom

1. Introduction

This chapter will review the roles of protein kinase C (PKC) isozymes in chronic lymphocytic leukaemia (CLL) cells. PKC family proteins are central to many signalling pathways within cells, and some have been implicated in the oncogenesis of numerous cancers (Benimetskaya, *et al.*, 2001;Keenan, *et al.*, 1999;O'Brian, 1998). In CLL, inhibitors of PKC signalling have been shown to have cytotoxic effects on the malignant cells, and the α, β and δ isoforms of PKC have been shown to have pathophysiological roles (Holler, *et al.*, 2009;Nakagawa, *et al.*, 2006;Ringshausen, *et al.*, 2002). The aim of this chapter is to discuss whether PKC can be considered a drug target in the treatment of this disease. We will examine how inhibitors of PKCs have been used in past preclinical studies of CLL, and will discuss the roles of various PKC isozymes (namely PKCβII, PKCα, PKCδ and PKCε) in the pathology of CLL. This chapter will end with the proposal that inhibition of PKC may be useful in combination therapy through a potential role in regulating Mcl-1 expression.

2. PKC in CLL

Survival and expansion of the malignant clone in CLL involves a myriad of intrinsic and extrinsic signals and most, if not all of these signals will involve the kinase function of PKC. For example, chronic antigen stimulation of the B-cell receptor (BCR) is thought to play a key role in CLL cell survival (Chiorazzi, *et al.*, 2005), and the β isoform of PKC (PKCβ) is known to play an important role in BCR signalling (Kang, *et al.*, 2001;Saijo, *et al.*, 2002). In this context, specific targeting of PKCβ in CLL cells may either enhance or inhibit the pro-survival signals that BCR engagement provides.

A role for PKC function in CLL cell survival was first suggested in experiments using PKC agonists such as the phorbol ester 12-*0* tetradecanoylphorbol 13-acetate (TPA) and bryostatin (al-Katib, *et al.*, 1993;Drexler, *et al.*, 1989;Forbes, *et al.*, 1992;Totterman, *et al.*, 1980). These compounds are natural product analogues of diacylglycerol, which is the ligand of PKC within cells, and act to stimulate kinase activity of PKC. Initial observations showed that treatment of CLL cells with either TPA or bryostatin-1 resulted in the induction of differentiation and inhibition of spontaneous apoptosis (al-Katib, *et*

al., 1993;Barragan, *et al.*, 2002;Drexler, *et al.*, 1989;Forbes, *et al.*, 1992;Totterman, *et al.*, 1980;Varterasian, *et al.*, 2000). Exploration of the mechanism through which TPA and bryostatin induced CLL cell differentiation showed that this was likely due to PKC-mediated activation of the ERK pathway (Figure 1A). These early experiments prompted a phase I (Varterasian, *et al.*, 1998) and phase II (Varterasian, *et al.*, 2000) clinical trial of bryostatin in CLL. The findings of these studies showed that bryostatin could induce *in vivo* differentiation of the malignant cells in CLL patients (Varterasian, *et al.*, 2000). Combination of bryostatin with 2-chlorodeoxyadenosine showed promise in treating CLL in both an animal model of CLL (Mohammad, *et al.*, 1998) as well as a case report of a single patient (Ahmad, *et al.*, 2000), however, the use of bryostatin as a therapeutic agent has not been followed up. This could be because other studies have shown that TPA and bryostatin provide protection against dexamethasone- and fludarabine-induced apoptosis of CLL cells (Bellosillo, *et al.*, 1997;Kitada, *et al.*, 1999). Investigation of the mechanism through which this protection is provided showed that these compounds stimulate upregulation of the anti-apoptotic proteins Mcl-1 and XIAP (Thomas, *et al.*, 2004) (Figure 1A).

A second approach to address the role of PKC in CLL cell survival has used inhibitors of this enzyme. Thus, compounds such as UCN01 (Byrd, *et al.*, 2001;Kitada, *et al.*, 2000), PKC412 (Ganeshaguru, *et al.*, 2002), LY379196 (Abrams, *et al.*, 2007) and Bisindolymaleimide (Barragan, *et al.*, 2002;Snowden, *et al.*, 2003) have all been shown to potently induce apoptosis of CLL cells *in vitro*. Interestingly, treatment of CLL cells with UCN01 or Bisindolylmaleimide reduces the expression of Mcl-1 and XIAP (Kitada, *et al.*, 2000;Snowden, *et al.*, 2003), thereby making treated cells more susceptible to apoptosis (Figure 1B). This observation, when taken together with others showing that activation of PKC results in an upregulation of Mcl-1 and XIAP, strongly suggest that PKC is an important mediator of CLL cell survival signals.

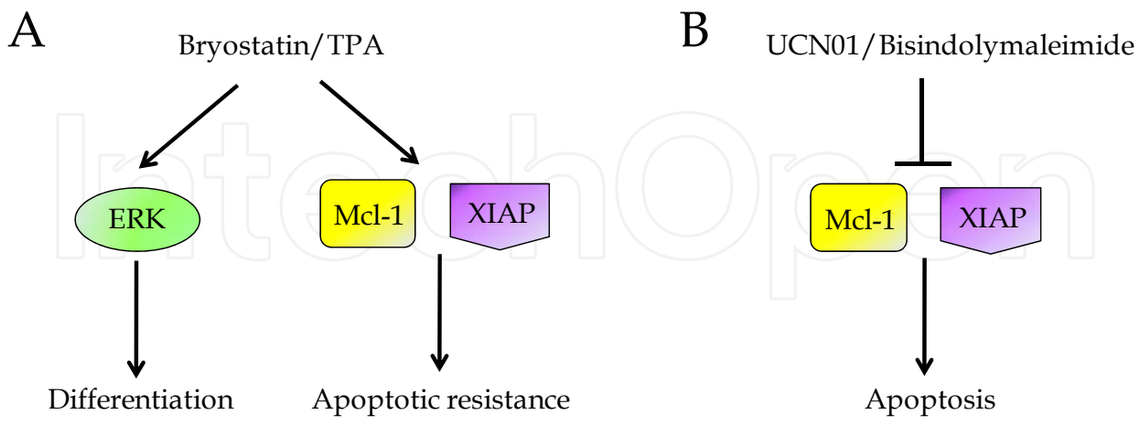


Fig. 1. Effects of PKC agonists and antagonists on CLL cells. (A) PKC agonists such as TPA and bryostatin induce ERK-mediated differentiation in CLL cells, and inhibit spontaneous apoptosis by stimulating the expression of Mcl-1 and XIAP. (B) PKC antagonists such as UCN01 or Bisindolymaleimide reduce the expression of Mcl-1 and XIAP in CLL cells thereby increasing the potential of CLL cells to undergo apoptosis.

2.1 PKC structure and function

PKCs are a family of serine/threonine kinases that share extensive structural homologies between different isotypes. Despite this homology, PKCs regulate different cellular functions in a variety of cell types, including proliferation, differentiation, apoptosis and cell survival (Tan & Parker, 2003). PKCs are divided into three subfamilies based on their regulatory domain composition, which determines what co-factors help induce their activation. Classical PKCs (PKC α, βI, βII, and γ) require the presence of DAG and calcium for activation, while novel PKCs (PKC δ, ε, η, θ) require only the presence of DAG. In contrast, atypical PKCs (PKC ζ, λ/ι) are both calcium and diacylglycerol-independent (Mellor & Parker, 1998).

The structure of all PKC family members is comprised of a C-terminal kinase domain linked by a flexible hinge segment to an N-terminal regulatory domain (Parker & Murray-Rust, 2004) (Figure 2). The kinase domain of PKC is highly conserved among isoforms and shows homology to the AGC superfamily of serine/threonine protein kinases. This domain contains the ATP- and substrate-binding sites, and also serves as a phosphorylation-dependent docking site for the regulatory molecules that interact with PKC (Newton, 2010).

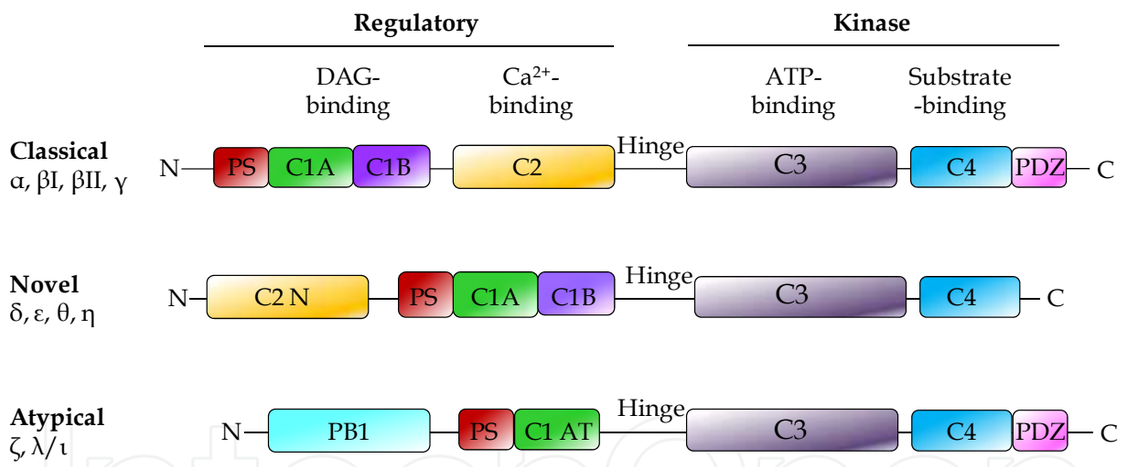


Fig. 2. Schematic representation of PKC isoform structure. The regulatory domain of PKC isoforms contain the regions necessary for membrane association and activation of the kinase. The C1 domain binds DAG/phorbol esters, and also contains a pseudosubstrate (PS) sequence at its N-terminus. The PS binds to the substrate-binding site within the catalytic domain to hold PKC in an inactive state. Atypical PKCs have a unique C1 region (C1 AT) as well as a Phox and Bem1 (PB1) region which are likely responsible for protein interaction resulting in kinase activation. The C2 domain regulates Ca^{2+}-mediated phospholipid binding in classical PKCs. Novel PKCs have a C2-like domain that does not bind Ca^{2+} (C2 N). The catalytic domain of all PKCs is conserved and contains the regions necessary for ATP-binding (the C3 domain) and for binding to substrate (the C4 domain). A PDZ region is also present in some PKC isoforms, and is responsible for protein-protein interactions following kinase activation.

The regulatory domain of PKC is divided into two regions. At the N-terminus there is a pseudosubstrate (PS) sequence that is responsible for binding the catalytic domain and maintaining the enzyme in an inactive conformation when it is in the cytoplasm (House & Kemp, 1987). This domain of PKC also contains the regions responsible for membrane targeting. Thus, classical PKCs contain motifs, termed C1 domains, that are able to bind DAG as well as phorbol esters (Newton, 1995a). Classical PKCs also have motifs, termed C2 domains, that are responsible for binding membrane phospholipids such as phosphatidylserine (PtS) and phosphatidylinositol-4-5-biphosphate (PIP_2) in a Ca^{2+}-dependent manner (Newton, 1995a). Novel and atypical PKC isoforms have a different regulatory domain structure. Novel PKCs contain tandem C1 domains that bind DAG with an affinity that is high enough to induce translocation to the membrane (Giorgione, *et al.*, 2006), and use a C2-like domain to bind phospholipids in a Ca^{2+}-independent manner (Newton, 1995a). In contrast, atypical PKCs lack a C2 domain in any format, and contain an impaired C1 domain that does not bind diacylglycerol (Newton, 1995a). Instead, atypical PKC isoforms depend largely upon protein-protein interactions for activation. For this purpose, these isoforms contain an N-terminal PB1 domain and a C-terminal PDZ ligand binding domain.

The flexible hinge region of PKCs is important in as much as it allows the close apposition of the regulatory and catalytic domains when PKC is in an inactive state. When PKC becomes activated, the hinge region allows the protein to unfold to the extent needed for the catalytic domain to interact with substrates and regulatory proteins.

2.2 PKC regulation

PKC is regulated by four key mechanisms: phosphorylation, co-factor binding, protein-protein interactions and regulated degradation. All help regulate the subcellular localisation, structure, and function of the enzyme.

2.2.1 Processing of PKC

Newly synthesised PKC is associated with membrane fractions where it is processed by a series of tightly coupled phosphorylations on serine and/or threonine residues in the catalytic domain (Newton, 2010) (Figure 3). These phosphorylations are essential before PKC can become activated, and the series in which they take place is analogous to other AGC protein kinases such as Akt. The binding of the chaperone protein heat shock protein 90 (HSP90) was identified as an initial step in the maturation of both classical and novel PKC isoforms (Gould, *et al.*, 2009). It binds to the catalytic domain of PKC and primes the enzyme for phosphorylation within the activation loop of the catalytic domain (Figure 3A). Failure of PKC to bind HSP90 results in inhibited phosphorylation at this site, misfolding of the entire protein and its consequent degradation (Balendran, *et al.*, 2000;Gould, *et al.*, 2009). Phosphorylation of the activation loop of PKC is catalyzed by 3-phosphoinositide-dependent kinase (PDK)-1, which binds to the exposed C-terminus of newly synthesised PKC that is in complex with HSP90 (Chou, *et al.*, 1998;Dutil, *et al.*, 1998;Dutil & Newton, 2000) (Figure 3A). This is followed by phosphorylation of the turn motif by the mTORC2 complex (Ikenoue, *et al.*, 2008) (Figure 3B). Phosphorylation of the turn motif stabilises the active conformation of PKC prior to autophosphorylation of the hydrophobic motif and

generation of catalytically competent PKC (Behn-Krappa & Newton, 1999). Whether this latter step results from autophosphorylation is controversial because phosphorylation of the hydrophobic motif does not take place in mTORC2 deficient cells (Newton, 2010). However, because phosphorylation of the turn motif must take place before phosphorylation of the hydrophobic motif, it is likely to be very difficult to fully define the kinase(s) responsible. It is important to note here that phosphorylation of the activation loop, turn and hydrophobic motifs within PKC only results in an enzyme that is fully matured and catalytically competent, it should not be mistaken for active PKC as these sites will be phosphorylated on inactive PKC located within the cytoplasm of cells.

2.2.2 Mechanism(s) of activation

Fully matured PKC is predominantly localised to the cytosol, where it is likely maintained in specific microenvironments by interacting with regulatory proteins (Schechtman & Mochly-Rosen, 2001). Here, the enzyme is held in an inactive conformation by the N-terminal PS binding to the substrate-binding site of the catalytic domain (House & Kemp, 1987). Processes that result in a structural change in the protein so that the N-terminus of PKC is no longer in close proximity to the C-terminus result in activation of the enzyme. Typically, activation of classical isoforms of PKC occurs following the induction of PIP_2 hydrolysis within certain pathways of intracellular signalling. This generates Ca^{2+} and DAG, two second messengers crucial for the activation of classical PKCs (Beaven, 1996;Nishizuka, 1988). Ca^{2+} binds to the C2 domain of classical PKCs causing their translocation to the plasma membrane where they bind phospholipids such as PtS and PIP_2 (Cho, 2001;Newton, 1995b) (Figure 3C). Once at the membrane the C1 domain of PKC binds to membrane-bound DAG, an interaction aided by the binding of PtS (Bolsover, *et al.*, 2003;Cho, 2001). The engagement of both the C1 and C2 domains then causes a structural change in PKC that induces the release of the PS from the substrate-binding site of the catalytic domain, freeing PKC to catalyze the phosphorylation of downstream substrates (Newton, 1995a). The greater affinity of the C1 domain of novel PKC isoforms for DAG (Giorgione, *et al.*, 2006) allows the recruitment of these isoforms to membranes without the need for Ca^{2+}. Once at the membrane, novel PKC isozymes, like classical ones, unfold the regulatory domains from the catalytic domains and kinase activities ensue.

However, there are additional mechanisms of activation involving post-translational modification. Tyrosine kinases such as $pp60^{src}$ are able to bind some PKC isoforms, such as PKCδ, and catalyze their tyrosine phosphorylation (Joseloff, *et al.*, 2002;Kronfeld, *et al.*, 2000;Yuan, *et al.*, 1998). Phosphorylated tyrosine residues within PKCδ then act as docking sites for SH2 domain-containing proteins, which can further regulate the function of this PKC isoform (Leitges, *et al.*, 2002). The specific tyrosine residue where phosphorylation occurs dictates the response induced by PKCδ. The location of this phosphorylation and resultant cellular response is largely dependent upon the inciting stimulus and cell type. For example, the use of a mutant form of PKCδ containing several tyrosine residue mutations found that phosphorylation of Y^{64} and Y^{187} were important sites for regulating etoposide-induced apoptosis in C6 glioma cells (Blass, *et al.*, 2002). In contrast, viral infection of PC12 cells induced the phosphorylation of Y^{52}, Y^{64} and Y^{155} in PKCδ and these sites proved essential in mediating the antiapoptotic effects of this PKC isoform (Wert & Palfrey, 2000).

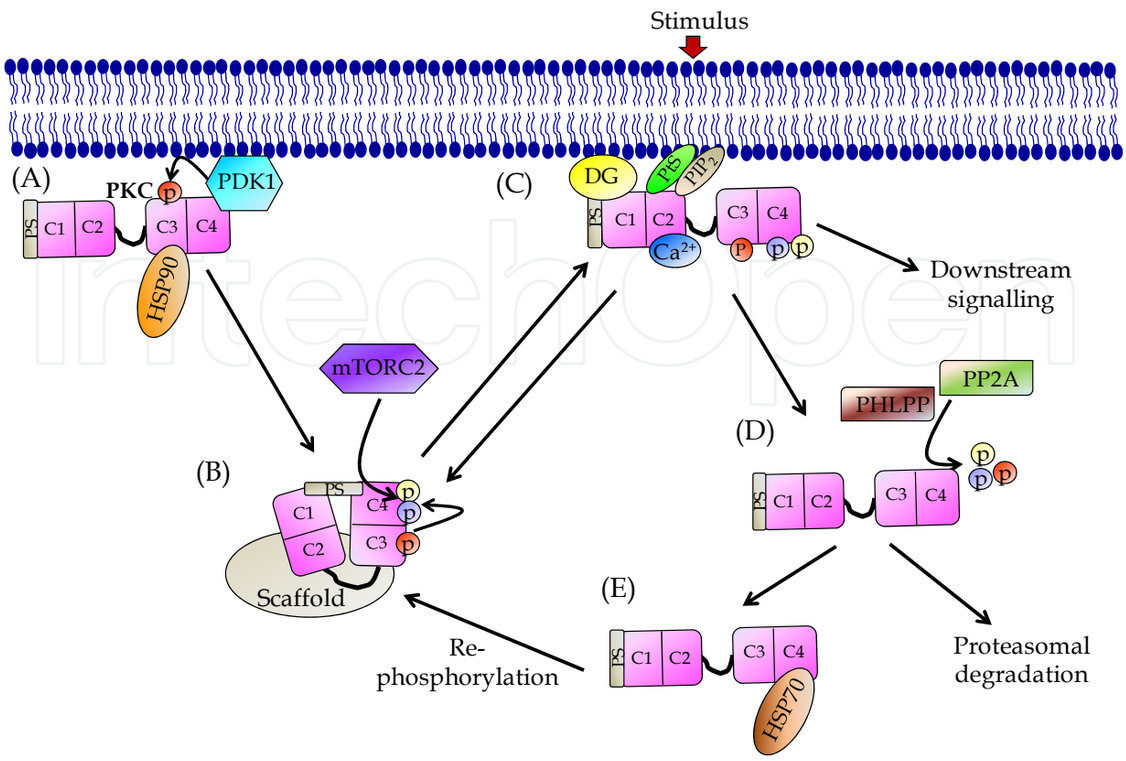


Fig. 3. PKC regulation (adapted from Newton, *et al.*, 2010). (A) HSP90 binds the kinase domain of newly synthesised PKC within the C3 region and primes it for phosphorylation of the activation motif by PDK-1. (B) mTORC2 and/or autophosphorylation is then responsible for phosphorylating the turn and then hydrophobic motifs within the C4 region of the catalytic domain. This results in fully-matured PKC which is then maintained in different cytosolic locations by interacting with scaffold proteins, and in an inactive state through interaction of the pseudosubstrate (PS) in the C1 region of the regulatory domain with the substrate binding site (C4) in the catalytic domain. (**C**) Specific stimuli induce the production of PIP_2, DAG and Ca^{2+}. This causes the recruitment of PKC to the membrane where the C1 domain binds DAG and the C2 domain binds phospholipids such as PIP_2 and phosphotidylserine (PtS) in a Ca^{2+}-dependant way. Membrane association of PKC releases the PS from the substrate-binding site to allow the protein to assume an active conformation and induce downstream signalling. (**D**) Active PKC is prone to dephosphorylation by phosphatases. PHLPP (PH domain leucine-rich repeat protein phosphatase) dephosphorylates the hydrophobic motif, while the activation and turn motifs are likely dephosphorylated by PP2A. (**E**) Dephosphorylated PKC is then either degraded, or HSP70 can bind the unphosphorylated turn motif and allow rephosphorylation of PKC to occur.

Other examples of post-translational modification include the oxidation of cysteines within the C1 domain. This causes a conformational change similar to that induced by lipid binding to result in increased PKC activity (Knapp & Klann, 2000). This phenomenon has been observed for PKCα, -βII, -γ and ε isoforms following exposure to superoxide anions (Knapp & Klann, 2000). Finally, nitrosylation of tyrosine residues can also activate certain PKC

isozymes. Tyrosine nitrosylation occurs in the presence of peroxynitrite and can affect PKCε activation (Balafanova, *et al.*, 2002). Pathologically, this is important for constitutive activation of ERK pathway signalling in hairy cell leukaemia cells (Slupsky, *et al.*, 2007).

Another mechanism of PKC activation is through the generation of an autonomous kinase by caspase-cleavage of the hinge domain. This form of the enzyme lacks the regulatory PS and is therefore maintained in an active conformation. This mechanism is best exemplified by PKCδ, which can be cleaved by caspases following the onset of apoptosis. Such cleavage causes the now autonomous catalytic domain of PKCδ to translocate to the nucleus where it catalyzes histone phosphorylation and chromosomal decondensation to aid in the production of DNA ladders that are characteristic of apoptotic cell death (Brodie & Blumberg, 2003;Kikkawa, *et al.*, 2002).

2.2.3 Co-factor binding

The activity of all PKC isoforms is tightly coordinated by interacting with different scaffold proteins. These interactions help localise the enzyme to different microenvironments where they are in proximity to particular lipid regulators, key proteins and substrates. The C2 (Brandman, *et al.*, 2007) , PB1 (Hirano, *et al.*, 2004;Moscat & Diaz-Meco, 2000) and PDZ (Staudinger, *et al.*, 1997) binding domains of PKCs are specifically engineered for this purpose, and define the individual functions of each isozyme. Examples of such proteins include receptors for activated C kinase (RACKs). RACK1 and RACK2 can competitively bind PKC isozymes, trapping them in active conformations by relieving autoinhibition by the N-terminus (Ron & Mochly-Rosen, 1995). Such interactions have the potential of localising active PKC in areas where sustained ligand activation is not possible. PKC can also interact with the cytoskeleton, either directly through protein-protein interaction or by binding to cytoskeleton-associated proteins (Larsson, 2006). Like PKCs interaction with RACK proteins, these interactions can replace the need for lipid second messengers and induce an active PKC conformation.

Co-factor binding to PKC can also prevent activation of this enzyme. For example, the overexpression of 14-3-3 in jurkat cells inhibits phorbol ester-induced PKCθ translocation from the cytosol to the membrane (Meller, *et al.*, 1996). Taking this into consideration it is important to note that there are different scaffold proteins for all conformations of PKC, all helping to regulate PKC from the moment it is synthesised and activated, until when it is deactivated and degraded.

2.2.4 Downregulation and degradation

Despite having a long half-life in the absence of stimulation, sustained activation of PKC, such as that achieved when cells are treated with phorbol esters, results in its rapid degradation (Hansra, *et al.*, 1999;Huang, *et al.*, 1989;Szallasi, *et al.*, 1994). Active PKC adopts a membrane-bound open conformation that is vulnerable to dephosphorylation by phosphatases (Dutil, *et al.*, 1994). PH domain leucine-rich repeat protein phosphatases (PHLPP) are able to dephosphorylate the hydrophobic motif of novel and classical PKC isoforms when they are in this open membrane-bound conformation (Figure 3D). This dephosphorylation causes these PKC isozymes to shunt to a detergent-insoluble cell fraction where they are then further dephosphorylated on the turn motif, possibly by PP2A, before

being degraded (Brognard & Newton, 2008;Gao, *et al.*, 2008). However, in some instances HSP70 can rescue PKC from this mechanism of degradation. Like HSP90, HSP70 can bind to the dephosphorylated turn motif of PKC and stabilise its conformation, and, in turn, promote its rephosphorylation and catalytic competence (Gao & Newton, 2002) (Figure 3E). This may be important because HSP70 is upregulated in cells undergoing stress, such as in response to chemotherapeutic agents (Jensen, *et al.*, 2009).

2.3 The role of different PKC isoforms in CLL

To gain a greater insight into the function of PKC in CLL pathobiology it is first important to determine the expression profile of this enzyme in CLL cells and to define the specific role each isoform plays in CLL signalling. Together, these findings may help design more customised clinical therapies targeting specific PKC isoforms.

2.3.1 Expression profile

Work in our lab discovered that CLL cells express PKCβI, -βII, -α, -δ, –ε, -ζ and PKC λ/ι (Abrams, *et al.*, 2007;Alkan, *et al.*, 2005). Furthermore, upon comparing the expression levels of these isoforms to the levels expressed in normal B cells, we discovered that CLL cells express less PKCβI and PKCα, and more PKCδ. However, what clearly distinguished CLL cells from normal B cells and other B-lymphoid malignancies was an overexpression of PKCβII equating to 0.53% ± 0.25% of total cellular protein (Abrams, *et al.*, 2007).

2.3.2 PKCβ

The PRKCB gene is transcribed as a single mRNA that is then alternatively spliced to produce PKCβI and PKCβII (Ono, *et al.*, 1986). In CLL cells, PKCβII is the predominant isoform and its elevated expression is thought to be due to increased transcription of the PKC gene by autocrine VEGF stimulation (Abrams, *et al.*, 2010). Furthermore, PKCβII is constitutively active in CLL cells and contributes to cell survival by protecting the cells from pro-apoptotic BCR signalling (Abrams, *et al.*, 2007). The importance of PKCβ in CLL development and propagation was more recently shown in a study using a CLL mouse model where the T-cell leukaemia (TCL1) protein is specifically overexpressed in B cells (Holler, *et al.*, 2009). This particular mouse model of CLL develops an aggressive disease that is similar to the aggressive form of CLL in humans (Yan, *et al.*, 2006). Thus, when this TCL1 transgenic mouse model of CLL was crossed with mice in which PKCβ was disrupted it was found that the CLL-like disease did not develop (Holler, *et al.*, 2009) (Figure 4A). Interestingly, in this same study the TCL1 transgenic PKCβ(+/-) heterozygous mice developed the CLL like disease with a slower kinetic than did TCL1 transgenic PKCβ wild type mice. Taken together, these data indicate that not only is PKCβ expression important for the development of CLL, but the level of expression plays a key role too. This same study also showed that the specific PKCβ inhibitor enzastaurin induced apoptosis of human CLL cells *in vitro*, suggesting that PKCβ was important in maintaining CLL cell survival.

Signals through the BCR are important for CLL cell survival and PKCβII activity inversely correlates with CLL cell response to BCR engagement (Abrams, *et al.*, 2007). An important substrate of PKCβ in B cells is Bruton's tyrosine kinase (Btk). Phosphorylation of Btk on

serine 180 results in its removal from the cell membrane and downregulation of its contribution to BCR signal transduction (Kang, *et al.*, 2001) (Figure 4B). During BCR signalling PKCβ is downstream of Btk activation, therefore, PKCβ acts in a feedback fashion to provide inhibition of this signalling. In CLL cells, PKCβII activity provides inhibition of BCR-induced intracellular calcium release and other downstream signals. We believe that this effect is largely pro-survival because strong, pro-apoptotic BCR signals would be largely suppressed in these cells. However, in CLL cells with high levels of PKCβII activity the pro-survival effects of BCR signalling are lost. Experiments comparing cell survival and Mcl-1 protein levels have shown that both these parameters are increased in response to BCR signalling in CLL cells with low levels of PKCβII activity, whereas there was little effect on these parameters when CLL cells with high levels of PKCβII activity were stimulated by BCR engagement. The regulation of PKCβ activity in CLL cells is likely to involve factors such as VEGF and bFGF, which have been shown to increase PKCβ activity and downregulate BCR signalling (Abrams, *et al.*, 2010).

In addition to its role in downregulating BCR signalling in CLL cells, PKCβII has also been shown to augment anti-apoptotic BCR signalling pathways in CLL cells (Barragan, *et al.*, 2006;zum Buschenfelde, *et al.*, 2010). The expression of ZAP70 in CLL cells is associated with poor disease prognosis and it is thought to enhance BCR signal transduction by acting as a platform to recruit downstream signalling proteins (Chen, *et al.*, 2005). In CLL cells, ZAP70 was recently demonstrated to enhance the BCR signal by recruiting PKCβII into lipid raft domains (zum Buschenfelde, *et al.*, 2010). Here, PKCβII becomes active and is shuttled to the mitochondrial membrane where it is able to phosphorylate anti-apoptotic Bcl-2 and pro-apoptotic Bim_{EL} (Figure 4B). This process provides important pro-survival signals because phosphorylation of Bcl-2 increases its ability to sequester Bim_{EL} and promote cell survival, whilst the phosphorylation of Bim_{EL} results in its proteasomal degradation and protection from its pro-apoptotic effects (zum Buschenfelde, *et al.*, 2010). Another example of how PKCβII mediates BCR-induced survival signals in CLL cells is by activating Akt, a kinase that provides an important source of survival signals to CLL cells (Barragan, *et al.*, 2006;Longo, *et al.*, 2008) (Figure 4B).

Finally, one study has shown that PKCβII may provide pro-survival signals in B cells by inducing the activation of Akt following stimulation by B cell-activating factor (BAFF) (Patke, *et al.*, 2006). This may be important for the pathophysiology of CLL cells because both BAFF and Akt are important sources of pro-survival signals for CLL cells (Barragan, *et al.*, 2006;Nishio, *et al.*, 2005). In B cells, PKCβII also transmits BCR signals to the NFκB pathway by phosphorylating CARMA1, which, together with MALT1, Bcl10 and TAK1 acts to stimulate I-κB kinase activity and NFκB pathway activation (Shinohara, *et al.*, 2005, 2007). Again, this may be pathophysiologically important in CLL because constitutive activation of the NFκB pathway is a feature of the malignant cells of this disease (Hewamana, *et al.*, 2008). Support for this idea comes from studies of diffuse large B cell lymphoma. PKCβ has been shown to be a therapeutic target in the malignant cells of this disease that bear the activated B cell phenotype because of the role it plays in activating the NFκB pathway through the CARMA1/MALT1/Bcl10 complex (Naylor, *et al.*, 2011). Taken together, these studies provide strong support for PKCβII in maintaining CLL cell survival by decreasing pro-apoptotic signals and increasing anti-apoptotic signals.

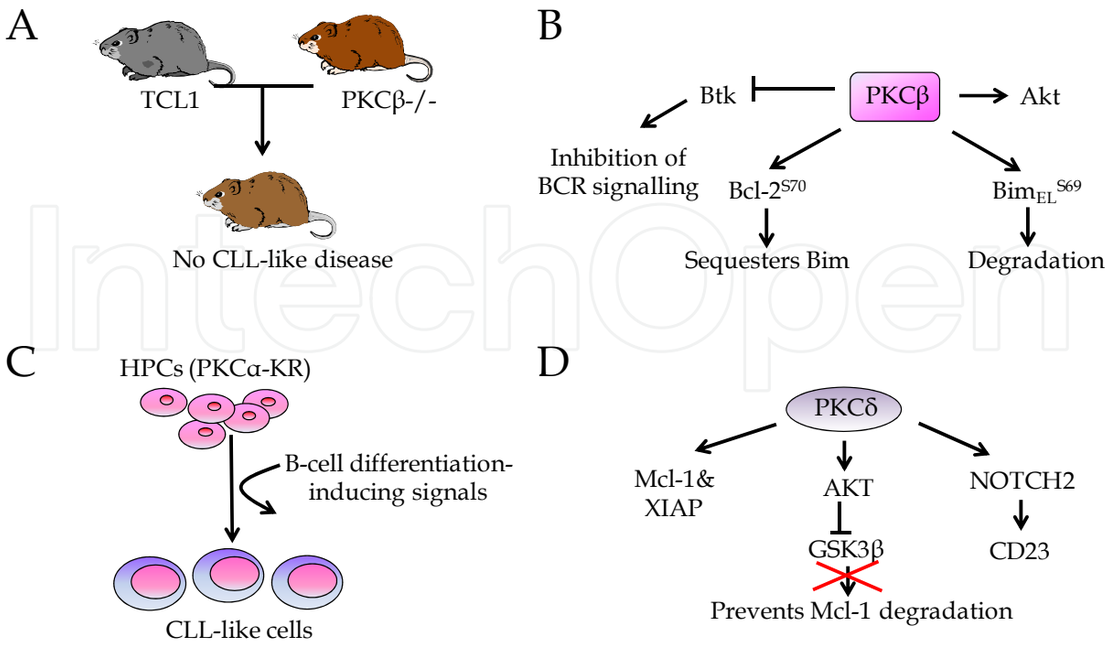


Fig. 4. PKC isoforms in CLL. (A) The T-cell leukaemia-1 (TCL1) mouse overexpresses TCL1 protein and develops an aggressive disease similar to aggressive CLL. TCL1 mice that do not express PKCβ (PKCβ-/-) do not develop the CLL-like disease. (B) PKCβII signalling. CLL cells express elevated levels of PKCβII, likely due to VEGF stimulation. High PKCβII-expressing CLL cases inhibit BCR-signalling by phosphorylating Btk on S^{180} which prevents its activation. Additionally, PKCβII augments antiapoptotic signalling by inducing S^{69} phosphorylation of Bim_{EL} and S^{70} phosphorylation of Bcl-2. Phosphorylation on these residues results in Bim_{EL} proteasomal degradation, and sequestration of Bim_{EL} by Bcl-2, respectively. PKCβII can also activate Akt which is an important mediator of CLL-cell survival. (**C**) Tumour suppressive effects of PKCα in CLL. When fetal-derived hematopoietic progenitor cells (HPCs) overexpressing a dominant negative form of PKCα (PKCα-KR) are induced to differentiate into B lineage cells, a population of CLL-like malignant cells is generated. (**D**) PKCδ signalling. PKCδ is constitutively active in CLL cells via a PI3Kδ-sensitive mechanism. Active PKCδ induces Akt phosphorylation which can then phosphorylate GSK3β. Hyperphosphorylated GSK3β is inactive, preventing it from phosphorylating Mcl-1 and inducing its proteasomal degradation. PKCδ may also induce the transcription of Mcl-1 and XIAP. More recent work has shown that PKCδ can induce the expression of CD23 by activating NOTCH2.

2.3.3 PKCα

Expression and function of PKCα is associated with both tumour promoting and tumour suppressing effects. For example, high levels of PKCα expression are associated with breast, prostrate, gastric and brain cancers (Griner & Kazanietz, 2007;Michie & Nakagawa, 2005) whilst low levels of PKCα expression are associated with cancers of epithelium, pancreas, colon and CLL (Abrams, *et al.*, 2007;Alvaro, *et al.*, 1997;Detjen, *et al.*, 2000;Neill, *et al.*, 2003). In its tumour promoting role PKCα is typically associated with anti-apoptotic signalling

achieved through the ability of this kinase to phosphorylate Bcl-2 at the mitochondrial membrane and increase its ability to sequester Bim (Jiffar, *et al.*, 2004;Ruvolo, *et al.*, 1998). The tumour suppressive functions of PKCα are unclear. It has been shown that PKCα knockout mice spontaneously develop intestinal lesions with greater frequency than wild type littermate controls, and that the mitotic index of the malignant cells derived from the PKCα knockout mice is greater than that of malignant cells derived from wild type mice (Oster & Leitges, 2006). However, the mechanism through which this happens remains undefined.

With respect to CLL, a very interesting study by Nakagawa *et al* (Nakagawa, *et al.*, 2006) has suggested that PKCα may have important tumour suppressive effects in this disease (Figure 4C). Using a system whereby fetal liver-derived hematopoietic progenitor cells (HPCs) are induced to differentiate into B lineage cells, this group show that stable overexpression of a dominant negative PKCα (PKCα-KR) leads to the generation of a population of malignant cells bearing a CLL phenotype ($CD19^{hi}$, $CD23^{+}$, $CD5^{+}$, $sIgM^{lo}$) (Figure 4C). This population of malignant cells, like human CLL cells, are arrested in G_0/G_1 phase of the cell cycle and have enhanced expression of Bcl-2 (Hanada, *et al.*, 1993;Kitada, *et al.*, 1998;Mariano, *et al.*, 1992). However, when these cells are injected into SCID mice they have an enhanced proliferative capacity over mock-transfected and non-transfected control populations. This effect was specific for PKCα-KR because expression of other kinase dead PKC isoforms within the same system did not produce cells bearing the same phenotype (Michie & Nakagawa, 2006).

This system as a model for CLL is interesting for the reason that the malignant cells it generates have a high resemblance to the human CLL phenotype, as well as to CLL-like cells in mouse models of the disease (Holler, *et al.*, 2009;Nakagawa, *et al.*, 2006). This is important because in virtually all mouse models of CLL there is expansion of the B1 population of cells prior to development of disease (Hamano, *et al.*, 1998), and B1 cells in the mouse mainly derive from progenitor cells within the fetal liver (Dorshkind & Montecino-Rodriguez, 2007). Importantly, where the B1 population is absent, such as in PKCβ knockout mice, CLL does not develop in mouse models of this disease (Holler, *et al.*, 2009). Thus, by subverting the function of PKCα, this group has created a system whereby malignant expansion of B1 cells is promoted at the haematopoietic stage. Although this system may not represent the actual mechanism of CLL pathogenesis, it does reveal some important aspects within this mechanism. Since this system is easily manipulated at a genetic level, further study will provide important information on the tumour suppressive function of PKCα not only in CLL cells, but in other cancers as well.

2.3.4 PKCδ

In normal B cells PKCδ plays a key role in mediating signals for cell survival in response to BAFF (Mecklenbrauker, *et al.*, 2004). BAFF signalling is important for maintaining B cell survival in the periphery, particularly with respect to B cells that have become tolerant to autoantigens (Ota, *et al.*, 2010). Thus, mice in which BAFF is overexpressed develop autoimmune diseases such as systemic lupus erythematosis because autoreactive B cells are able to escape tolerance (Stohl, *et al.*, 2005). A similar situation is observed in mice where PKCδ expression is disrupted (Mecklenbrauker, *et al.*, 2004), indicating a pro-apoptotic role

for this PKC isozyme within a process that maintains survival of tolerant B cells. The relationship of PKCδ to BAFF is illustrated by experiments showing that this cytokine prevents nuclear localization of this PKC isoform. The absence of BAFF-stimulated signals results in nuclear localisation of PKCδ where it contributes to phosphorylation of histone H2B at serine 14 and initiation of apoptosis (Mecklenbrauker, *et al.*, 2004). Whether BAFF-mediated signalling stimulates pro-survival functions of PKCδ or merely prevents nuclear localisation is not clear at the present time, however, the pro-survival signalling capabilities of PKCδ, particularly those potentially induced by BAFF, may be highly relevant for CLL cells.

A potential role of PKCδ in maintaining CLL cell survival was first proposed in a paper by Ringshausen *et al*. (Ringshausen, *et al.*, 2002, 2006). This paper demonstrated that PKCδ was constitutively active in a phosphatidylinositol 3 kinase (PI3K)-dependent manner in CLL cells, and that inhibition of this isozyme with rottlerin induced apoptosis of treated cells by reducing the expression of Mcl-1 and XIAP (Ringshausen, *et al.*, 2002, 2006) (Figure 4D). However, with respect to this latter aspect there are some controversies regarding the use of rottlerin. Rottlerin is described as a specific inhibitor of PKCδ, but its pro-apoptotic activity is associated with PKC-independent effects (Villalba, *et al.*, 1999), particularly with respect to the uncoupling and depolarization of mitochondrial membranes (Soltoff, 2007). This uncoupling in cells leads to a reduction in ATP levels and consequent activation of 5'-AMP-activated protein kinase (AMPK), with the end result resembling that produced by direct inhibition of PKCδ. Therefore, results using this inhibitor lack specificity and must be approached with caution. Nevertheless, there is evidence linking PI3K activity to PKCδ in B cells. BAFF signalling in B cells is impaired by the absence of the p110δ isoform of PI3K (Henley, *et al.*, 2008), and inhibition of PI3Kδ with a compound known as CAL101 has shown clinical efficacy both *in vitro* and *in vivo* in CLL cells (Herman, *et al.*, 2010, 2011;Hoellenriegel, *et al.*, 2011). Moreover, other recent findings have shown that PKCδ regulates CLL cell survival by activating the Akt pathway and stabilising the expression of Mcl-1 (Baudot, *et al.*, 2009) (Figure 4D). These findings provide confirmation that PKCδ may play an anti-apoptotic role in CLL cells.

Our own work has discovered that PKC may be important for the survival of CLL cells through its ability to phosphorylate STAT3 on serine 727, and cause increased transcription of the gene for Mcl-1 (Allen, *et al.*, 2011) (Figure 4D). We found that treatment of CLL cells with Bis-1, a specific inhibitor of the novel isoforms PKCδ and PKCε, inhibited the phosphorylation of $STAT3^{S727}$ and decreased the expression of Mcl-1. Conversely, treatment with bryostatin, to activate classical and novel PKC isoforms, induced $STAT3^{S727}$ phosphorylation and increased Mcl-1 expression in CLL cells. Of course, the identity of the PKC isoform phosphorylating STAT3 in CLL cells remains to be characterised by more specific investigations, such as siRNA-mediated knock down of specific PKC isoform expression. Indeed, investigation of the mechanism of Syk-mediated CLL cell survival used siRNA to knock down PKCδ expression and showed a concomitant downregulation of Mcl-1 expression (Baudot, *et al.*, 2009). This study does not address whether PKCδ can phosphorylate STAT3 in CLL cells, but studies using other cell types have shown that PKCδ and PKCε can perform this function (Aziz, *et al.*, 2010;Gartsbein, *et al.*, 2006). Thus, there is ample support for our findings that these PKC isoforms may promote CLL cell survival by activating STAT3-mediated Mcl-1 transcription. Such a mechanism may be useful

therapeutically. High expression levels of Mcl-1 correlate with more aggressive and poor prognosis disease in CLL by providing the malignant cells with protection against chemotherapy (Pepper, *et al.*, 2008). Inhibiting PKC may reduce Mcl-1 expression in CLL cells, thereby lowering the apoptosis threshold and making them more susceptible to other chemotherapeutic agents.

Another way in which PKCδ may contribute to CLL cell pathophysiology is through NOTCH2. One study has used RNAi to knock down PKCδ expression in CLL cells and found that this procedure antagonises PMA-induced NOTCH2 activation (Hubmann, *et al.*, 2010). This result is important because one characteristic of CLL cells is high expression of CD23, and NOTCH2 is known to regulate CD23 expression in these cells (Hubmann, *et al.*, 2002). Taken together, these results suggest that PKCδ and NOTCH2 are critically involved in maintaining the malignant phenotype of CLL cells.

2.3.5 Other PKC isoforms

The function of the remaining PKC isoforms in CLL cells remains poorly defined. However, their role in other cell types is well documented and may provide clues as to what function they have in CLL cells. Intriguingly, PKCε is known to phosphorylate and activate signalling proteins such as Akt (Matsumoto, *et al.*, 2001), PKD (Waldron & Rozengurt, 2003) and STAT3 (Aziz, *et al.*, 2010), pathways which all provide an important source of anti-apoptotic signals to CLL cells. Furthermore, in hairy cell leukaemia, PKCε is activated by nitration of a tyrosine residue causing it to co-localise with ERK1/2 at the mitochondrial membrane and induce activation of the MAPK pathway (Slupsky, *et al.*, 2007). Given that ERK1/2 has been shown to phosphorylate and stabilise the expression of Mcl-1 (Domina, *et al.*, 2004), its activation in CLL cells may provide additional anti-apoptotic signals to CLL cells. Moreover, knockout mouse models have highlighted the importance of the atypical PKCζ in B-cell survival and proliferation by regulating the activation of ERK and NFκB signalling pathways (Martin, *et al.*, 2002).

The above findings are important because Akt, NFκB and STAT3 signalling pathways are known to be constitutively active in CLL cells, and because these pathways are essential in maintaining CLL cell viability (Allen, *et al.*, 2011;de Frias, *et al.*, 2009;Hazan-Halevy, *et al.*, 2010;Hewamana, *et al.*, 2008;Zhuang, *et al.*, 2010). The level of NFκB activation is regarded as an essential component of CLL survival because it correlates with *in vitro* survival of CLL cells as well as with clinical disease progression (Hewamana, *et al.*, 2008). Furthermore, our own work has demonstrated that STAT3 mediates CLL cell survival by inducing Mcl-1 transcription (Allen, *et al.*, 2011), and more recent studies have shown that both pathways can work in concert to induce the expression of anti-apoptotic proteins (Liu, *et al.*, 2011). Collectively, these findings highlight that PKC has the potential to activate numerous anti-apoptotic pathways and that further work is now critical to help understand the specific role these isoforms play in CLL cell signalling.

2.4 CLL cell microenvironment and PKC

It is clear that PKC-mediated signalling pathways seem to provide important survival signals to CLL cells *in vitro*, but how close do these conditions mimic those of *in vivo*? The CLL microenvironment is a milieu rich in signals generated by the interaction of the

malignant cells with IL-6 (Moreno, *et al.*, 2001), IL-4 (Dancescu, *et al.*, 1992) , SDF-1 (Burger, *et al.*, 2000), BAFF and April (Endo, *et al.*, 2007). These cytokines have all been shown to have anti-apoptotic effects on CLL cells. Moreover, the interaction of CLL cells with bone marrow stromal cells (Lagneaux, *et al.*, 1998;Panayiotidis, *et al.*, 1996), follicular dendritic cells (Pedersen, *et al.*, 2002), endothelial cells (Moreno, *et al.*, 2001), nurse-like cells (Burger, *et al.*, 2000) and CD40L-expressing cells (Hallaert, *et al.*, 2008) results in an increase apoptotic threshold. This may be due to the induction of anti-apoptotic genes by these interactions; a comparison of the apoptosis regulatory genes and proteins in neoplastic B cells derived from CLL lymph node proliferation centres and peripheral blood found that lymph node-derived cells had increased expression of anti-apoptotic Mcl-1, Bcl-XL and A1/Bfl-1 (Smit, *et al.*, 2007). Moreover, co-culture of CLL cells on CD40L-expressing fibroblasts strongly induces the expression of these anti-apoptotic proteins, and this culminates in drug resistance (Hallaert, *et al.*, 2008). PKC activation is likely to play a role in all of the microenvironmental interactions CLL cells are likely to encounter in an *in vivo* setting. However, whether inhibition of PKC lowers the threshold of apoptosis in CLL cells within their microenvironment is unknown and requires proper assessment before PKC inhibition becomes a therapeutic target in the treatment of this disease. Recent studies have begun to address this area and have demonstrated the importance of PKC in the survival of CLL cells that have been co-cultured with accessory cells (Martins, *et al.*, 2011).

3. Conclusion

There are convincing demonstrations that PKC-mediated signalling is an important contributor to the development and propagation of the malignant clone in CLL. Inhibition of PKC, therefore, poses an attractive therapeutic approach for the treatment of this debilitating disease, particularly when we consider the roles of the individual PKC isozymes in CLL pathobiology. Within this review we have addressed the potential functions of PKCβ, α, δ and, to a lesser extent, PKCε and PKCζ. There is clear contribution of PKCβ to the pathogenesis of CLL, because disruption of PKCβ expression blocks the development of the CLL-like disease in TCL1-transgenic mice. This same type of experiment now needs to be done for the other PKC isoforms. Thus, disruption of PKCα may accelerate disease progression in TCL1 mice because of the tumour suppressive action of this PKC isoform. The effect of PKCδ disruption is harder to predict. On one hand, disruption of PKCδ should accelerate disease development because the pro-apoptotic effects of this isoform would be lost. However, this prediction does not take into account the pro-survival functions of PKCδ in CLL cells, particularly its potential role in regulating STAT3 phosphorylation and Mcl-1 protein expression. Finally, targeted disruption of PKC isoforms would potentially yield useful information on the role these isoforms play in CLL cell–microenvironment interactions.

Within this review we have not addressed opposing functions of different PKC isoforms. For example, PKCα and PKCδ can act antagonistically to regulate cellular proliferation and apoptosis in glioma cells (Mandil, *et al.*, 2001). It is conceivable that more general inhibitors of PKC, through their ability to inhibit tumour suppressive or pro-apoptotic functions of PKC, may have an adverse effect by actually promoting CLL cell survival and proliferation. Nevertheless, PKC inhibitors such as N-benzoyl-staurosporine (PKC412) have already been tested in clinical trials, and were found to be effective at inducing CLL cell apoptosis in

patients that were refractory to fludarabine and chlorambucil (Ganeshaguru, *et al.*, 2002). Furthermore, a more recent drug called sotrastaurin (AEB071) has been introduced as an immunosuppressant following organ transplant, and for the treatment of psoriasis. Early clinical trials suggest sotrastaurin has no clinically relevant side effects and has the potential to become a long term treatment option (Skvara, *et al.*, 2008). Another study has suggested that AEB071 may even be useful in the treatment of diffuse large B-cell lymphoma (DLCBL) through inhibition of BCR-mediated NFκB pathway activation (Naylor, *et al.*, 2011). Thus, given the potential role of PKC in regulating CLL cell survival and disease pathogenesis and that side effects associated with the use of some inhibitors can be adequately managed within a clinical setting, PKC inhibitors may have therapeutic application in the treatment of CLL.

4. Acknowledgement

JCA and JRS thank Leukaemia and Lymphoma Research U.K. for their support in publishing this article.

5. References

Abrams, S.T., Lakum, T., Lin, K., Jones, G.M., Treweeke, A.T., Farahani, M., Hughes, M., Zuzel, M. & Slupsky, J.R. (2007). B-cell receptor signaling in chronic lymphocytic leukemia cells is regulated by overexpressed active protein kinase CβII. *Blood*, Vol.109, No.3, (Feb 2007), pp. 1193-1201, ISSN 0006-4971

Abrams, S.T., Brown, B.R., Zuzel, M. & Slupsky, J.R. (2010). Vascular endothelial growth factor stimulates protein kinase CβII expression in chronic lymphocytic leukemia cells. *Blood*, Vol.115, No.22, (Jun 2010), pp. 4447-4454, ISSN 0006-4971

Ahmad, I., Al-Katib, A.M., Beck, F.W. & Mohammad, R.M. (2000). Sequential treatment of a resistant chronic lymphocytic leukemia patient with bryostatin 1 followed by 2-chlorodeoxyadenosine: case report. *Clinical Cancer Research*, Vol.6, No.4, (Apr 2000), pp. 1328-1332, ISSN 1078-0432

al-Katib, A., Mohammad, R.M., Dan, M., Hussein, M.E., Akhtar, A., Pettit, G.R. & Sensenbrenner, L.L. (1993). Bryostatin 1-induced hairy cell features on chronic lymphocytic leukemia cells in vitro. *Experimental Hematology*, Vol.21, No.1, (Jan 1993), pp. 61-65, ISSN 0301-472X

Alkan, S., Huang, Q., Ergin, M., Denning, M.F., Nand, S., Maududi, T., Paner, G.P., Ozpuyan, F. & Izban, K.F. (2005). Survival role of protein kinase C (PKC) in chronic lymphocytic leukemia and determination of isoform expression pattern and genes altered by PKC inhibition. *American Journal of Hematology*, Vol.79, No.2, (Jun 2005), pp. 97-106, ISSN 0361-8609

Allen, J.C., Talab, F., Zuzel, M., Lin, K. & Slupsky, J.R. (2011). c-Abl regulates Mcl-1 gene expression in chronic lymphocytic leukemia cells. *Blood*, Vol.117, No.8, (Feb 2011), pp. 2414-2422, ISSN 0006-4971

Alvaro, V., Prevostel, C., Joubert, D., Slosberg, E. & Weinstein, B.I. (1997). Ectopic expression of a mutant form of PKCα originally found in human tumors: aberrant subcellular translocation and effects on growth control. *Oncogene*, Vol.14, No.6, (Feb 1997), pp. 677-685, ISSN 0950-9232

Aziz, M.H., Hafeez, B.B., Sand, J.M., Pierce, D.B., Aziz, S.W., Dreckschmidt, N.E. & Verma, A.K. (2010). Protein kinase Cε mediates Stat3 Ser^{727} phosphorylation, Stat3-regulated gene expression, and cell invasion in various human cancer cell lines through integration with MAPK cascade (RAF-1, MEK1/2, and ERK1/2). *Oncogene*, Vol.29, No.21, (May 2010), pp. 3100-3109, ISSN 0950-9232

Balafanova, Z., Bolli, R., Zhang, J., Zheng, Y., Pass, J.M., Bhatnagar, A., Tang, X.L., Wang, O., Cardwell, E. & Ping, P. (2002). Nitric oxide (NO) induces nitration of protein kinase Cε (PKCε), facilitating PKCε translocation via enhanced PKCε -RACK2 interactions: a novel mechanism of no-triggered activation of PKCε. *The Journal of Biological Chemistry*, Vol.277, No.17, (Apr 2002), pp. 15021-15027, ISSN 0021-9258

Balendran, A., Hare, G.R., Kieloch, A., Williams, M.R. & Alessi, D.R. (2000). Further evidence that 3-phosphoinositide-dependent protein kinase-1 (PDK1) is required for the stability and phosphorylation of protein kinase C (PKC) isoforms. *FEBS Letters*, Vol.484, No.3, (Nov 2000), pp. 217-223, ISSN 0014-5793

Barragan, M., Bellosillo, B., Campas, C., Colomer, D., Pons, G. & Gil, J. (2002). Involvement of protein kinase C and phosphatidylinositol 3-kinase pathways in the survival of B-cell chronic lymphocytic leukemia cells. *Blood*, Vol.99, No.8, (Apr 2002), pp. 2969-2976, ISSN 0006-4971

Barragan, M., de Frias, M., Iglesias-Serret, D., Campas, C., Castano, E., Santidrian, A.F., Coll-Mulet, L., Cosialls, A.M., Domingo, A., Pons, G. & Gil, J. (2006). Regulation of Akt/PKB by phosphatidylinositol 3-kinase-dependent and -independent pathways in B-cell chronic lymphocytic leukemia cells: role of protein kinase Cβ. *Journal of Leukocyte Biology*, Vol.80, No.6, (Dec 2006), pp. 1473-1479, ISSN 0741-5400

Baudot, A.D., Jeandel, P.Y., Mouska, X., Maurer, U., Tartare-Deckert, S., Raynaud, S.D., Cassuto, J.P., Ticchioni, M. & Deckert, M. (2009). The tyrosine kinase Syk regulates the survival of chronic lymphocytic leukemia B cells through PKCδ and proteasome-dependent regulation of Mcl-1 expression. *Oncogene*, Vol.28, No.37, (Sep 2009), pp. 3261-3273, ISSN 0950-9232

Beaven, M.A. (1996). Calcium signalling: sphingosine kinase versus phospholipase C? *Current Biology*, Vol.6, No.7, (Jul 1996), pp. 798-801, ISSN 0960-9822

Behn-Krappa, A. & Newton, A.C. (1999). The hydrophobic phosphorylation motif of conventional protein kinase C is regulated by autophosphorylation. *Current Biology*, Vol.9, No.14, (Jul 1999), pp. 728-737, ISSN 0960-9822

Bellosillo, B., Dalmau, M., Colomer, D. & Gil, J. (1997). Involvement of CED-3/ICE proteases in the apoptosis of B-chronic lymphocytic leukemia cells. *Blood*, Vol.89, No.9, (May 1997), pp. 3378-3384, ISSN 0006-4971

Benimetskaya, L., Miller, P., Benimetsky, S., Maciaszek, A., Guga, P., Beaucage, S.L., Wilk, A., Grajkowski, A., Halperin, A.L. & Stein, C.A. (2001). Inhibition of potentially anti-apoptotic proteins by antisense protein kinase C-α (Isis 3521) and antisense bcl-2 (G3139) phosphorothioate oligodeoxynucleotides: relationship to the decreased viability of T24 bladder and PC3 prostate cancer cells. *Molecular Pharmacology*, Vol.60, No.6, (Dec 2001), pp. 1296-1307, ISSN 0026-895X

Blass, M., Kronfeld, I., Kazimirsky, G., Blumberg, P.M. & Brodie, C. (2002). Tyrosine phosphorylation of protein kinase Cδ is essential for its apoptotic effect in response to etoposide. *Molecular Cell Biology*, Vol.22, No.1, (Jan), pp. 182-195, ISSN 0270-7306

Bolsover, S.R., Gomez-Fernandez, J.C. & Corbalan-Garcia, S. (2003). Role of the Ca^{2+}/phosphatidylserine binding region of the C2 domain in the translocation of protein kinase Cα to the plasma membrane. *Journal of Biological Chemistry*, Vol.278, No.12, (Mar 2003), pp. 10282-10290, ISSN 0021-9258

Brandman, R., Disatnik, M.H., Churchill, E. & Mochly-Rosen, D. (2007). Peptides derived from the C2 domain of protein kinase C ε (ε PKC) modulate ε PKC activity and identify potential protein-protein interaction surfaces. *Journal of Biological Chemistry*, Vol.282, No.6, (Feb 2007), pp. 4113-4123, ISSN 0021-9258

Brodie, C. & Blumberg, P.M. (2003). Regulation of cell apoptosis by protein kinase c δ. *Apoptosis*, Vol.8, No.1, (Jan 2003), pp. 19-27, ISSN 1360-8185

Brognard, J. & Newton, A.C. (2008). PHLiPPing the switch on Akt and protein kinase C signaling. *Trends in Endocrinology and Metabolism*, Vol.19, No.6, (Aug 2008), pp. 223-230, ISSN 1043-2760

Burger, J.A., Tsukada, N., Burger, M., Zvaifler, N.J., Dell'Aquila, M. & Kipps, T.J. (2000). Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. *Blood*, Vol.96, No.8, (Oct 2000), pp. 2655-2663, ISSN 0006-4971

Byrd, J.C., Shinn, C., Willis, C.R., Flinn, I.W., Lehman, T., Sausville, E., Lucas, D. & Grever, M.R. (2001). UCN-01 induces cytotoxicity toward human CLL cells through a p53-independent mechanism. *Experimental Hematology*, Vol.29, No.6, (Jun 2001), pp. 703-708, ISSN 0301-472X

Chen, L., Apgar, J., Huynh, L., Dicker, F., Giago-McGahan, T., Rassenti, L., Weiss, A. & Kipps, T.J. (2005). ZAP-70 directly enhances IgM signaling in chronic lymphocytic leukemia. *Blood*, Vol.105, No.5, (Mar 2005), pp. 2036-2041, ISSN 0006-4971

Chiorazzi, N., Rai, K.R. & Ferrarini, M. (2005). Chronic lymphocytic leukemia. The *New England Journal of Medicine*, Vol.352, No.8, (Feb 2005), pp. 804-815, ISSN 0028-4793

Cho, W. (2001). Membrane targeting by C1 and C2 domains. *Journal of Biological Chemistry*, Vol.276, No.35, (Aug 2001), pp. 32407-32410, ISSN 0021-9258

Chou, M.M., Hou, W., Johnson, J., Graham, L.K., Lee, M.H., Chen, C.S., Newton, A.C., Schaffhausen, B.S. & Toker, A. (1998). Regulation of protein kinase C ζ by PI 3-kinase and PDK-1. *Current Biology*, Vol.8, No.19, (Sep 1998), pp. 1069-1077, ISSN 0960-9822

Dancescu, M., Rubio-Trujillo, M., Biron, G., Bron, D., Delespesse, G. & Sarfati, M. (1992). Interleukin 4 protects chronic lymphocytic leukemic B cells from death by apoptosis and upregulates Bcl-2 expression. *The Journal of Experimental Medicine*, Vol.176, No.5, (Nov 1992), pp. 1319-1326, ISSN 0022-1007

de Frias, M., Iglesias-Serret, D., Cosialls, A.M., Coll-Mulet, L., Santidrian, A.F., Gonzalez-Girones, D.M., de la Banda, E., Pons, G. & Gil, J. (2009). Akt inhibitors induce apoptosis in chronic lymphocytic leukemia cells. *Haematologica*, Vol.94, No.12, (Dec 2009), pp. 1698-1707, ISSN 0390-6078

Detjen, K.M., Brembeck, F.H., Welzel, M., Kaiser, A., Haller, H., Wiedenmann, B. & Rosewicz, S. (2000). Activation of protein kinase Cα inhibits growth of pancreatic cancer cells via p21(cip)-mediated G(1) arrest. *Journal of Cell Science*, Vol.113 (Pt 17), (Sep 2000), pp. 3025-3035, ISSN 0021-9533

Domina, A.M., Vrana, J.A., Gregory, M.A., Hann, S.R. & Craig, R.W. (2004). MCL1 is phosphorylated in the PEST region and stabilized upon ERK activation in viable

cells, and at additional sites with cytotoxic okadaic acid or taxol. *Oncogene*, Vol.23, No.31, (Jul 2004), pp. 5301-5315, ISSN 0950-9232

Dorshkind, K. & Montecino-Rodriguez, E. (2007). Fetal B-cell lymphopoiesis and the emergence of B-1-cell potential. *Nature reviews. Immunology*, Vol.7, No.3, (Mar 2007), pp. 213-219, ISSN 1474-1733

Drexler, H.G., Gignac, S.M., Jones, R.A., Scott, C.S., Pettit, G.R. & Hoffbrand, A.V. (1989). Bryostatin 1 induces differentiation of B-chronic lymphocytic leukemia cells. *Blood*, Vol.74, No.5, (Oct 1989), pp. 1747-1757, ISSN 0006-4971

Dutil, E.M., Keranen, L.M., DePaoli-Roach, A.A. & Newton, A.C. (1994). In vivo regulation of protein kinase C by trans-phosphorylation followed by autophosphorylation. *Journal of Biological Chemistry*, Vol.269, No.47, (Nov 1994), pp. 29359-29362, ISSN 0021-9258

Dutil, E.M., Toker, A. & Newton, A.C. (1998). Regulation of conventional protein kinase C isozymes by phosphoinositide-dependent kinase 1 (PDK-1). *Current Biology*, Vol.8, No.25, (Dec 1998), pp. 1366-1375, ISSN 0960-9822

Dutil, E.M. & Newton, A.C. (2000). Dual role of pseudosubstrate in the coordinated regulation of protein kinase C by phosphorylation and diacylglycerol. *Journal of Biological Chemistry*, Vol.275, No.14, (Apr 2000), pp. 10697-10701, ISSN 0021-9258

Endo, T., Nishio, M., Enzler, T., Cottam, H.B., Fukuda, T., James, D.F., Karin, M. & Kipps, T.J. (2007). BAFF and APRIL support chronic lymphocytic leukemia B-cell survival through activation of the canonical NF-κB pathway. *Blood*, Vol.109, No.2, (Jan 2007), pp. 703-710, ISSN 0006-4971

Forbes, I.J., Zalewski, P.D., Giannakis, C. & Cowled, P.A. (1992). Induction of apoptosis in chronic lymphocytic leukemia cells and its prevention by phorbol ester. *Experimental Cell Research*, Vol.198, No.2, (Feb 1992), pp. 367-372, ISSN 0014-4827

Ganeshaguru, K., Wickremasinghe, R.G., Jones, D.T., Gordon, M., Hart, S.M., Virchis, A.E., Prentice, H.G., Hoffbrand, A.V., Man, A., Champain, K., Csermak, K. & Mehta, A.B. (2002). Actions of the selective protein kinase C inhibitor PKC412 on B-chronic lymphocytic leukemia cells in vitro. *Haematologica*, Vol.87, No.2, (Feb 2002), pp. 167-176, ISSN 0390-6078

Gao, T. & Newton, A.C. (2002). The turn motif is a phosphorylation switch that regulates the binding of Hsp70 to protein kinase C. *Journal of Biological Chemistry*, Vol.277, No.35, (Aug 2002), pp. 31585-31592, ISSN 0021-9258

Gao, T., Brognard, J. & Newton, A.C. (2008). The phosphatase PHLPP controls the cellular levels of protein kinase C. *Journal of Biological Chemistry*, Vol.283, No.10, (Mar 2008), pp. 6300-6311, ISSN 0021-9258

Gartsbein, M., Alt, A., Hashimoto, K., Nakajima, K., Kuroki, T. & Tennenbaum, T. (2006). The role of protein kinase C δ activation and STAT3 Ser^{727} phosphorylation in insulin-induced keratinocyte proliferation. *Journal of Cell Science* Vol.119, No.Pt 3, (Feb 2006), pp. 470-481, ISSN 0021-9533

Giorgione, J.R., Lin, J.H., McCammon, J.A. & Newton, A.C. (2006). Increased membrane affinity of the C1 domain of protein kinase Cδ compensates for the lack of involvement of its C2 domain in membrane recruitment. *Journal of Biological Chemistry*, Vol.281, No.3, (Jan 2006), pp. 1660-1669, ISSN 0021-9258

Gould, C.M., Kannan, N., Taylor, S.S. & Newton, A.C. (2009). The chaperones Hsp90 and Cdc37 mediate the maturation and stabilization of protein kinase C through a

conserved PXXP motif in the C-terminal tail. *Journal of Biological Chemistry*, Vol.284, No.8, (Feb 2009), pp. 4921-4935, ISSN 0021-9258

Griner, E.M. & Kazanietz, M.G. (2007). Protein kinase C and other diacylglycerol effectors in cancer. *Nat Rev Cancer*, Vol.7, No.4, (Apr 2007), pp. 281-294, ISSN 1474-175X

Hallaert, D.Y., Jaspers, A., van Noesel, C.J., van Oers, M.H., Kater, A.P. & Eldering, E. (2008). c-Abl kinase inhibitors overcome CD40-mediated drug resistance in CLL: implications for therapeutic targeting of chemoresistant niches. *Blood*, Vol.112, No.13, (Dec 2008), pp. 5141-5149, ISSN 0006-4971

Hamano, Y., Hirose, S., Ida, A., Abe, M., Zhang, D., Kodera, S., Jiang, Y., Shirai, J., Miura, Y., Nishimura, H. & Shirai, T. (1998). Susceptibility alleles for aberrant B-1 cell proliferation involved in spontaneously occurring B-cell chronic lymphocytic leukemia in a model of New Zealand white mice. *Blood*, Vol.92, No.10, (Nov 1998), pp. 3772-3779, ISSN 0006-4971

Hanada, M., Delia, D., Aiello, A., Stadtmauer, E. & Reed, J.C. (1993). bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. *Blood*, Vol.82, No.6, (Sep 1993), pp. 1820-1828, ISSN 0006-4971

Hansra, G., Garcia-Paramio, P., Prevostel, C., Whelan, R.D., Bornancin, F. & Parker, P.J. (1999). Multisite dephosphorylation and desensitization of conventional protein kinase C isotypes. *The Biochemical Journal*, Vol.342 (Pt 2), (Sep 1999), pp. 337-344, ISSN 0264-6021

Hazan-Halevy, I., Harris, D., Liu, Z., Liu, J., Li, P., Chen, X., Shanker, S., Ferrajoli, A., Keating, M.J. & Estrov, Z. (2010). STAT3 is constitutively phosphorylated on serine 727 residues, binds DNA, and activates transcription in CLL cells. *Blood*, Vol.115, No.14, (Apr 2010), pp. 2852-2863, ISSN 0006-4971

Henley, T., Kovesdi, D. & Turner, M. (2008). B-cell responses to B-cell activation factor of the TNF family (BAFF) are impaired in the absence of PI3K δ. *European Journal of Immunology* Vol.38, No.12, (Dec 2008), pp. 3543-3548, ISSN 0014-2980

Herman, S.E., Lapalombella, R., Gordon, A.L., Ramanunni, A., Blum, K.A., Jones, J., Zhang, X., Lannutti, B.J., Puri, K.D., Muthusamy, N., Byrd, J.C. & Johnson, A.J. (2011). The role of phosphatidylinositol 3-kinase-δ in the immunomodulatory effects of lenalidomide in chronic lymphocytic leukemia.. *Blood*, Vol.117, No.16, (Apr 2011), pp. 4323-4327, ISSN 0006-4971

Herman, S.E., Gordon, A.L., Wagner, A.J., Heerema, N.A., Zhao, W., Flynn, J.M., Jones, J., Andritsos, L., Puri, K.D., Lannutti, B.J., Giese, N.A., Zhang, X., Wei, L., Byrd, J.C. & Johnson, A.J. (2010). Phosphatidylinositol 3-kinase-δ inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. *Blood*, Vol.116, No.12, (Sep 2010), pp. 2078-2088, ISSN 0006-4971

Hewamana, S., Alghazal, S., Lin, T.T., Clement, M., Jenkins, C., Guzman, M.L., Jordan, C.T., Neelakantan, S., Crooks, P.A., Burnett, A.K., Pratt, G., Fegan, C., Rowntree, C., Brennan, P. & Pepper, C. (2008). The NF-κB subunit Rel A is associated with in vitro survival and clinical disease progression in chronic lymphocytic leukemia and represents a promising therapeutic target. *Blood*, Vol.111, No.9, (May 2008), pp. 4681-4689, ISSN 0006-4971

Hirano, Y., Yoshinaga, S., Ogura, K., Yokochi, M., Noda, Y., Sumimoto, H. & Inagaki, F. (2004). Solution structure of atypical protein kinase C PB1 domain and its mode of

interaction with ZIP/p62 and MEK5. *Journal of Biological Chemistry*, Vol.279, No.30, (Jul 2004), pp. 31883-31890, ISSN 0021-9258

Hoellenriegel, J., Meadows, S.A., Sivina, M., Wierda, W.G., Kantarjian, H., Keating, M.J., Giese, N., O'Brien, S., Yu, A., Miller, L.L., Lannutti, B.J. & Burger, J.A. (2011). The phosphoinositide 3'-kinase δ inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. *Blood*, (Jul 2011), pp. ISSN 0006-4971

Holler, C., Pinon, J.D., Denk, U., Heyder, C., Hofbauer, S., Greil, R. & Egle, A. (2009). PKCβ is essential for the development of chronic lymphocytic leukemia in the TCL1 transgenic mouse model: validation of PKCβ as a therapeutic target in chronic lymphocytic leukemia. *Blood*, Vol.113, No.12, (Mar 2009), pp. 2791-2794, ISSN 0006-4971

House, C. & Kemp, B.E. (1987). Protein kinase C contains a pseudosubstrate prototope in its regulatory domain. *Science*, Vol.238, No.4834, (Dec 1987), pp. 1726-1728, ISSN 0036-8075

Huang, F.L., Yoshida, Y., Cunha-Melo, J.R., Beaven, M.A. & Huang, K.P. (1989). Differential down-regulation of protein kinase C isozymes. *Journal of Biological Chemistry*, Vol.264, No.7, (Mar 1989), pp. 4238-4243, ISSN 0021-9258

Hubmann, R., Schwarzmeier, J.D., Shehata, M., Hilgarth, M., Duechler, M., Dettke, M. & Berger, R. (2002). Notch2 is involved in the overexpression of CD23 in B-cell chronic lymphocytic leukemia. *Blood*, Vol.99, No.10, (May 2002), pp. 3742-3747, ISSN 0006-4971

Hubmann, R., Duchler, M., Schnabl, S., Hilgarth, M., Demirtas, D., Mitteregger, D., Holbl, A., Vanura, K., Le, T., Look, T., Schwarzmeier, J.D., Valent, P., Jager, U. & Shehata, M. (2010). NOTCH2 links protein kinase C δ to the expression of CD23 in chronic lymphocytic leukaemia (CLL) cells. *British Journal of Haematology*, Vol.148, No.6, (Mar 2010), pp. 868-878, ISSN 0007-1048

Ikenoue, T., Inoki, K., Yang, Q., Zhou, X. & Guan, K.L. (2008). Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *The EMBO Journal*, Vol.27, No.14, (Jul 2008), pp. 1919-1931, ISSN 0261-4189

Jensen, H., Andresen, L., Hansen, K.A. & Skov, S. (2009). Cell-surface expression of Hsp70 on hematopoietic cancer cells after inhibition of HDAC activity. *Journal of Leukocyte Biology* Vol.86, No.4, (Oct 2009), pp. 923-932, ISSN 0741-5400

Jiffar, T., Kurinna, S., Suck, G., Carlson-Bremer, D., Ricciardi, M.R., Konopleva, M., Andreeff, M. & Ruvolo, P.P. (2004). PKC α mediates chemoresistance in acute lymphoblastic leukemia through effects on Bcl2 phosphorylation. *Leukemia*, Vol.18, No.3, (Mar 2004), pp. 505-512, ISSN 0887-6924

Joseloff, E., Cataisson, C., Aamodt, H., Ocheni, H., Blumberg, P., Kraker, A.J. & Yuspa, S.H. (2002). Src family kinases phosphorylate protein kinase C δ on tyrosine residues and modify the neoplastic phenotype of skin keratinocytes. *Journal of Biological Chemistry*, Vol.277, No.14, (Apr 2002), pp. 12318-12323, ISSN 0021-9258

Kang, S.W., Wahl, M.I., Chu, J., Kitaura, J., Kawakami, Y., Kato, R.M., Tabuchi, R., Tarakhovsky, A., Kawakami, T., Turck, C.W., Witte, O.N. & Rawlings, D.J. (2001). PKCβ modulates antigen receptor signaling via regulation of Btk membrane localization. *The EMBO Journal*, Vol.20, No.20, (Oct 2001), pp. 5692-5702, ISSN 0261-4189

Keenan, C., Thompson, S., Knox, K. & Pears, C. (1999). Protein kinase C-α is essential for Ramos-BL B cell survival. *Cellular Immunology*, Vol.196, No.2, (Sep 1999), pp. 104-109, ISSN 0008-8749

Kikkawa, U., Matsuzaki, H. & Yamamoto, T. (2002). Protein kinase C δ (PKC δ): activation mechanisms and functions. *Journal of Biochemistry*, Vol.132, No.6, (Dec 2002), pp. 831-839, ISSN 0021-924X

Kitada, S., Andersen, J., Akar, S., Zapata, J.M., Takayama, S., Krajewski, S., Wang, H.G., Zhang, X., Bullrich, F., Croce, C.M., Rai, K., Hines, J. & Reed, J.C. (1998). Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with In vitro and In vivo chemoresponses. *Blood*, Vol.91, No.9, (May 1998), pp. 3379-3389, ISSN 0006-4971

Kitada, S., Zapata, J.M., Andreeff, M. & Reed, J.C. (1999). Bryostatin and CD40-ligand enhance apoptosis resistance and induce expression of cell survival genes in B-cell chronic lymphocytic leukaemia. *British Journal of Haematology*, Vol.106, No.4, (Sep 1999), pp. 995-1004, ISSN 0007-1048

Kitada, S., Zapata, J.M., Andreeff, M. & Reed, J.C. (2000). Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. *Blood*, Vol.96, No.2, (Jul 2000), pp. 393-397, ISSN 0006-4971

Knapp, L.T. & Klann, E. (2000). Superoxide-induced stimulation of protein kinase C via thiol modification and modulation of zinc content. *Journal of Biological Chemistry*, Vol.275, No.31, (Aug 2000), pp. 24136-24145, ISSN 0021-9258

Kronfeld, I., Kazimirsky, G., Lorenzo, P.S., Garfield, S.H., Blumberg, P.M. & Brodie, C. (2000). Phosphorylation of protein kinase Cδ on distinct tyrosine residues regulates specific cellular functions. *Journal of Biological Chemistry*, Vol.275, No.45, (Nov 2000), pp. 35491-35498, ISSN 0021-9258

Lagneaux, L., Delforge, A., Bron, D., De Bruyn, C. & Stryckmans, P. (1998). Chronic lymphocytic leukemic B cells but not normal B cells are rescued from apoptosis by contact with normal bone marrow stromal cells. *Blood*, Vol.91, No.7, (Apr 1998), pp. 2387-2396, ISSN 0006-4971

Larsson, C. (2006). Protein kinase C and the regulation of the actin cytoskeleton. *Cell Signal*, Vol.18, No.3, (Mar 2006), pp. 276-284, ISSN 0898-6568

Leitges, M., Gimborn, K., Elis, W., Kalesnikoff, J., Hughes, M.R., Krystal, G. & Huber, M. (2002). Protein kinase C-δ is a negative regulator of antigen-induced mast cell degranulation. *Molecular and Cellular Biology*, Vol.22, No.12, (Jun 2002), pp. 3970-3980, ISSN 0270-7306

Liu, Z., Hazan-Halevy, I., Harris, D.M., Li, P., Ferrajoli, A., Faderl, S., Keating, M.J. & Estrov, Z. (2011). STAT-3 activates NF-κB in chronic lymphocytic leukemia cells. *Mol Cancer Research*, Vol.9, No.4, (Apr 2011), pp. 507-515, ISSN 1541-7786

Longo, P.G., Laurenti, L., Gobessi, S., Sica, S., Leone, G. & Efremov, D.G. (2008). The Akt/Mcl-1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B-cell receptor in chronic lymphocytic leukemia B cells. *Blood*, Vol.111, No.2, (Jan 2008), pp. 846-855, ISSN 0006-4971

Mandil, R., Ashkenazi, E., Blass, M., Kronfeld, I., Kazimirsky, G., Rosenthal, G., Umansky, F., Lorenzo, P.S., Blumberg, P.M. & Brodie, C. (2001). Protein kinase Cα and protein

kinase Cδ play opposite roles in the proliferation and apoptosis of glioma cells. *Cancer Research*, Vol.61, No.11, (Jun 2001), pp. 4612-4619, ISSN 0008-5472

Mariano, M.T., Moretti, L., Donelli, A., Grantini, M., Montagnani, G., Di Prisco, A.U., Torelli, G., Torelli, U. & Narni, F. (1992). bcl-2 gene expression in hematopoietic cell differentiation. *Blood*, Vol.80, No.3, (Aug 1992), pp. 768-775, ISSN 0006-4971

Martin, P., Duran, A., Minguet, S., Gaspar, M.L., Diaz-Meco, M.T., Rennert, P., Leitges, M. & Moscat, J. (2002). Role of ζ PKC in B-cell signaling and function. *The EMBO Journal*, Vol.21, No.15, (Aug 2002), pp. 4049-4057, ISSN 0261-4189

Martins, L.R., Lucio, P., Silva, M.C., Gameiro, P., Silva, M.G. & Barata, J.T. (2011). On CK2 regulation of chronic lymphocytic leukemia cell viability. *Molecular and Cellular Biochemistry*, (Jul 2001), pp. ISSN 0300-8177

Matsumoto, M., Ogawa, W., Hino, Y., Furukawa, K., Ono, Y., Takahashi, M., Ohba, M., Kuroki, T. & Kasuga, M. (2001). Inhibition of insulin-induced activation of Akt by a kinase-deficient mutant of the epsilon isozyme of protein kinase C. *Journal of Biological Chemistry*, Vol.276, No.17, (Apr 2001), pp. 14400-14406, ISSN 0021-9258

Mecklenbrauker, I., Kalled, S.L., Leitges, M., Mackay, F. & Tarakhovsky, A. (2004). Regulation of B-cell survival by BAFF-dependent PKCδ-mediated nuclear signalling. *Nature*, Vol.431, No.7007, (Sep 2004), pp. 456-461, ISSN 0028-0836

Meller, N., Liu, Y.C., Collins, T.L., Bonnefoy-Berard, N., Baier, G., Isakov, N. & Altman, A. (1996). Direct interaction between protein kinase C θ (PKC θ) and 14-3-3 τ in T cells: 14-3-3 overexpression results in inhibition of PKC θ translocation and function. *Molecular and Cellular Biology*, Vol.16, No.10, (Oct 1996), pp. 5782-5791, ISSN 0270-7306

Mellor, H. & Parker, P.J. (1998). The extended protein kinase C superfamily. *The Biochemical Journal*, Vol.332 (Pt 2), (Jun 1998), pp. 281-292, ISSN 0264-6021

Michie, A.M. & Nakagawa, R. (2005). The link between PKCα regulation and cellular transformation. Immunology Letters, Vol.96, No.2, (Jan 2005), pp. 155-162, ISSN 0165-2478

Michie, A.M. & Nakagawa, R. (2006). Elucidating the role of protein kinase C in chronic lymphocytic leukaemia. *Hematolgical Oncology*, Vol.24, No.3, (Sep 2006), pp. 134-138, ISSN 0278-0232

Mohammad, R.M., Katato, K., Almatchy, V.P., Wall, N., Liu, K.Z., Schultz, C.P., Mantsch, H.H., Varterasian, M. & al-Katib, A.M. (1998). Sequential treatment of human chronic lymphocytic leukemia with bryostatin 1 followed by 2-chlorodeoxyadenosine: preclinical studies. *Clinical Cancer Research*, Vol.4, No.2, (Feb 1998), pp. 445-453, ISSN 1078-0432

Moreno, A., Villar, M.L., Camara, C., Luque, R., Cespon, C., Gonzalez-Porque, P., Roy, G., Lopez-Jimenez, J., Bootello, A. & Santiago, E.R. (2001). Interleukin-6 dimers produced by endothelial cells inhibit apoptosis of B-chronic lymphocytic leukemia cells. *Blood*, Vol.97, No.1, (Jan 2001), pp. 242-249, ISSN 0006-4971

Moscat, J. & Diaz-Meco, M.T. (2000). The atypical protein kinase Cs. Functional specificity mediated by specific protein adapters. *EMBO Reports* Vol.1, No.5, (Nov 2000), pp. 399-403, ISSN 1469-221X

Nakagawa, R., Soh, J.W. & Michie, A.M. (2006). Subversion of protein kinase C α signaling in hematopoietic progenitor cells results in the generation of a B-cell chronic

lymphocytic leukemia-like population in vivo. *Cancer Research*, Vol.66, No.1, (Jan 2006), pp. 527-534, ISSN 0008-5472

Naylor, T.L., Tang, H., Ratsch, B.A., Enns, A., Loo, A., Chen, L., Lenz, P., Waters, N.J., Schuler, W., Dorken, B., Yao, Y.M., Warmuth, M., Lenz, G. & Stegmeier, F. (2011). Protein kinase C inhibitor sotrastaurin selectively inhibits the growth of CD79 mutant diffuse large B-cell lymphomas. *Cancer Research*, Vol.71, No.7, (Apr 2011), pp. 2643-2653, ISSN 0008-5472

Neill, G.W., Ghali, L.R., Green, J.L., Ikram, M.S., Philpott, M.P. & Quinn, A.G. (2003). Loss of protein kinase Cα expression may enhance the tumorigenic potential of Gli1 in basal cell carcinoma. *Cancer Research*, Vol.63, No.15, (Aug 2003), pp. 4692-4697, ISSN 0008-5472

Newton, A.C. (1995a). Protein kinase C: structure, function, and regulation. *Journal of Biological Chemistry*, Vol.270, No.48, (Dec 1995), pp. 28495-28498, ISSN 0021-9258

Newton, A.C. (1995b). Protein kinase C. Seeing two domains. *Current Biology*, Vol.5, No.9, (Sep 1995), pp. 973-976, ISSN 0960-9822

Newton, A.C. (2010). Protein kinase C: poised to signal. *American Journal of Physiology. Endocrinology and Metabolism* Vol.298, No.3, (Mar 2010), pp. E395-402, ISSN 0193-1849

Nishio, M., Endo, T., Tsukada, N., Ohata, J., Kitada, S., Reed, J.C., Zvaifler, N.J. & Kipps, T.J. (2005). Nurselike cells express BAFF and APRIL, which can promote survival of chronic lymphocytic leukemia cells via a paracrine pathway distinct from that of SDF-1α. *Blood*, Vol.106, No.3, (Aug 2005), pp. 1012-1020, ISSN 0006-4971

Nishizuka, Y. (1988). The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature*, Vol.334, No.6184, (Aug 1998), pp. 661-665, ISSN 0028-0836

O'Brian, C.A. (1998). Protein kinase C-α: a novel target for the therapy of androgen-independent prostate cancer? (Review-hypothesis). *Oncology Reports*, Vol.5, No.2, (Mar-Apr 1998), pp. 305-309, ISSN 1021-335X

Ono, Y., Kurokawa, T., Fujii, T., Kawahara, K., Igarashi, K., Kikkawa, U., Ogita, K. & Nishizuka, Y. (1986). Two types of complementary DNAs of rat brain protein kinase C. Heterogeneity determined by alternative splicing. *FEBS Letters*, Vol.206, No.2, (Oct 1986), pp. 347-352, ISSN 0014-5793

Oster, H. & Leitges, M. (2006). Protein kinase C α but not PKCζ suppresses intestinal tumor formation in $Apc^{Min/+}$ mice. *Cancer Research*, Vol.66, No.14, (Jul 2006), pp. 6955-6963, ISSN 0008-5472

Ota, M., Duong, B.H., Torkamani, A., Doyle, C.M., Gavin, A.L., Ota, T. & Nemazee, D. (2010). Regulation of the B cell receptor repertoire and self-reactivity by BAFF. Journal of Immunology, Vol.185, No.7, (Oct 2010), pp. 4128-4136, ISSN 0022-1767

Panayiotidis, P., Jones, D., Ganeshaguru, K., Foroni, L. & Hoffbrand, A.V. (1996). Human bone marrow stromal cells prevent apoptosis and support the survival of chronic lymphocytic leukaemia cells in vitro. *British Journal of Haematology*, Vol.92, No.1, (Jan 1996), pp. 97-103, ISSN 0007-1048

Parker, P.J. & Murray-Rust, J. (2004). PKC at a glance. *Journal of Cell Science* Vol.117, No.Pt 2, (Jan 2004), pp. 131-132, ISSN 0021-9533

Patke, A., Mecklenbrauker, I., Erdjument-Bromage, H., Tempst, P. & Tarakhovsky, A. (2006). BAFF controls B cell metabolic fitness through a PKC β- and Akt-dependent

mechanism. *The Journal of Experimental Medicine*, Vol.203, No.11, (Oct 2006), pp. 2551-2562, ISSN 0022-1007

Pedersen, I.M., Kitada, S., Leoni, L.M., Zapata, J.M., Karras, J.G., Tsukada, N., Kipps, T.J., Choi, Y.S., Bennett, F. & Reed, J.C. (2002). Protection of CLL B cells by a follicular dendritic cell line is dependent on induction of Mcl-1. *Blood*, Vol.100, No.5, (Sep 2002), pp. 1795-1801, ISSN 0006-4971

Pepper, C., Lin, T.T., Pratt, G., Hewamana, S., Brennan, P., Hiller, L., Hills, R., Ward, R., Starczynski, J., Austen, B., Hooper, L., Stankovic, T. & Fegan, C. (2008). Mcl-1 expression has in vitro and in vivo significance in chronic lymphocytic leukemia and is associated with other poor prognostic markers. *Blood*, Vol.112, No.9, (Nov 2008), pp. 3807-3817, ISSN 0006-4971 (Linking)

Ringshausen, I., Schneller, F., Bogner, C., Hipp, S., Duyster, J., Peschel, C. & Decker, T. (2002). Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: association with protein kinase Cδ. *Blood*, Vol.100, No.10, (Nov 2002), pp. 3741-3748, ISSN 0006-4971

Ringshausen, I., Oelsner, M., Weick, K., Bogner, C., Peschel, C. & Decker, T. (2006). Mechanisms of apoptosis-induction by rottlerin: therapeutic implications for B-CLL. *Leukemia*, Vol.20, No.3, (Mar 2006), pp. 514-520, ISSN 0887-6924

Ron, D. & Mochly-Rosen, D. (1995). An autoregulatory region in protein kinase C: the pseudoanchoring site. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.92, No.2, (Jan 1995), pp. 492-496, ISSN 0027-8424

Ruvolo, P.P., Deng, X., Carr, B.K. & May, W.S. (1998). A functional role for mitochondrial protein kinase Cα in Bcl2 phosphorylation and suppression of apoptosis. *Journal of Biological Chemistry*, Vol.273, No.39, (Sep 1998), pp. 25436-25442, ISSN 0021-9258 (Print)

Saijo, K., Mecklenbrauker, I., Santana, A., Leitger, M., Schmedt, C. & Tarakhovsky, A. (2002). Protein kinase C β controls nuclear factor κB activation in B cells through selective regulation of the IκB kinase α. *The Journal of Experimental Medicine*, Vol.195, No.12, (Jun 2002), pp. 1647-1652, ISSN 0022-1007

Schechtman, D. & Mochly-Rosen, D. (2001). Adaptor proteins in protein kinase C-mediated signal transduction. *Oncogene*, Vol.20, No.44, (Oct 2001), pp. 6339-6347, ISSN 0950-9232

Shinohara, H., Yasuda, T., Aiba, Y., Sanjo, H., Hamadate, M., Watarai, H., Sakurai, H. & Kurosaki, T. (2005). PKC β regulates BCR-mediated IKK activation by facilitating the interaction between TAK1 and CARMA1. *The Journal of Experimental Medicine*, Vol.202, No.10, (Nov 2005), pp. 1423-1431, ISSN 0022-1007

Shinohara, H., Maeda, S., Watarai, H. & Kurosaki, T. (2007). IκB kinase β-induced phosphorylation of CARMA1 contributes to CARMA1 Bcl10 MALT1 complex formation in B cells. *The Journal of Experimental Medicine*, Vol.204, No.13, (Dec 2007), pp. 3285-3293, ISSN 0022-1007

Skvara, H., Dawid, M., Kleyn, E., Wolff, B., Meingassner, J.G., Knight, H., Dumortier, T., Kopp, T., Fallahi, N., Stary, G., Burkhart, C., Grenet, O., Wagner, J., Hijazi, Y., Morris, R.E., McGeown, C., Rordorf, C., Griffiths, C.E., Stingl, G. & Jung, T. (2008). The PKC inhibitor AEB071 may be a therapeutic option for psoriasis. *The Journal of Clinical Investigation*, Vol.118, No.9, (Sep 2008), pp. 3151-3159, ISSN 0021-9738

Slupsky, J.R., Kamiguti, A.S., Harris, R.J., Cawley, J.C. & Zuzel, M. (2007). Central role of protein kinase Cε in constitutive activation of ERK1/2 and Rac1 in the malignant cells of hairy cell leukemia. *The American Journal of Pathology*, Vol.170, No.2, (Feb 2007), pp. 745-754, ISSN 0002-9440

Smit, L.A., Hallaert, D.Y., Spijker, R., de Goeij, B., Jaspers, A., Kater, A.P., van Oers, M.H., van Noesel, C.J. & Eldering, E. (2007). Differential Noxa/Mcl-1 balance in peripheral versus lymph node chronic lymphocytic leukemia cells correlates with survival capacity. *Blood*, Vol.109, No.4, (Feb 2007), pp. 1660-1668, ISSN 0006-4971

Snowden, R.T., Sun, X.M., Dyer, M.J. & Cohen, G.M. (2003). Bisindolylmaleimide IX is a potent inducer of apoptosis in chronic lymphocytic leukaemic cells and activates cleavage of Mcl-1. *Leukemia*, Vol.17, No.10, (Oct 2003), pp. 1981-1989, ISSN 0887-6924

Soltoff, S.P. (2007). Rottlerin: an inappropriate and ineffective inhibitor of PKCδ. *Trends Pharmacol Sci*, Vol.28, No.9, (Sep 2007), pp. 453-458, ISSN 0165-6147

Staudinger, J., Lu, J. & Olson, E.N. (1997). Specific interaction of the PDZ domain protein PICK1 with the COOH terminus of protein kinase C-α. *Journal of Biological Chemistry*, Vol.272, No.51, (Dec 1997), pp. 32019-32024, ISSN 0021-9258

Stohl, W., Xu, D., Kim, K.S., Koss, M.N., Jorgensen, T.N., Deocharan, B., Metzger, T.E., Bixler, S.A., Hong, Y.S., Ambrose, C.M., Mackay, F., Morel, L., Putterman, C., Kotzin, B.L. & Kalled, S.L. (2005). BAFF overexpression and accelerated glomerular disease in mice with an incomplete genetic predisposition to systemic lupus erythematosus. *Arthritis and rheumatism*, Vol.52, No.7, (Jul 2005), pp. 2080-2091, ISSN 0004-3591

Szallasi, Z., Smith, C.B., Pettit, G.R. & Blumberg, P.M. (1994). Differential regulation of protein kinase C isozymes by bryostatin 1 and phorbol 12-myristate 13-acetate in NIH 3T3 fibroblasts. *Journal of Biological Chemistry*, Vol.269, No.3, (Jan 1994), pp. 2118-2124, ISSN 0021-9258

Tan, S.L. & Parker, P.J. (2003). Emerging and diverse roles of protein kinase C in immune cell signalling. *The Biochemical Journal*, Vol.376, No.Pt 3, (Dec 2003), pp. 545-552, ISSN 0264-6021

Thomas, A., Pepper, C., Hoy, T. & Bentley, P. (2004). Bryostatin induces protein kinase C modulation, Mcl-1 up-regulation and phosphorylation of Bcl-2 resulting in cellular differentiation and resistance to drug-induced apoptosis in B-cell chronic lymphocytic leukemia cells. *Leukemia & Lymphoma*, Vol.45, No.5, (May 2004), pp. 997-1008, ISSN 1026-8022

Totterman, T.H., Nilsson, K. & Sundstrom, C. (1980). Phorbol ester-induced differentiation of chronic lymphocytic leukaemia cells. *Nature*, Vol.288, No.5787, (Nov 1980), pp. 176-178, ISSN 0028-0836

Varterasian, M.L., Mohammad, R.M., Eilender, D.S., Hulburd, K., Rodriguez, D.H., Pemberton, P.A., Pluda, J.M., Dan, M.D., Pettit, G.R., Chen, B.D. & Al-Katib, A.M. (1998). Phase I study of bryostatin 1 in patients with relapsed non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *Journal of Clinical Oncology*, Vol.16, No.1, (Jan 1998), pp. 56-62, ISSN 0732-183X

Varterasian, M.L., Mohammad, R.M., Shurafa, M.S., Hulburd, K., Pemberton, P.A., Rodriguez, D.H., Spadoni, V., Eilender, D.S., Murgo, A., Wall, N., Dan, M. & Al-Katib, A.M. (2000). Phase II trial of bryostatin 1 in patients with relapsed low-grade

non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *Clinical Cancer Research*, Vol.6, No.3, (Mar 2000), pp. 825-828, ISSN 1078-0432

Villalba, M., Kasibhatla, S., Genestier, L., Mahboubi, A., Green, D.R. & Altman, A. (1999). Protein kinase cθ cooperates with calcineurin to induce Fas ligand expression during activation-induced T cell death. *Journal of Immunology*, Vol.163, No.11, (Dec 1999), pp. 5813-5819, ISSN 0022-1767

Waldron, R.T. & Rozengurt, E. (2003). Protein kinase C phosphorylates protein kinase D activation loop Ser744 and Ser748 and releases autoinhibition by the pleckstrin homology domain. *Journal of Biological Chemistry*, Vol.278, No.1, (Jan 2003), pp. 154-163, ISSN 0021-9258

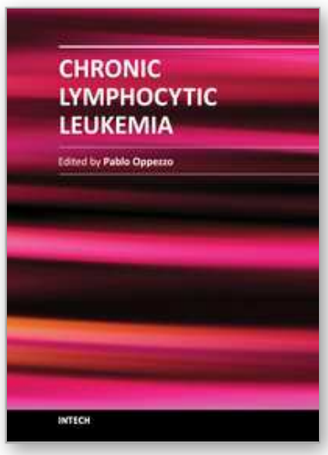
Wert, M.M. & Palfrey, H.C. (2000). Divergence in the anti-apoptotic signalling pathways used by nerve growth factor and basic fibroblast growth factor (bFGF) in PC12 cells: rescue by bFGF involves protein kinase C δ. *The Biochemical Journal*, Vol.352 Pt 1, (Nov 2000), pp. 175-182, ISSN 0264-6021

Yan, X.J., Albesiano, E., Zanesi, N., Yancopoulos, S., Sawyer, A., Romano, E., Petlickovski, A., Efremov, D.G., Croce, C.M. & Chiorazzi, N. (2006). B cell receptors in TCL1 transgenic mice resemble those of aggressive, treatment-resistant human chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America* Vol.103, No.31, (Aug 2006), pp. 11713-11718, ISSN 0027-8424

Yuan, Z.M., Utsugisawa, T., Ishiko, T., Nakada, S., Huang, Y., Kharbanda, S., Weichselbaum, R. & Kufe, D. (1998). Activation of protein kinase C δ by the c-Abl tyrosine kinase in response to ionizing radiation. *Oncogene*, Vol.16, No.13, (Apr 1998), pp. 1643-1648, ISSN 0950-9232

Zhuang, J., Hawkins, S.F., Glenn, M.A., Lin, K., Johnson, G.G., Carter, A., Cawley, J.C. & Pettitt, A.R. (2010). Akt is activated in chronic lymphocytic leukemia cells and delivers a pro-survival signal: the therapeutic potential of Akt inhibition. *Haematologica*, Vol.95, No.1, (Jan 2010), pp. 110-118, ISSN 0390-6078

zum Buschenfelde, C.M., Wagner, M., Lutzny, G., Oelsner, M., Feuerstacke, Y., Decker, T., Bogner, C., Peschel, C. & Ringshausen, I. (2010). Recruitment of PKC-βII to lipid rafts mediates apoptosis-resistance in chronic lymphocytic leukemia expressing ZAP-70. *Leukemia*, Vol.24, No.1, (Jan 2010), pp. 141-152, ISSN 0887-6924



Chronic Lymphocytic Leukemia
Edited by Dr. Pablo Oppezzo

ISBN 978-953-307-881-6
Hard cover, 448 pages
Publisher InTech
Published online 10, February, 2012
Published in print edition February, 2012

B-cell chronic lymphocytic leukemia (CLL) is considered a single disease with extremely variable course, and survival rates ranging from months to decades. It is clear that clinical heterogeneity reflects biologic diversity with at least two major subtypes in terms of cellular proliferation, clinical aggressiveness and prognosis. As CLL progresses, abnormal hematopoiesis results in pancitopenia and decreased immunoglobulin production, followed by nonspecific symptoms such as fatigue or malaise. A cure is usually not possible, and delayed treatment (until symptoms develop) is aimed at lengthening life and decreasing symptoms. Researchers are playing a lead role in investigating CLL's cause and the role of genetics in the pathogenesis of this disorder. Research programs are dedicated towards understanding the basic mechanisms underlying CLL with the hope of improving treatment options.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

John C. Allen and Joseph R. Slupsky (2012). Pathophysiology of Protein Kinase C Isozymes in Chronic Lymphocytic Leukaemia, Chronic Lymphocytic Leukemia, Dr. Pablo Oppezzo (Ed.), ISBN: 978-953-307-881-6, InTech, Available from: http://www.intechopen.com/books/chronic-lymphocytic-leukemia/pathophysiology-of-protein-kinase-c-isozymes-in-chronic-lymphocytic-leukaemia

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen