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Atypical Protein Kinase C α as a human oncogene and therapeutic target

Peter J. Parker^{†,*,}, Verline Justilien[#], Philippe Riou[†], Mark Linch^{^,†}, and Alan P. Fields^{#,*}

[#]Department of Cancer Biology, Mayo Clinic Comprehensive Cancer Center, Jacksonville, Florida

[†]London Research Institute, Lincoln's Inn Fields, London, UK

[@]King's College London, Guy's Campus, London, UK

[^]Royal Marsden Hospital, Fulham Road, London, UK

Abstract

Protein kinase inhibitors represent a major class of targeted therapeutics that has made a positive impact on treatment of cancer and other disease indications. Among the promising kinase targets for further therapeutic development are members of the Protein Kinase C (PKC) family. The PKCs are central components of many signaling pathways that regulate diverse cellular functions including proliferation, cell cycle, differentiation, survival, cell migration, and polarity. Genetic manipulation of individual PKC isoforms has demonstrated that they often fulfill distinct, nonredundant cellular functions.¹¹ Participation of PKC members in different intracellular signaling pathways reflects responses to varying extracellular stimuli, intracellular localization, tissue distribution, phosphorylation status, and intermolecular interactions. PKC activity, localization, phosphorylation, and/or expression are often altered in human tumors, and PKC isoforms have been implicated in various aspects of transformation, including uncontrolled proliferation, migration, invasion, metastasis, angiogenesis, and resistance to apoptosis. Despite the strong relationship between PKC isoforms and cancer, to date only atypical PKC α has been shown to function as a bona fide oncogene, and as such is a particularly attractive therapeutic target for cancer treatment. In this review, we discuss the role of PKC α in transformation and describe mechanism-based approaches to therapeutically target oncogenic PKC α signaling in cancer.

Keywords

Atypical Protein Kinase C α ; cellular transformation; Phox-Bem1 (PB1) Domain; Mechanism-based therapeutics; oncogenic signaling

Introduction

Protein kinase inhibitors represent a major class of targeted therapeutics that has made a positive impact on treatment of cancer and other disease indications. There are currently 23

*To whom correspondence should be addressed: Alan P. Fields, Ph.D. Department of Cancer Biology Mayo Clinic 45400 San Pablo Road Jacksonville, Florida 32224 Peter J. Parker, Ph.D. London Research Institute Lincoln's Inn Fields London WC2A 3LY UK.

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FDA-approved kinase inhibitors in use for various cancer and immune indications (see <http://www.brimr.org/PKI/PKIs.htm>). In addition, there is a rich pipeline of novel agents targeting disease-associated kinase targets in the clinic or at various stages of pre-clinical development (http://www.insightpharmareports.com/uploadedFiles/Executive_Summary%282%29.pdf). Among the promising kinase targets for further therapeutic development are members of the Protein Kinase C (PKC) family.

PKCs are a family of lipid-dependent serine/threonine kinases that represent a branch of the ACG kinase group.¹ The PKC family is subdivided into 4 classes based on unique structural and regulatory properties. The conventional PKCs (cPKCs) are comprised of the PKC α , alternatively spliced PKC β I and β II, and PKC γ isozymes. These PKC isozymes bind diacylglycerol (DAG) and are activated by phosphatidylserine (PS) in a Ca²⁺-dependent manner, properties consistent with the initial description of PKC regulation.² The novel PKCs (nPKCs) include the PKC δ , ϵ , η , θ , and μ isozymes. These PKC isozymes require DAG and PS for activation but are insensitive to Ca²⁺.³ The atypical PKCs (aPKCs), comprised of ζ and ι (also known as λ in rodents), are Ca²⁺-independent and do not respond to DAG.^{4,5} Finally, the three PRK members (PRK 1–3) are also insensitive to Ca²⁺ and DAG.^{6,7}

The PKCs are central components of many signaling pathways that regulate diverse cellular functions including proliferation, cell cycle, differentiation, survival, cell migration, and polarity (recently reviewed in^{8–10}). Genetic manipulation of individual PKC isozymes has demonstrated that they often fulfill distinct, non-redundant cellular functions.¹¹ Participation of PKC members in different intracellular signaling pathways reflects responses to varying extracellular stimuli, intracellular localization, tissue distribution, phosphorylation status, and intermolecular interactions (recently reviewed in¹²). PKC activity, localization, phosphorylation, and/or expression are often altered in human tumors, and PKC isozymes have been implicated in various aspects of transformation, including uncontrolled proliferation, migration, invasion, metastasis, angiogenesis, and resistance to apoptosis (reviewed in^{10,13}). Despite the strong relationship between PKC isozymes and cancer, to date only atypical PKC ι has been shown to function as a bona fide oncogene,^{14,15} and as such is a particularly attractive therapeutic target for cancer treatment. In this review, we discuss the role of PKC ι in transformation and describe mechanism-based approaches to therapeutically target oncogenic PKC ι signaling in cancer.

General PKC Activation Mechanisms

Since Nishizuka's initial report of a proteolytically-activated protein kinase,¹⁶ it has been apparent that PKCs are present in cells in an inhibited basal state. Subsequent studies have identified upstream signaling pathways that allow PKCs to assume a fully “primed”, catalytically competent, inhibited basal state, which can be acutely activated to trigger PKC-dependent processes (reviewed in^{17,18}). These upstream signaling pathways lead to multi-site PKC phosphorylation on three conserved kinase domain serine/threonine phosphorylation sites – the activation loop, C-terminal turn, and hydrophobic motif sites – which in combination act to stabilize a fully active catalytic domain. In aPKC isoforms, the hydrophobic motif phosphorylation site acceptor amino acid is replaced with the partial phospho-mimetic glutamic acid. In the phosphorylated, primed state, the kinase and regulatory domains bind in an auto-inhibited conformation. Auto-inhibition appears to involve both a pseudosubstrate site present in all PKC family members, and other regulatory-kinase domain packing interactions^{19,20} creating a ‘closed’ inactive conformation.

Though no structures have been determined for an inactive full length PKC, a structure for a partially activated PKC β structure has been described.²¹ A key feature of this structure is the orientation of the “NFD” motif within the kinase domain such that the Phe side chain makes contact with the ATP adenine ring²² to form a structure incompatible with an active, nucleotide-bound kinase domain. This structure likely reflects the fully-inhibited state, which is probably not nucleotide-bound, although the protein substrate binding pocket is expected to be occupied by the pseudosubstrate motif.

From this primed, inhibited state, acute activation involves loss of inhibitory regulatory-catalytic domain interactions and subsequent binding of Mg-ATP and protein substrates in the nucleotide and substrate binding pockets, respectively. For the classical PKC isoforms (α , β , γ), activation involves Ca^{2+} -dependent recruitment of the kinase to anionic phospholipids, typically at the plasma membrane, through the Ca^{2+} binding C2 domains. Once at the membrane, the now proximal tandem C1A–C1B domain, is able to sample the membrane for DAG, a process that involves a conformation change exposing the otherwise obscured, intrinsically rather rigid DAG binding sites. The K/R-rich pseudosubstrate site, which is contiguous with the N-terminus of the C1A domain, interacts directly with anionic phospholipids to further stabilize membrane association. The nPKC isoforms are also activated by DAG binding to their C1A–C1B domains, but they do not display Ca^{2+} dependence for membrane recruitment. The C2 domains of certain nPKCs also have been shown to possess phosphotyrosine binding activity involved in localizing PKC δ to CDCP1, and in activation of PKC θ .²³

Following activation, several PKC isoforms can bind to RACKs – receptors for activated C-kinases (reviewed in²⁴). It has been proposed that RACK interactions can lock the kinase in an open conformation thereby contributing to maintenance of the active state. Studies on PKC-RACK interactions have led to the idea that expression of short synthetic forms of the domains responsible for these interactions can disrupt RACK-PKC interactions and serve as isoform-selective PKC inhibitors. Such inhibitors have been reported for many PKC isoforms, but to date not for the aPKC family. Similarly, protein scaffolds that direct localization of PKC isoforms prior to activation have been identified, among which are members of the A-Kinase Activating Proteins (AKAPs) (recently reviewed in²⁵). AKAPs can bind specific PKC isoforms and link them to particular pathway outputs (eg. PKC η >PKD). Inhibitors of AKAP function in the form of short oligopeptides that compete for PKC-AKAP interactions have been described, however there is little evidence for a role of AKAPs in aPKC action.

aPKC Activation Mechanisms

The aPKCs (PKC ζ and PKC ι) are structurally and functionally distinct from other PKCs. The catalytic activity of the aPKCs does not require DAG, PS, or calcium, and the aPKCs do not serve as cellular receptors for phorbol esters.^{4, 26} Rather, PKC ι can be activated by various acidic phospholipids including phosphatidylinositol 3,4,5 trisphosphate,^{7, 27} through specific protein–protein interactions mediated by a Phox Bem1 (PB1) domain within the N-terminal regulatory domain of the aPKCs (reviewed in²⁸; discussed in detail below), and potentially through phosphorylation events that can regulate PKC ι and its localization.

In addition to the priming phosphorylation events described above, PKC ι has been reported to be phosphorylated by Src at tyrosines 256, 271, 325 in response to NGF stimulation.²⁹ Site-directed mutagenesis indicates that Y325 phosphorylation is required for NGF-mediated PKC ι activation and subsequent NF κ B activation,²⁹ whereas T256 phosphorylation regulates nuclear-cytoplasmic PKC ι localization by modulating a canonical nuclear localization sequence (NLS).³⁰ Src-mediated phosphorylation is also necessary for

PKC ζ -dependent recruitment of β -COP to pre-golgi intermediates³¹ and the movement of PKC ζ into endosomes where it binds p62.³² Mass spectrometry analysis has revealed several other potential phosphorylation sites on PKC ζ ,³³ however, to date, neither the responsible kinase(s), nor the functional importance of these phosphorylation events, have been elucidated.

Protein-protein interactions regulate PKC ζ localization and activity

The aPKCs possess a region within their regulatory domain that is unique to the atypical PKCs, the Phox-Bem (PB)1 domain. The PB1 domain is a structurally conserved motif found on a family of signaling molecules (reviewed in²⁸) that mediates their homo- and heterotypic interactions through specific interaction codes. The PB1 domain interactions formed between aPKCs and other PB1 domain containing proteins such as ZIP/p62, Par6 and MEK5 [MAPK(mitogen-activated protein kinase)/ ERK(extracellular-signal-regulated kinase)kinase 5] are critical for aPKC activation, localization and function in several contexts, including cell polarity, cell proliferation, cell survival, and more recently cell transformation.^{14, 34–38}

Perhaps the best studied PB1-PB1 domain interaction involving PKC ζ is that between PKC ζ and the polarity protein Par6, which is essential for epithelial cell polarity (recently reviewed in³⁹). PKC ζ interacts with Par6 to form a heterodimeric complex that is activated when Par6 binds to GTP-loaded cdc42/Rac1. Par6 binds PKC ζ through a characteristic PB1-PB1 interaction, for which a crystal structure has been solved.⁴⁰ Par3 subsequently binds the PKC ζ -Par6 complex through a Par3 PDZ domain interaction with the kinase domain of PKC ζ , and a PDZ:PDZ domain interaction between Par3 and Par6. Par3 functions to localize PKC ζ /Par6 to the apical compartment of polarized cell membranes through a network of interactions involving nectin-willitin-afadin (recently reviewed in³⁹). The isoprenylated small GTPases cdc42/Rac1 are typically membrane-associated in their active GTP-bound states, contributing to the association of the cdc42/Rac-Par6-PKC ζ -Par3 complex with the apical membrane. Interestingly, a frequent mechanism by which human tumors lose polarity, a hallmark of cancer, is through loss of Par3 and/or other components of tight junctions such as E-cadherin.^{41, 42} As a consequence, PKC ζ is frequently mis-localized within the cytoplasm and nucleus of transformed cells and human tumors^{14, 15, 43, 44} Despite the loss of membrane restricted localization, recent studies have demonstrated that PKC ζ retains its association with Par6 in tumor cells,^{35, 36, 45} and this complex, and PKC ζ activity, is required for maintenance of the transformed phenotype.^{34–36, 45–47}

PKC ζ is Oncogenic in Human Tumors

The PKC ζ gene (*PRKCI*) is frequently amplified in human cancers

The PKC ζ gene, *PRKCI*, resides on chromosome 3q26, one of the most frequently amplified genomic regions in human cancers. Compelling evidence across multiple tumor types demonstrate that *PRKCI* is a relevant target of 3q26 amplification. *PRKCI* copy number gains are observed in ~80% of human primary lung squamous cell carcinomas (LSCC),¹⁴ ~70% of serous epithelial ovarian cancer¹⁵ and ~53% of ESCC tumors.⁴⁸ Consistent with these published findings, analysis of human tumor genomic datasets from the Cancer Genome Atlas, and other large scale sequencing projects (compiled at <http://www.cbioportal.org/public-portal/>), demonstrates that *PRKCI* copy number gains are prominent in many major forms of human cancer, being most prevalent in cervical, head and neck, lung squamous and ovarian serous cancers (Figure 1A). Surprisingly however, other major tumor types such as bladder, breast, kidney, lung adenocarcinoma, stomach and uterine cancers also harbor frequent *PRKCI* copy number gains, albeit less frequently than the tumor types mentioned above. Interestingly, gene expression data from these same tumor

datasets reveal a strong positive correlation between *PRKCI* copy number gains and elevated PKC ζ mRNA expression across these tumor types (Figure 1B). Thus, tumor-specific gene copy number gain in *PRKCI* is a major genetic mechanism driving PKC ζ expression in human tumors. In contrast, *PRKCI* is rarely mutated with frequencies ranging from 0–3% across tumor types. Of approximately 9,000 human tumor samples derived from different tissues analyzed for *PRKCI* somatic mutations in the COSMIC database (<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>), only 0.81% of tumors harbor a *PRKCI* mutation. Although of low frequency, one mutated site (R471), which has been reported on multiple occasions, maps to a substrate docking domain required for PKC ζ to support cellular polarization, while not influencing catalytic capacity per se.⁴⁹ Thus, R471 mutation mediates a change of function wherein one aspect of PKC ζ output potential is selectively compromised.

PKC ζ is over-express and mislocalized in human tumors—PKC ζ is frequently overexpressed in the majority of tumor types examined (recently reviewed in⁵⁰ (Figure 1). PKC ζ is overexpressed at the mRNA and protein level in NSCLC, ovarian, brain, breast, rhabdomyosarcomas,⁵¹ melanomas, esophageal, gastric, colon, liver, pancreas, and bile duct tumors. Interestingly, immunohistochemical analyses have reveal PKC ζ overexpression in tumor cells, with little to no staining in tumor-associated stroma and adjacent matched normal lung epithelium, indicating that PKC ζ overexpression is largely restricted to tumor cells. PKC ζ is frequently mislocalized in several tumor types. Whereas PKC ζ is localized to the apical and not the basal membrane of normal ovarian surface epithelial cells and benign serous and mucinous cysts, apical membrane staining was lost in 85% of serous low malignant samples and in all serous epithelial ovarian cancers analyzed where staining was diffuse throughout the tumor cells.¹⁵ NSCLC tumors reveal intense PKC ζ cytoplasmic and nuclear staining whereas adjacent normal lung epithelial cells exhibited membrane staining.¹⁴ Similarly, PKC ζ localized throughout the cytoplasm of primary breast tumor cells, whereas it localized to the apical membrane of normal breast epithelial cells.⁴⁴ Cytoplasmic PKC ζ in hepatocellular carcinoma (HCC) correlated with reduced cell-cell contact, loss of both adherens and tight junction formation, reduced E-cadherin expression, and an increase in cytoplasmic beta-catenin.⁴³ Taken together, these findings indicate that PKC ζ overexpression and intracellular mislocalization is a frequent event in cancer cells that is associated with loss of cellular polarity.

Although *PRKCI* gene copy gain is a major mechanism that drives PKC ζ overexpression in some human tumor types, alternative mechanisms may also promote PKC ζ overexpression. PKC ζ is frequently overexpressed in tumor types that harbor infrequent 3q26 amplification including colon cancer,³⁷ pancreatic cancer,^{52, 53} and chronic myelogenous leukemia (CML).⁵⁴ In CML cells, a Bcr-Abl/Mek/Erk signaling axis transcriptionally activates PKC ζ expression through an Elk1 element within the promoter region of *PRKCI*.⁵⁴ Whether this or similar transcriptional mechanisms are relevant in other tumor types remains undetermined. Furthermore, PKC ζ activity has been shown to be primed by phosphorylation and, at least in the related PKC α it is documented that dephosphorylation at these sites can trigger PKC degradation.⁵⁵ However, it is presently unclear whether PKC ζ phosphorylation and other post-translational mechanisms serve to regulate PKC ζ stability in tumor cells.

PKC ζ is required for the cancer cell phenotype

PKC ζ is required for maintenance of the transformed phenotype of cancer cells, regulating transformed growth, survival and chemoresistance of several tumor types including CML, prostate cancer, NSCLC, glioma, and ESCC (reviewed in⁵⁰). Genetic or pharmacologic inhibition of PKC ζ blocks transformed growth of NSCLC, ovarian cancer, ESCC,⁵⁶ colon carcinoma, PDAC,⁵³ and rhabdomyosarcoma⁵¹ cells. In addition, PKC ζ promotes cellular

migration and or invasion of NSCLC, ESCC,⁵⁶ PDAC, and glioma cells. PKC ι is also critical for tumorigenesis *in vivo*. Orthotopic or subcutaneous implantation of NSCLC, ESCC,⁵⁶ and PDAC cells in which PKC ι has been genetically-depleted into immune deficient mice results in impaired tumor initiation, growth and metastatic potential. Transgenic mice expressing constitutively active PKC ι (caPKC ι) in the colonic epithelium exhibit a higher incidence of colon tumors when compared to transgenic mice expressing kdPKC ι or non-transgenic littermates after exposure to the colon-specific carcinogen azoxymethane.³⁷ Furthermore, transgenic caPKC ι mice develop mostly malignant carcinomas whereas non-transgenic mice develop mainly benign tubular adenomas.³⁷ Consistent with the requirements of PKC ι for cellular invasion, ESCC⁵⁶ and PDAC⁵³ cells with reduced PKC ι expression exhibit a decrease in metastasis. These findings demonstrate that PKC ι is functionally required for the transformed behavior of cancer cells and indicate roles for PKC ι in tumor initiation, progression and metastatic behavior.

PKC ι expression predicts survival and is associated with tumor aggressiveness

Reflecting its functional role in cellular transformation, PKC ι expression profiling may be of prognostic value to predict survival in patients with various tumors types (reviewed in⁵⁰). Elevated PKC ι expression is associated with decreased survival in patients with NSCLC, cholangiocarcinoma, ovarian, bile duct, and prostate tumors. In other cancer types such as ESCC and breast, PKC ι expression is greater in later stage tumors suggesting a role for PKC ι in tumor aggressiveness, progression and metastasis. Thus, PKC ι expression may be useful for identifying patients that are more likely to relapse and those who may benefit from more aggressive adjuvant chemotherapeutic treatment. In this regard, patients with high PKC ι expressing gastric tumors are more likely to experience relapse,⁵⁷ and elevated PKC ι in early stage NSCLC tumors is associated with poor outcome.¹⁴

Oncogenic PKC ι Signaling

Upstream activators of PKC ι

PKC ι participates in a number of oncogenic signaling pathways that promote transformation (reviewed in^{10, 50, 58}). PKC ι plays a critical role in oncogenic *Ras*-mediated transformation. Exogenous over-expression of PKC ι increases the size and number of soft agar colonies formed by oncogenic *Ras*-transformed ovarian surface epithelial cells.⁵⁹ Likewise, PKC ι activity is elevated in *Ras*-transformed intestinal epithelial cells, and is required for invasion and anchorage independent growth of these cells *in vitro*. The importance of PKC ι as a downstream effector of oncogenic *Ras* *in vivo* has been demonstrated in several mouse models of *K-Ras*-mediated tumorigenesis.^{37, 60} In a mouse model of *K-Ras*-mediated colon cancer, K-Ras^{LA2} transgenic mice, which contain a latent oncogenic *K-Ras* G12D allele that is activated by spontaneous recombination, develop oncogenic *K-Ras*-dependent aberrant crypt foci (ACF) in the colonic epithelium.³⁷ ACFs are likely precursors to colon cancer⁶¹ and harbor many of the same genetic and biochemical alterations found in colon tumors, including activating *K-Ras* mutations. KrasLA/kdPKC ι mice, which express kinase deficient PKC ι in the colonic epithelium, develop significantly fewer ACF than KrasLA mice.⁶² A mouse model in which oncogenic *Kras*^{G12D} is activated by Cre-mediated recombination in the lung with or without simultaneous genetic loss of the mouse PKC ι gene (*Prkci*) was used to assess whether PKC ι is involved in lung tumor development.⁶⁰ Genetic disruption of *Prkci* blocked *K-Ras*-mediated lung tumor formation *in vivo*, a phenotype that was traced to a defect in *K-Ras*-mediated expansion of bronchioalveolar stem cells (BASCs) in *Prkci* deficient mice.⁶⁰ Interestingly, *Kras* induced uncontrolled proliferation and morphological transformation of BASCs maintained in three dimensional culture.⁶⁰ Whereas non-transformed BASCs form acinar structures characterized by a single large central lumen and a single layer of polarized epithelial cells, *Kras*-mediated transformation is characterized by

disruption of cellular polarity, loss of the central lumen and uncontrolled growth of BASC cells as an amorphous mass.⁶⁰ These morphological and proliferative changes induced by oncogenic *Kras* are strictly dependent upon PKC ζ expression.⁶⁰ These data provide direct evidence that PKC ζ -dependent, *Kras*-mediated transformation involves both disruption of cellular polarity and stimulation of transformed growth.⁶⁰ BASCs exhibit stem-like properties and are thought to be the tumor-initiating cells in *KRas*-mediated lung adenocarcinoma formation.⁶³ Thus, *Prkci* is required for the earliest identifiable events in *KRas* mediated lung tumorigenesis *in vivo*. PKC ζ is also required for maintenance of the transformed phenotype and *in vivo* tumorigenic potential of established human cancer cells harboring oncogenic *KRAS* mutations.³⁶ Expression of kdPKC ζ or RNAi knockdown of PKC ζ in NSCLC cells that harbor an oncogenic *KRas* mutation resulted in reduced formation and growth of xenograft tumors in immune-deficient mice.³⁶ Greater than 90% of pancreatic tumors harbor oncogenic *KRas* mutation, and orthotopic implantation of *KRas* mutant pancreatic cancer cells depleted of PKC ζ into the pancreas of nude mice led to decreased tumor burden, reduced markers of angiogenesis and fewer metastases.⁵³

Several studies have implicated aPKCs in *Src*-mediated transformation.^{30, 64} *Src* is reported to directly bind and phosphorylate aPKCs at multiple sites to promote their activation.^{30, 64} As noted above, *Src*-mediated phosphorylation of tyrosine 325 was shown to be functionally required for *Src* activation of PKC ζ in response to NGF.²⁹ In NSCLC cells, NNK has been reported to increase *c-Src*-activated PKC ζ , which in turn phosphorylates Bad resulting in reduced Bad/Bcl-XL interaction.⁶⁴ PKC ζ -mediated phosphorylation abrogates the pro-apoptotic function of Bad, and enhances cell survival and decreased sensitivity of NNK treated NSCLC cells to VP-16 and cisplatin.⁶⁴ Genetic or pharmacological inhibition of *Src* led to decreased aPKC activation loop phosphorylation and reduced proliferation of androgen dependent prostate cancer cells.⁶⁵ Additionally, inhibition of aPKC decreased *v-Src*-mediated morphological transformation, migration, matrix degradation and invasion of transformed NIH3T3 cells.⁶⁶

Phosphoinositide 3-kinase (PI3K) has been implicated in the activation of aPKC. The PI3K lipid metabolite, PIP3, may promote aPKC activation through 3-phosphoinositide dependent protein kinase-1 (PDK-1) T403 loop phosphorylation.⁶⁷ Consistently, RNAi-mediated knockdown of PDK1 inhibited priming phosphorylation of PKC ζ at T555 and reduced PKC ζ expression in glioma cells.⁶⁸ Recently PI3K/PKC ζ signaling has been linked to the alternative splicing of Bcl-x pre-mRNA to promote cell survival in NSCLC cells.⁶⁹ NSCLC, breast, cervical, and esophageal tumors exhibit increased expression of anti-apoptotic Bcl-x(L) when compared to their respective matched normal tissues. Interestingly, treatment of NSCLC cells with PI3K or pan-PKC inhibitors resulted in a reduced ratio of Bcl-x(L)/(s) splice variants as did knockdown of PKC ζ .⁶⁹ Furthermore, over-expression of Bcl-x(L) could rescue PKC ζ knockdown mediated inhibition of NSCLC cell growth in soft agar. Thus, PI3K/PKC ζ promote an antiapoptotic/prosurvival phenotype in NSCLC cells through modulation of Bcl-x(L)/(s) splice variant ratios.

PKC ζ is a downstream effector of *Bcr-Abl*-mediated chronic myelogenous leukemia (CML) cell survival and resistance to chemotherapeutic drugs.^{38, 70} In K562 CML cells, *Bcr-Abl* activates a Ras/Mek/Erk signaling pathway that stimulates PKC ζ expression through an Elk1 transcription site within the PKC ζ promoter.⁵⁴ PKC ζ is activated upon treating K562 cells with taxol,³⁸ and overexpression of PKC ζ leads to enhanced resistance of K562 cells to taxol-induced apoptosis, whereas inhibition of PKC ζ expression enhances taxol mediated apoptotic cell death of K562 cells.⁷⁰ Furthermore, inhibition of Bcr-Abl blocked taxol-induced PKC ζ activation and sensitized K562 cells to taxol mediated apoptosis. In contrast, expression of caPKC ζ protects K562 cells from chemotherapeutic drug-mediated apoptosis.⁷⁰ Interestingly, in *Bcr-Abl* negative HL60 promyelocytic leukemia cells, taxol did

not induce sustained PKC ζ activation.³⁸ Thus activation of PKC ζ is an important downstream mediator of *Bcr-Abl*-induced chemotherapeutic resistance.

Effectors of PKC ζ signaling

PKC ζ promotes cell survival in some tumor cells through activation of NF κ B signaling. PKC ζ associates with IKK $\alpha\beta$ and I κ B α in TNF α -treated DU-145 prostate carcinoma cells where it is thought to phosphorylate IKK $\alpha\beta$, which subsequently phosphorylates I κ B α resulting in NF κ B/p65 translocation into the nucleus and transcriptional activation of targets.⁷¹ Interestingly, PKC ζ does not associate with IKK $\alpha\beta$ and I κ B α in transformed non-tumorigenic RWPE-1 prostate cells. Rather, TNF α treatment of RWPE-1 induces association of PKC ζ with both IKK $\alpha\beta$ and I κ B α suggesting that PKC ζ may be preferentially used to activate NF κ B pathways in tumorigenic prostate cancer cells.⁷¹ As described above, CML cells expressing *Bcr-Abl* are resistant to chemotherapy-induced apoptosis as a result of increased PKC ζ activation.^{38, 70, 72} Treatment of CML cells with taxol induces I κ B α phosphorylation and NF κ B nuclear translocation and transcriptional activation and disruption of PKC ζ function sensitizes K562 cells to taxol-induced apoptosis and inhibits RelA transcriptional activity.⁷² Overexpression of NF κ B in K562 cells with disrupted PKC ζ function, rescues taxol-induced apoptosis. In addition, overexpression of constitutively active PKC ζ further upregulates NF κ B transcriptional activity. Thus, PKC ζ induction of NF κ B transactivation is important for *Bcr-Abl*-dependent resistance to taxol-induced apoptosis.^{70, 72} Glioblastoma multiforme are highly resistant to most standard cancer chemotherapeutics.⁷³ RNAi-mediated depletion of PKC ζ results in sensitization of U87MG glioblastoma cells to cisplatin.⁷³ Interestingly, unlike CML and prostate cancer cells, activation of the NF κ B pathway does not appear to be a major mechanism driving PKC ζ dependent chemoresistance in glioblastoma. Rather, PKC ζ -mediated survival appears to result from PKC ζ -mediated attenuation of p38 mitogen-activated protein kinase signaling that protects these cells against cytotoxicity to chemotherapeutic agents.⁷³ Thus, PKC ζ appears to function in several signaling pathways that promote cell survival in different tumor cell types.

Given the crucial role that polarity plays in maintaining the highly organized epithelial architecture, it is perhaps not surprising that several components of the polarity complex, particularly PKC ζ , Par6, Par3 and Rac1 have been directly implicated in oncogenesis. Aberrant expression of genes associated with cellular transformation such as *ErbB2*, *Ras*, *NOTCH* and TGF β disrupt cell polarity and cause mislocalization of polarity complex components from the apical-lateral membrane. Activation of ErbB2 disrupts cell polarity through a mechanism in which ErbB2 causes mislocalization of Par6 from the apical-lateral border and disassociates Par3 from the aPKC/Par6 complex.⁴⁶ Likewise, in rat proximal epithelial cells, TGF β disrupts cell polarity through downregulation of Par3 and redistribution of aPKC/Par6 complex from the cell membrane to the cytoplasm.⁷⁴ A recent report demonstrated that loss of Par3 cooperates with the intracellular domain of NOTCH (NICD) or *H-Ras* to induce breast tumorigenesis, tumor invasion and metastasis.⁷⁵ A model was proposed in which the loss of Par3 in transformed cells triggers mislocalization and activation of aPKC.⁷⁵ aPKC in turn activates a JAK/Stat3/MMP signaling cascade that induces changes in the ECM that promote tumor cell invasion and metastasis.⁷⁵

Rac1 is a key downstream intermediate in oncogenic PKC ζ signal in multiple tumor types including the colon,³⁷ lung,^{35, 36} and pancreas.⁵³ In NSCLC cells, the PB1:PB1 interaction between PKC ζ and Par6 is required for the transformed phenotype and Rac1 activation.^{34, 36, 53} RNAi mediated depletion of PKC ζ , Par6 or Rac1 blocks transformation.³⁵ Expression of PB1 domain mutants of PKC ζ or Par6 that inhibit the PKC ζ -Par6 interaction fail to restore transformation in PKC ζ and Par6 knockdown cells

respectively,³⁵ and either decreased PKC ϵ or Par6 expression inhibits activation of Rac1, whereas expression of a constitutively active Rac1 allele (RacV12) in either PKC ϵ - or Par6-depleted NSCLC cells restores transformation.³⁵ Thus, Rac1 is a key effector of PKC ϵ -mediated transformation in NSCLC. Proteomics analysis of proteins that associate with the PKC ϵ -Par6 complex in NSCLC cells identified ECT2, a Rho family GTPase guanine nucleotide exchange factor (GEF), as a component of the PKC ϵ -Par6 complex.^{34, 47} RNAi-mediated knock down of ECT2 inhibits Rac1 activity and blocks transformed growth, invasion, and tumorigenicity of NSCLC cells.³⁴ Interestingly, the role of ECT2 in NSCLC transformation is distinct from its well established role in cytokinesis.³⁴ In NSCLC cells ECT2 is mislocalized to the cytoplasm where it binds the PKC ϵ -Par6 complex. Knock down of either PKC ϵ or Par6 causes redistribution of ECT2 to the nucleus and loss of transformed growth and invasion.³⁴ PKC ϵ directly phosphorylates ECT2 at a single site, T328, *in vitro* and modulates T328 phosphorylation in NSCLC cells.⁴⁷ T328 ECT2 phosphorylation is required for efficient binding of ECT2 to the PKC ϵ -Par6 complex, activation of Rac1, and the transformed phenotype of NSCLC cells.⁴⁷ Interestingly, the *ECT2* gene resides on chromosome 3q26 in close proximity to *PRKCI*. Expression analysis in primary NSCLC tumors demonstrated that *PRKCI* and *ECT2* are co-amplified and overexpressed in NSCLC.³⁴ Thus, ECT2 and PKC ϵ are genetically linked through coordinate gene amplification in NSCLC tumors, and biochemically and functionally linked in NSCLC transformation through formation of an oncogenic PKC ϵ -Par6- ECT2 complex that drives NSCLC cell transformation.

The PKC ϵ /Par6 complex has also been implicated in transforming growth factor β (TGF- β) induced epithelial-to-mesenchymal transition (EMT).⁷⁶ TGF- β induces PKC ϵ activation and T β RII and PKC ϵ cooperate to phosphorylate Par6 at S345, a phosphorylation event required for TGF- β EMT and subsequent migration and invasion of NSCLC cells.⁷⁶

In addition to Rac1, RhoB, another small GTPase, has been implicated in oncogenic PKC ϵ signaling. Microarray analysis of PKC ϵ -regulated genes in glioblastoma cells identified RhoB as being up-regulated in PKC ϵ knockdown cells.⁷⁷ Overexpression of RhoB in glioblastoma cells led to decreased proliferation, migration and invasion.⁷⁷ Interestingly, constitutive expression of RhoB can repress PKC ϵ activation implying a model whereby PKC ϵ represses RhoB to promote an invasive phenotype, and RhoB repression of PKC ϵ activity may lead to a noninvasive phenotype in glioblastoma cells.

In order to identify potential transcriptional targets of PKC ϵ required for transformation, a genome-wide gene expression analysis has been carried out in H1703 NSCLC cells expressing either control or PKC ϵ RNAi.³⁵ The matrix metalloproteinase 10/stromelysin 2 (MMP10) emerged from this analysis as a major PKC ϵ target.³⁵ RNAi mediated depletion of PKC ϵ , Par6, or Rac1 inhibits MMP10 expression in NSCLC cells,³⁵ and similar to PKC ϵ and Par6 knockdown, RNAi mediated knockdown of MMP10 blocks anchorage-independent growth and cell invasion in NSCLC cells.³⁵ In addition, loss of transformed growth and invasion in PKC ϵ KD or Par6 KD NSCLC cells is rescued by addition of catalytically-active MMP10.³⁵ MMP10 is also a critical downstream effector of PKC ϵ in *Kras*-transformed BASCs.⁶⁰ Similar to *Prkci*, *Mmp10* is required for BASC transformation and tumor initiation *in vivo*.⁷⁸ Analysis of primary tumors revealed that *MMP10* is overexpressed in NSCLC and MMP10 expression correlates positively with PKC ϵ expression.^{35, 78} Gene set enhancement analysis of publicly-available gene expression profiles showed that elevated *Mmp10* expression correlates strongly with both cancer stem cell and tumor metastasis gene signatures.⁷⁸ Thus, expression of MMP10 is regulated through the PKC ϵ -Par6-Rac1 signaling axis, and represents a key downstream effector in PKC ϵ -mediated transformation in lung cancer cells. In addition to MMP10, a gene expression meta-analysis in primary lung adenocarcinomas identified the genes COPB2,

ELF3, RFC4, and PLS1 as PKC ϵ transcriptional targets.⁷⁹ RNAi-mediated knock down of PKC ϵ in lung adenocarcinoma (LAC) cell lines led to a significant reduction in expression of each of the four target genes, indicating that PKC ϵ regulates the expression of these four genes in LAC cells.⁷⁹ RNAi-mediated knock down of each of these genes led to significant inhibition of anchorage independent growth and cellular invasion demonstrating that each of them play a role in LAC transformation.⁷⁹ Several of these PKC ϵ -regulated genes are coordinately overexpressed with PKC ϵ in other major tumor types including lung squamous cell carcinoma, breast, colon prostate, pancreatic, and glioblastomas.⁷⁹ suggesting that these PKC ϵ regulated genes may serve as useful biomarkers in determining the effectiveness of PKC ϵ -directed therapies and may themselves serve as targets for the development of novel prognostic markers and/or therapeutic agents.

Therapeutic Targeting of aPKC ϵ

As described above there is compelling evidence for aPKC as a target for intervention in cancer. In principle, interventions in aPKC could be afforded by: (i) modulating transcription/translation, for instance through an anti-sense approach as has been pursued for PKC α (ii) blocking upstream priming events; (iii) interfering with acute activation processes; (iv) modifying protein substrate/scaffold interactions; (v) inhibiting catalytic activity (Figure 2). We will comment here on those avenues where progress has been made in aPKC intervention.

Priming processes

The priming pathways have been reported to involve PI3kinase, PDK1 and TORC2 (see above) and all these have been the object of drug development programs. For the class I PI3kinases (α , β , γ , δ) there are multiple inhibitors of varying isoform selectivity under investigation clinically see⁸⁰. However it remains moot as to whether the product of this class I PI3 kinase pathway, phosphatidylinositol 3,4,5 trisphosphate, is generally rate limiting for the priming of aPKC (either directed at aPKC itself or through the PDK1 PH domain). By contrast the inhibition of PDK1 or mTOR catalytic activity might be expected to influence aPKC priming directly, albeit as a chronic and not an acute effect.¹⁷ However as discussed above, the turnover of these sites in PKCs is generally much slower than found in for example Akt/PKB. Thus, it is unclear whether even chronic inhibition will greatly influence the steady state phosphorylation of aPKC which will tend to ratchet towards an occupied state.¹⁷ Multiple agents have been characterized retrospectively as targeting PDK1 (eg UCN-01 a broad specificity kinase inhibitor which potently inhibits PDK1⁸¹ or targeted to PDK1 by design (eg AR-12, GSK2334470).⁸² A number of these agents have been in, are in, or are approved for clinical testing. Whether any of these agents influence the aPKC pathway in patients has not been reported. A more selective approach to intervention in the PDK1-aPKC cascade has come from targeting the aPKC PIF pocket which is involved in the PDK1-PKC ϵ interaction (see^{19, 83}). Several small molecule inhibitors that act at the PKC ϵ PIF pocket have been identified.^{19, 84} The application and efficacy of such approaches to PKC ϵ awaits further developments. The immunosuppressive agent rapamycin, acting via TOR⁸⁵ has been used to target mammalian TOR in the context of TORC1. Rapamycin and derivatives with improved PK properties have been in clinical trials and some single agent activity has been documented. However the evidence indicates that PKC isoforms are targeted directly by TORC2 and not TORC1 and it is evident that TORC2 itself can contribute to the transformed phenotype.⁸⁶ Agents which block both TORC1 and TORC2 activity have been developed and, interestingly, show greater activity *in vitro* and *in vivo* than TORC1 selective agents.⁸⁷ It will be of interest to monitor whether biomarkers of aPKC action are modulated in patient studies with these agents, although as noted above the

kinetics of steady state change in aPKC priming might not afford much leverage through these upstream pathways.

Partner interactions

As noted above there are no RACK-interaction directed inhibitors of the aPKCs and indeed there are no RACKs formally described for this branch of the family. However there are agents that suppress partner interactions. Interference of one such aPKC partner interaction has been proposed as a mechanisms of action of the triterpenoid ursolic acid,⁸⁸ a compound present in many fruits, enriched in apple peel and touted as a body-building supplement. Ursolic acid has been reported to block aPKC ζ -sequestosome 1 interaction resulting in and associated with this is a net priming dephosphorylation of aPKC ζ ;⁸⁸ this drug may well influence both aPKC isoforms, since antibodies detecting these priming site phosphorylations not distinguish aPKC ι and aPKC ζ . Derivatives of ursolic acid have been reported to display increased cytotoxicity in a range of tumor cell lines,⁸⁹ however the structure activity relationship for these derivatives in relation to aPKC-sequestosome1 interaction have not been determined, nor have they been shown to modulate any of the other pathways affected by ursolic acid (for example NF κ B, STAT3; reviewed in⁹⁰). It remains to be seen whether ursolic is the basis of an aPKC intervention.

PB1 domain inhibitors

The PKC ι PB1 domain is uniquely present in atypical PKCs, and the PB1 domain interaction formed between PKC ι and Par6 is required for the oncogenic PKC ι -Par6-Rac1-MMP10 signaling axis that promotes anchorage-independent growth and invasion of human NSCLC cells in vitro and tumorigenicity *in vivo*.³⁵ Therefore, compounds that can disrupt the PB1-PB1 domain interaction between PKC ι and Par6 may serve as a novel mechanism-based therapeutic intervention. A high throughput screen for small molecular weight compounds that can disrupt the PB1-PB1 domain interaction between PKC ι and Par6 identified gold-containing compounds such as aurothioglucose (ATG), aurothiomalate (ATM) and auranofin (ANF) as selective and relatively potent inhibitors of PKC ι and Par6 binding.⁹¹ These compounds are FDA-approved for treatment of rheumatoid arthritis patients,⁹² and ANF is still used clinically. These compounds exhibit dose dependent inhibition of PKC ι -Par6 binding with IC₅₀s of ~1 μ M, good antitumor activity, and they inhibit PKC ι -mediated Rac1 activation and anchorage independent growth of NSCLC cells *in vitro* and tumorigenicity *in vivo*.⁹¹ The inhibitory efficacy of ATM has been tested on a panel of cell lines that represent the major subtypes of lung cancer including lung adenocarcinoma (LAC), lung squamous cell carcinoma (LSCC), large cell carcinoma (LCC), and small cell lung carcinoma (SCLC).⁹³ ATM inhibited transformed growth in all cell lines tested with IC₅₀s ranging from ~300 nM – 100 μ M.⁹³ Interestingly, ATM sensitivity among this group of cell lines did not correlate with tumor sub-type, *KRas* status, or sensitivity to a panel of standard chemotherapeutic agents frequently used to treat lung cancer patients, including cis-platin, platinaxel, and gemcitabine.⁹³ Rather, PKC ι expression (measured by mRNA or protein) was the major molecular characteristic of lung cancer cells corresponding to ATM responsiveness.⁹³ ATM inhibits tumorigenicity of lung cell tumors *in vivo* at plasma drug concentrations consistent with the IC₅₀ of the cell lines to ATM in vitro and ATM plasma drug concentrations routinely achieved in RA patients.⁹³ Given the correlation between PKC ι expression and ATM sensitivity, PKC ι expression profiling in lung tumor samples may be useful in identifying lung cancer patients most likely to respond to these agents. In addition to NSCLC, the use of ATM and other gold-compound PKC ι targeted therapy has also shown promise in other tumor types. Treatment of primary rhabdomyosarcoma cell cultures with ATM resulted in decreased interaction between PKC ι and Par6, decreased Rac1 activity and reduced cell viability at clinically relevant

concentrations.⁵¹ Combination therapy studies of ATM and vincristine (VCR), a microtubule inhibitor currently used in treatment regimens of rhabdomyosarcoma, exhibited synergistic anti-proliferative effects in cells and a trend towards further sensitizing tumors to VCR treatment in vivo.⁵¹ ATM mediated inhibition of PKC ϵ in the prostate cancer cell line, PC3U, induced apoptosis, but ATM treatment did not affect the growth of human primary epithelial prostate cells, PrEC.⁹⁴ A phase I dose escalation trial to determine an appropriate dosing regimen and describe toxicities associated with ATM in patients with advanced NSCLC, ovarian cancer, and pancreatic cancer was recently completed.⁹⁵ ATM was well tolerated at the MTD of 50mg IM/weekly, and exhibited modest signs of tumor response (stable disease in 2/15 patients while on therapy) in this heavily pretreated patient population. Further trials are ongoing with the related compound auranofin (ANF), which exhibits higher potency for PKC ϵ -Par6 inhibition (unpublished observations).

The precise mechanism by which ATG, ATM and ANF elicit their inflammatory activities in RA is unknown. A proposed mechanism of action is the formation of gold-cysteine adducts with target cellular proteins (reviewed in⁵⁰). The PB1 domain of the aPKCs contains a cysteine residue, (Cys69) that is not present in other PB1 domain-containing proteins. The crystal structure of the PKC ϵ -Par6 complex reveals that Cys69 is located within the conserved OPR, PC, and AID (OPCA) motif of PKC ϵ at the binding interface between PKC ϵ and Par6 in the complex.⁴⁵ Molecular modeling of the interaction between ATM and the PKC ϵ PB1 domain, predicts formation of an adduct between Cys69 and ATM that would cause steric hindrance to Par6 binding.⁴⁵ Mutation of PKC ϵ Cys69 to isoleucine (C69I) or valine (C69V), amino acids that frequently exist at this position in other PB1 domains, does not alter Par6 binding but makes PKC ϵ resistant to ATM inhibitory effects on PKC ϵ -Par6 binding in vitro.⁴⁵ Consistent with these findings, expression of the C69I PKC ϵ mutant in NSCLC cells supports transformed growth and renders these cells resistant to the inhibitory effects of ATM on transformed growth.⁴⁵

Substrate interactions

The regulatory domain pseudosubstrate sites from individual isoforms have been exploited as natural inhibitors to generate short oligopeptide inhibitors.⁹⁶ Thus cell permeable forms of synthetic pseudosubstrate site sequences have been employed to block function of their cognate target eg PKC α derived pseudosubstrate site targeting PKC α . A critical issue for use of these peptide inhibitors is specificity. Typically these peptides are employed in the 10–100 μ M range and it is questionable as to whether they display specificity at these concentrations. Binding of one PKC isoform to a non-cognate pseudosubstrate site sequence is well established, as evidenced by the use of synthetic pseudosubstrate site oligopeptides with A>S changes that converts them to good substrates.⁹⁷ These oligopeptides exhibit poor differentiating ability between PKC isoforms. A more specific inhibitor that is protein substrate competitive has been identified for aPKC ϵ and that is [4-(5-amino-4-carbamoylimidazol-1-yl)-2,3-dihydroxycyclopentyl] methyl dihydrogen phosphate.⁶⁸ This drug was shown to inhibit PKC ϵ competitively with respect to a protein substrate (myelin basic protein) *in vitro* and to block proliferation of neuroblastoma cells potentially via CDK7.⁶⁸ The specific site of action will be of interest for this class of compounds, moreover their selectivity for aPKC ϵ and potentially for a subset of its substrates offers some interesting opportunities for fine tuning interventions.

The majority of PKC inhibitors are typical protein kinase small molecule inhibitors that compete for the ATP binding pocket. Notably, until very recently, no compounds had shown any selectivity for the aPKC isoforms and much pharmacological dissection of the PKC family had exploited differences between compounds of decreasing specificity: Gö6976 (cPKC specific;⁹⁸), bis-indolyl maleimide I (BIMI; cPKC and nPKC selective;⁹⁹) and

Gö6983 (cPKC, nPKC and aPKC selective);¹⁰⁰ so, for example, a process inhibited by Gö6983 but not BIM1 provides consistent evidence on aPKC involvement (certainly not proof given the lack of absolute specificity for these kinases).

More recently aPKC selective ATP-competitive inhibitors have been described.¹⁰¹ The compound CRT0066854 is a thieno[2',3-d] pyrimidine compound, which has been shown to display good kinase selectivity across a broad kinase panel (selectivity score S(80) at 1 μ M is ~0.02) with excellent selectivity for aPKC ζ within the PKC family. This aPKC inhibitor selectively blocks aPKC ζ substrate phosphorylation in cells and inhibits proliferation in 2D and 3D cultures, suppresses polarized morphogenesis and directional migration – effects all mimicking aPKC intervention through siRNA.¹⁰² The basis of specificity for CRT0066854 has been established through a high resolution inhibitor bound complex of the aPKC ζ kinase domain. Here it has been shown that the fused tricyclic scaffold forms hydrophobic contacts with the glycine loop, while the substituents at the 2 and 3 positions, respectively contribute hinge-binding and a combination of charge and hydrophobic interactions. Notably the CRT0066854 benzyl group forming part of the substituent in the 3 position displaces Phe⁵⁴³ of the PKC ζ “NFD motif”. As seen for the domain interaction dependent displacement of the NFD motif in the PKC β structure (discussed above), the benzyl group confers an inactive conformation on the PKC ζ kinase domain contributing to its specificity. CRT0066854 as noted above inhibits growth in soft agar and suppresses migration, and can suppress also the transformed morphology associated with mutant Ras expression.⁴⁹ This compound provides clear evidence of an ability to selectively target the aPKC isoform catalytic activity with consequences consistent with characterized behaviors of aPKC pathways. How such agents play out in the clinical setting remains to be seen.

Perspectives

Studies to date provide compelling evidence that PKC ζ is oncogenic in a variety of human cancers. Functionally, PKC ζ is required for multiple aspects of the transformed phenotype and appears to participate in the initiation, progression and metastatic stages of cancer. Furthermore, PKC ζ participates downstream of other key oncogenes that have proven difficult to target therapeutically such as oncogenic *Kras*. Acquisition of tumor-specific mutations in targeted molecules has become a recurrent theme and presents a major hurdle in achieving curative therapy in cancer patients. Interestingly, PKC ζ has been shown to promote chemoresistance in a number of cancer types and inhibition of PKC ζ sensitizes these tumor cells to chemotherapeutics. There is growing evidence that human tumors contain a population of cells that exhibit properties of tumor-initiating or cancer stem cells (CSCs) that are thought to be responsible for lung tumor initiation, maintenance, relapse and chemoresistance. These findings have led to the hypothesis that CSCs must be effectively targeted to elicit effective and long lasting therapeutic treatment of cancer patients. Genetic ablation of PKC ζ in a lung tumorigenesis model indicates that PKC ζ plays a critical role in transformation of lung CSCs. Despite the critical requirement for PKC ζ in tumor cell function, normal cells as well as tumor cells grown in adherent non-transformed growth conditions can better tolerate PKC ζ inhibition, thus providing a therapeutic window. For these reasons PKC ζ is an attractive therapeutic target for novel cancer treatment.

As discussed above, several approaches have been taken to modulate oncogenic PKC ζ function for cancer therapeutics. The use of non-selective PKC inhibitors in the clinic have given mixed results, and have not been very effective in treating tumors. This in part is due to the high homology between the PKC isozymes and the relative lack of specificity in previously designed PKC inhibitors. In this regard, PKC ζ and PKC ξ are highly related, exhibiting 72% overall amino acid sequence homology and 86% identity within the kinase domain. While PKC ζ appears to consistently play a promotive role in transformation PKC ξ

is reported to have tumor suppressive functions in some contexts. Therefore, PKC ι isozyme specific inhibitors may need to be utilized in order to circumvent the possible cross target effects on aPKC ζ . Future work is also required to identify distinct targets of PKC ι signaling that can be developed as robust, sensitive markers of therapeutic response to PKC ι inhibition in clinical trials.

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Figure 1. *PRKCI* copy number gain is a major genetic mechanism driving *PRKCI* overexpression in human tumors

A. The percentage of each listed tumor type harboring copy number gains, somatic mutation or multiple alterations in the indicated tumor types is shown. Data were compiled from the cBioPortal for Cancer Genomics (<http://www.cbioportal.org/public-portal/>). **B.** *PRKCI* copy number gain correlates with *PRKCI* overexpression across human tumor types indicating that copy number gain is a major causative genetic mechanism driving *PRKCI* expression in these tumors. For each of the tumor types shown in **A**, the % of tumors harboring *PRKCI* gene copy number gains was plotted against the % of tumors exhibiting *PRKCI* overexpression. A direct relationship exists between *PRKCI* copy number gain and overexpression across tumor types (correlation co-efficient $R^2=0.7756$).

Figure 2. Potential modes of *PRKCI*-directed therapeutic intervention

Five potential modes of therapeutic intervention are outlined: **(I)** control of *PRKCI* transcription, **(II)** disruption of PB1 domain protein-protein interactions between $\text{PKC}\iota$ and its oncogenic partner Par6; **(III)** inhibition of upstream kinase cascades that regulate $\text{PKC}\iota$ priming; **(IV)** inhibition of $\text{PKC}\iota$ -scaffold/protein substrate interactions; **(V)** inhibition of $\text{PKC}\iota$ catalytic activity through competitive inhibition of ATP binding. See text for discussion and details.