Birkbeck
UNIVERSITY OF LONDON

BIROn - Birkbeck Institutional Research Online

Parker, P.J. and Brown, S.J. and Calleja, V. and Chakravarty, P. and Cobbaut, M. and Linch, M. and Marshall, J.J.T. and Martini, S. and McDonald, Neil Q. and Soliman, T. and Watson, L. (2021) Equivocal, explicit and emergent actions of PKC isoforms in Cancer. Nature Reviews Cancer 21 , pp. 51-63. ISSN 1474-175X.

Downloaded from: https://eprints.bbk.ac.uk/id/eprint/41063/

Usage Guidelines:
Please refer to usage guidelines at https://eprints.bbk.ac.uk/policies.html or alternatively contact lib-eprints@bbk.ac.uk.

Equivocal, Explicit and Emergent Actions of PKC isoforms in Cancer

Peter J. Parker[1,2]*, Sophie J. Brown[1], Veronique Calleja[1], Probir Chakravarty[3], Mathias Cobbaut[1], Mark Linch[4], Jacqueline J. T. Marshall[1], Silvia Martini[1], Neil Q. McDonald[5,6], Tanya Soliman[7], Lisa Watson[1].

[1]Protein Phosphorylation Laboratory, Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK; [2]School of Cancer and Pharmaceutical Sciences, King's College London, Guy's campus, London SE1 1UL, UK; [3]Bioinformatics, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK; [4]UCL Cancer Institute, University College London, 74 Huntley Street, London WC1E 6BT, UK; [5]Signalling and Structural Biology Laboratory, Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK; [6]Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck College, Malet Street, London, WC1E 7HX, UK, [7]Bart's Cancer Institute, Charterhouse Square, London EC1M 6BE, UK.

*email: peter.parker@crick.ac.uk

Abstract

The maturing mutational landscape of cancer genomes, the development and application of clinical interventions, and evolving insights into tumour-associated functions, reveal unexpected features of the protein kinase C (PKC) family of serine/threonine protein kinases. These advances include recent work showing gain or loss-of-function mutations relating to driver or bystander roles, how conformational constraints and plasticity impact this class of proteins and how emergent cancer-associated properties may offer opportunities for intervention. The profound impact of the tumour microenvironment, reflected in the efficacy of immune checkpoint interventions, further prompts to incorporate PKC family actions and interventions in this eco-system, informed by insights into the control of stromal and immune cell functions. Drugging PKC isoforms has offered much promise, but the when and how is not obvious.

[H1] Introduction

The protein kinase C (PKC) family of serine/threonine protein kinases, comprising the 'classical' PKC (cPKC), 'novel' PKC (nPKC), 'atypical' PKC (aPKC) and PKN

35 subfamilies, are one of the defining families of the AGC kinase class[1]. They retain a
36 modular structure, consisting of domain permutations in their N-terminal regulatory
37 regions, linked via variable sequences to highly conserved C-terminal kinase
38 domains[2]. In *Saccharomyces cerevisiae*, the singular *PKC1* gene encodes a protein
39 retaining domains characteristic of the greatly expanded mammalian family[3]. A
40 subset of PKCs (cPKCs, nPKCs) are responsive to the second messenger 1,2-
41 diacylglycerol **[G]** (DAG) and feature in many signalling cascades downstream of the
42 broad class of phosphoinositide-specific phospholipases (reviewed[4]), which are
43 themselves linked to a spectrum of G-protein and tyrosine kinase associated
44 receptors (see[5]). Other family members respond directly (PKNs) or through partner
45 proteins (aPKCs) to membrane active, small G-proteins, downstream of the
46 exchange factors that control them (recently reviewed[6]).
47
48 The potential impact of PKCs on cancer has been the subject of extensive
49 investigation, greatly influenced by the pioneering work from Nishizuka's laboratory
50 that identified 'PKC' as a target for certain tumour promoters[7]. What has emerged in
51 the intervening decades informed by cancer genomics, *ex vivo* studies and *in vivo*
52 models, is a complex picture that presents practical and conceptual challenges to the
53 field. Here, we will provide an overview of PKC functional attributes, elaborating on
54 properties that influence target validation in cancer. The review will then focus on
55 cPKC and nPKC families as DAG and/or tumour promoter responsive kinases,
56 discussing promoter and suppressor activities in experimental studies and
57 associated with cancer genomics. Finally, we comment on PKC pharmacology and
58 clinical trials. To note, there are over 12,000 publications in the PKC-cancer area
59 and not all will be referenced, rather exemplars of critical findings and commentaries
60 will be featured, so we beg indulgence of those in the field who have contributed
61 greatly, but are conspicuous by their absence.
62
63
64 **[H1] Regulation and function of PKCs**
65
66 *[H2] Turning PKCs on*

Canonical activation of cPKCs, which include PKCα, β and γ and nPKCs, which include PKCδ, ε, η and θ, involves the binding of membrane resident DAG, inducing conformational changes and the release of the autoinhibitory pseudosubstrate site, triggering catalytic activity-dependent downstream events[8] (Figure 1A). A similar conformational principle operates for aPKCs, which include PKCζ and ι, albeit effected physiologically through the protein binding of CDC42 and PAR6 or p62[9] to their regulatory domains in a spatially constrained manner (see recent review[10]). A related scenario pertains to the activation of proteins of the PKN subfamily, which include PKN1-3, responding to RHO or RAC[11], however this is likely complicated by autoinhibitory dimerization in the basal state as reported for PKN2[12]. In all cases the membrane recruited PKCs take on a de-inhibited, open conformation, competent to phosphorylate substrates and associate with conformation-dependent partners[13,14]. There is the potential for dissociation from the membrane of scaffold-bound, active PKC, but evidence for this is scarce[15]. Experimentally, cPKC, aPKC or nPKC isoforms can be expressed as open-conformer, gain-of-function mutants through mutation of the gene regions encoding their autoinhibitory pseudosubstrate sites (see[16]).

The catalytic potential of PKCs is dictated by 'priming' phosphorylations in their catalytic domains that are largely conserved in AGC family members and executed by common PDK1 and mTORC2 pathways[17-20] (see animated model for PKCε; supplementary video). Autophosphorylation of the hydrophobic priming site has been proposed also (reviewed in[21]), but this does not appear to dominate behaviour in cells[22]. Integrity of the kinase domain for priming is nevertheless a necessity, requiring competence to bind nucleotide which acts to protect the phosphorylated kinase domain from dephosphorylation [22-24].

Open PKC conformers are required for the upstream kinases to act upon them, for example, the action of PDK1 on PKN1 requires RAC and/or RHO in cells[25] and similarly PKC recruitment to membranes appears critical for PDK1 input (reviewed in[26]). PKC kinase domain priming phosphorylations are typically retained under autoinhibited conditions, such that PDK1 and/or mTORC2 activity is not required to impact short-term actions. Acute inhibition of these upstream kinases has limited

effect on PKC isoform phosphorylation, however knock-out of the gene encoding PDK1 has a more profound effect[27], as does prolonged inhibition of mTORC2 function[20]. This relative stability of priming phosphorylation contrasts starkly with the related AGC kinases of the AKT-PKB family (see[28]) and makes these priming modifications poor readouts of PKC activity (see 2.3 below).

PKCs are basophilic kinases[29] with overlapping substrate recognition as demonstrated in *drosophila*[30] and in mammalian cells[31]. This overlapping specificity has profound functional consequences as evident in a double *Prkce* (encoding PKCε) and *Prkcd* (encoding PKCδ) knockout mouse, which is embryonic lethal, while neither individual knockout displays a developmental phenotype[32]. Beyond their intrinsic specificities, many isoforms have extensive interactomes, associating with scaffolds and partners that impact localisation (reviewed[13,14]) as well as substrate docking, as documented for aPKC[33].

[H2] Turning PKCs off

The inactivation of PKC is in part dictated by the loss of the typically transient triggers that switch them on. Metabolism of DAG will lead to membrane dissociation of cPKC or nPKC, then the regulatory domain will re-associate with the catalytic domain through interaction between the pseudosubstrate and substrate binding pocket [34] and likely other inter-domain interactions[35], leading to the accumulation of the primed, latent protein in the cytosol. Beyond this simple reversal of activation, activation-associated downregulation of PKC protein levels has been characterised for the DAG-responsive cPKC and nPKC isoforms. However, how acute or chronic activation impacts regulation of protein levels of aPKC and PKN isoforms is not clear. Downregulation of PKC isoform protein levels is associated with cell-type specific patterns of endomembrane trafficking, dephosphorylation, ubiquitination and degradation of the respective PKC isoform (Figure 1B). The extent to which one or other degradative pathway dominates, the activity of specific protein phosphatases, E3 ligase(s) and endocytic requirements, reflects the cell model and the PKC isoform that is affected.

Activation-induced downregulation of PKCα protein levels was originally linked to degradation of PKC protein[36]. Subsequently, evidence indicated that PKC downregulation (α, δ, ε) was associated with ubiquitination[37-39] and also with dephosphorylation and caveolin-dependent endocytosis[40,41]. Two distinct pathways acting in parallel were later reported for PKCα, one involving the ubiquitination of plasma membrane active, primed protein and its degradation through the proteasome; the second engaging caveolin-dependent traffic and non-proteasomal degradation[42]. Two separate endocytic pathways were reported by Lum and colleagues[43] and the sequential operation of cholesterol-dependent endocytosis of ubiquitinated PKCα with delivery to the proteasome provides yet another route to downregulation[44].

Various E3 ligases have been proposed to drive PKC ubiquitination and proteasomal degradation in different contexts, including RINCK, LUBAC and MDM2[45-47]. Interestingly, the LUBAC complex preferentially bound activated cPKC, consistent with the observed activation-induced ubiquitination[47]. Contrasting with these emerging players, molecular details of membrane traffic-dependent, non-proteasomal degradation are limited.

Priming site dephosphorylation of PKCs is a prelude to degradation in many contexts. In the inactive state, PKC priming site dephosphorylation is limited by the interaction between the regulatory and catalytic domains as indicated by the finding that the phosphatase PHLPP1 suppresses the accumulation of primed PKCβ when there are mutations in the inhibitory pseudosubstrate site[48]. In the membrane-associated active state, dephosphorylation is governed by nucleotide pocket occupation[22]. cPKCs may require peptidyl-prolyl isomerisation of the turn motif priming site (phosphoThr-Pro) by PIN1, to enable dephosphorylation and ubiquitination[49]. The often transient nature of DAG production physiologically means that under many circumstances, activation-induced dephosphorylation may have a limited impact on cPKCs and nPKCs. However there are contexts in which dephosphorylated PKCs accumulate, reflecting either reduced action of upstream kinases or the increased dephosphorylation of primed PKCs under conditions of protection from degradation (see for example[40,50,51]).

[H2] Challenges for target validation

Consideration of PKC isoforms as drug targets sits squarely with the generic demands of any intervention programme – what is the clinical evidence for action or inaction playing a critical role in a given disease setting and what is the expectation of a suitable therapeutic index? For cancer patients, target validation draws in part upon the evidence of observed somatic changes impacting function (Section 4), transcriptional/protein level changes that also may reflect gain or loss of function and evidence of downstream pathway dysregulation. For PKC genes, like any other, this patient-derived data needs interpretation in the context of our understanding of the intrinsic isoform properties, their physiological roles and experimental tumour models (Section 3).

A substantial gap in addressing these validation issues is the lack of biomarker evidence that speaks to PKC (in)activation in tumour settings (see Figure 2A). In an experimental context, isoform activation has been monitored through rapid fractionation protocols (e.g.[52]), fluorescently tagged isoforms as initially reported by Saito and colleagues[53] and direct compartment-directed activity monitors[54]. However these approaches do not lend themselves to pathology. As detailed above, the levels of priming phosphorylations required for function do not typically correlate with levels of activation and chronic activation can actually induce dephosphorylation and degradation. Thus, measurements that are related to PKC protein levels do not of themselves provide insight into pathway function.

Intramolecular events have been investigated as activation markers, specifically autophosphorylation[55-58]. This has been exploited in pathological samples for PKCα using imaging methodologies not easily adapted to routine use[56]. It also transpires that in cells, “autophosphorylation” for PKCε while potentially dependent upon membrane recruitment and conformational activation, is executed in *trans*, limiting biomarker utility[57].

There is a wealth of data on higher or lower levels of expression of PKC isoforms in cancers (Supplementary Figure 1), but this is not coupled to defined downstream events that provides insight into (in)action. For these highly regulated signalling proteins that do not themselves appear to signal via concentration-dependent oligomerisation (aPKC might be an exception in some circumstances[59]), variations in expression alone may not impact signal output without other contributing factors that influence signal input or downstream signal termination. Is the increased expression of PKCι and ζ, and the reduction of PKCβ and θ meaningful in pancreatic ductal adenocarcinoma and is a reverse functional interpretation valid for the inverse pattern of expression reported for renal clear cell carcinoma (Supplementary Figure 1), or are these in fact bystander transcriptomic changes that reflect programming within the tumour, which might be prognostic signatures, but do not assert gain/loss-of-function?

Ultimately, understanding the context-dependent molecular mechanisms of PKC isoform action will provide the much-needed biomarkers that give insight into pathway operation in tumours and pharmacodynamic biomarkers for trials; specifically, pathophysiological mechanisms, which do not always reflect amplified or muted physiology. This is well exemplified by PKCι which is an established regulator of cell polarity, a property considered tumour suppressive and characteristically lost in transformed cells[60]. PKCι operates in a sweet spot to control polarity, too little or too much activity prevents polarisation; this is not a concentration dependent titration of interacting partners, but a property that can be reversed by catalytic inhibitors[61]. Such behaviour likely underlies the aPKC suppressor - promoter question (see Box 1).

[H1] cPKC and nPKC in tumour models

Against a backdrop of cPKC and nPKC roles as mediators of downstream signalling for tumour growth promoting signals, or tumour promoters, numerous cell transformation and *in vivo* mouse models have been assessed for the tumour promoters' dependence upon PKC family members. This has created a varied and sometimes conflicting profile of promoter and suppressor actions.

230

231 *[H2] Phorbol ester-mediated tumour promotion*

232 In mouse skin pretreated with a sub-threshold dose of a carcinogen such as DMBA,
233 phorbol esters will promote the formation of papillomas followed by conversion to
234 overt carcinomas on continued exposure (reviewed in[62]). The initiation event is
235 stable, requiring DMBA metabolism to a genotoxic form (reviewed in [63]) and it has
236 been established that this genotoxic form frequently induces *Ras* mutations[64]. The
237 tumour promotion process elicited by phorbol esters itself has multiple stages, with
238 an irreversible first step, a chronic phase that is at least initially reversible and a
239 progression phase that is irreversible[63]. Phorbol esters represent only one class of
240 tumour promoters and impact both the presumptive tumour as well as the tumour
241 microenvironment where a clear inflammatory driver is involved[65].

242

243 The DAG-responsive cPKC and nPKC isoforms are the founding members of the
244 class of targets for the phorbol esters[7,66]. Molecularly, phorbol esters act in a
245 membrane context by mimicking DAG to engage the C1 domains of cPKCs and
246 nPKCs causing activation[67]. Underlining their importance as targets, structurally
247 unrelated tumour promoters also act on PKCs, including mezerin, teleocidin and
248 aplysiatoxin[68-70]. However, not all tumour promoters in this particular mouse skin
249 model target PKC; additional targets of mouse skin tumour promoters include the ER
250 Calcium-ATPase (the target of thapsigargin)[71] and protein phosphatase 1/2A
251 (inhibited by okadaic acid[72]).

252

253 Phorbol ester-mediated downregulation of PKC protein levels in the mouse skin
254 promotion model has been documented [73,74]. That phorbol esters induced acute
255 activation of PKCs, followed by chronic downregulation of PKC protein levels, begs
256 the question of whether cPKC and nPKCs in this context function as oncogenic
257 drivers and/or as tumour suppressors. This complexity in interpreting causation is
258 heightened by two further considerations. Firstly, PKC isoforms are not the only C1
259 domain containing proteins in the human genome (discussed in[75]) and although not
260 all C1 domains bind phorbol esters with high affinity, the tumour promotion response
261 to these C1-binding promoting agents is likely a complex pattern of action on multiple
262 targets. Secondly, the behaviour of these initiation-promotion models reflects an
263 interplay of both the somatically altered target cell (e.g. H-RAS mutant target cell[64])

264 and the inflammatory cellular environment elicited by these promoters (discussed[65]).
265 Notably, PKC isoforms and other tumour promoter targets are expressed both in the
266 emerging tumour and in the infiltrating inflammatory cells, stroma and vasculature,
267 questioning the combinatorial nature of C1 domain protein engagement in these
268 individual cell types and making resolution of essential promoter or suppressor
269 actions more difficult to dissect.

270

271 *[H2] Distinguishing suppressors and promoters*

272 Constitutive knock-out of genes encoding PKC isoforms in mice (all are viable except
273 *Prkci* or *Pkn2* knockout mice[76,77]) do not predispose to cancer in the manner of a
274 classic tumour suppressor (e.g. p53 or APC), although in *Prkca*$^{-/-}$ mice, an increase
275 in spontaneous colorectal lesions has been reported[78]. The impact of changes in
276 PKC activity or expression has been assessed more widely in mouse models of
277 cancer in the context of other treatments/driver mutations and here, gain-of-function
278 and loss-of-function alterations indicate a mixed pattern of behaviours, as
279 exemplified below.

280

281 For *Prkca*, transgenic expression in the basal layer of the epidermis sensitises to
282 phorbol ester driven inflammatory responses and to papilloma-carcinoma conversion
283 in mice[79,80]. However, in the *Prkca* knock-out mouse, while the absence of PKCα
284 reduces the inflammatory response to phorbol ester promotion, the knock-out also
285 leads to enhanced tumour formation[81]. These somewhat contradictory observations
286 likely reflect the complex interplay of diverse cellular responses and that the altered
287 PKCα expression impinges on different cell types in these models. In the *Apc*$^{+/Min}$
288 mouse model of CRC, *Prkca* knock-out increases tumour growth rate and
289 aggressiveness but not incidence[78]. It would be of interest to determine whether this
290 effect of PKCα deficiency is dependent on its specific loss in the follicle-derived
291 tumour cells or impacts through the microenvironment.

292

293 A tumour suppressive role for PKCδ has been reported[82]. In the mouse skin
294 promotion context, transgenic expression of *Prkcd* has a selective effect in
295 suppressing phorbol ester induced tumour formation but not that promoted by UV[83],
296 suggesting that there are distinct PKCδ dependent and independent signalling

pathways operating in this model. It would be informative to determine whether the phorbol ester effect (i.e. PKCδ activation) is dominant over UV action when co-administered in this model. Knock-out of *Prkcd* in mice leads to a lymphoproliferative response with altered B-cell self-tolerance[84,85]. Interestingly, in a patient with an autoimmune lymphoproliferative syndrome-like disease, a mutation in *PRKCD* was identified associated with a substantial loss of protein expression[86]. The lymphoproliferation phenotype of this germline alteration indicates specificity in the wiring of B-cell controls, with PKCδ acting in a tolerogenic, physiological feedback to promote B-cell anergy and in this cellular context to be proliferation-suppressive. However, suppressive actions cannot be attributed exclusively to PKCδ as shown in an MMTV-ErbB2 transformation model **[G]** [87] and also in urethane-induced lung tumours in mice[88] where PKCδ plays promoting roles.

[H2] Tumour or microenvironment action

The extent to which PKC activation or absence impacts the stroma, the innate, or the adaptive immune system, is germane to defining promotion and/or suppressor functions. These latter terms typically refer to the tumour autonomous behaviour and not to the tumour microenvironment (TME) dependencies, however experimentally we do not often distinguish the site of action.

Many isoforms control aspects of immune cell function. PKCβ is known to influence B-cell responses in mice[89] and was recently shown to regulate mTORC1 signalling in mouse B-cells, influencing gene expression and metabolic reprogramming[90]. PKCα regulates T-cell dependent interferon production and B-cell IgG2a/b class switching[91]; PKCε influences T-cell differentiation[92] and macrophage function[93]. PKCθ regulates T-cell receptor induced NFAT and NFκB activation[94,95] and prevents stabilisation of regulatory T-cells (Tregs)[96,97] supporting tumour immune recognition. Conversely, PKCη associates with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) at the Treg immune synapse enabling immune suppression[98]. This likely relates to the reported tumour suppressive effects of PKCη[99] and its broader regulation of adaptive and innate immune cell functions[100,101].

The influence of PKCs on immune cells and more generally the TME, questions where experimental organismal inactivation impacts tumourigenesis and there are few examples where this issue has been addressed directly. In the MMTV-PyMT model of breast cancer, PKCβ has been found to promote tumour formation[102]. Allograft of an MMTV-PyMT tumour (PKCβ replete) into a $Prkcb^{-/-}$ recipient mouse has shown that the requirement for PKCβ for tumour growth in this model operates through its expression in tumour-associated cells[102]. A similar tumour conducive effect of PKCβ in stroma has recently been described in a model of B-cell malignancy[103].

Allograft experiments have shown that the seeding of melanoma-derived lung tumours is compromised in *Pkn3* knockout mice[104], consistent with the siRNA-mediated knock-down of *Pkn3* inhibiting metastasis in vivo[105], although contrasting with the tumour-directed effects observed for *Pkn3* knockdown in an orthotopic prostate cancer mouse model[106].

Evidently the vasculature and tumour niche can be impacted by PKC isoform (in)action and this may also contribute to the distinctive responses observed with C1 domain targeting PKC activators employed clinically (see below), the bryostatins **[G]** and epoxytiglianes **[G]**. The bryostatins are PKC activators[107] with a context-dependent, variable ability to invoke PKC downregulation in cell culture[108,109]. Remarkably, bryostatin 1 can protect from phorbol ester-induced tumour promotion[110]. The target cell type(s) that mediate this tumour suppressive behaviour is not known. Using intratumoural injection, the PKC activators belonging to epoxytiglianes has been shown to have efficacy in treating mouse cancer models[111] and also in treating canine mast cell tumours[112]. As such, tigilanol tiglate has been approved for the treatment of canine mast cell tumours by the European Medicines Agency (EMA). Intratumoural injections produce high local concentrations and the extent to which the responses to epoxytiglianes are PKC-dependent rather than acting through other C1 domain targets and physicochemical effects remains to be seen. It is also noted that there is evidence of vasculature targeted effects for the haemorrhagic necrosis observed in response to tiglianes[111].

362
363 **[H1] PKC gene mutations in cancer**
364 The mutational landscape of human cancer, has provided some profound insights
365 into drivers of disease exemplified by the penetrant mutation of *BRAF* in
366 melanoma[113]. For PKC genes there is a spectrum of patient specific, private
367 mutations **[G]** across cancer genomes and some rare penetrant mutations.
368
369 *[H2] Private mutations in PKCs*
370 Recent studies have addressed the breadth of mutations found in PKC genes in
371 human cancers and concluded that these proteins play a suppressive role (reviewed
372 in [21]). Direct analysis of a number of the private *PRKCB* mutations indicated that
373 they are loss-of-function mutations and one studied in detail (A509T) was shown to
374 be dominant, rationalising the heterozygous nature of these mutations[114]. The
375 reversion of this *PRKCB* A509T mutation in a naturally occurring cancer cell setting
376 (DLD1 colon cancer cells) and the associated tumour growth rate reduction, supports
377 a tumour suppressive role of PKCβ and reinforces the idea that specific genetic
378 context is critical in these functional assessments.
379
380 While consistent with a tumour suppressor role, the penetration and pattern of these
381 diverse PKC mutations begs the question of whether, in patients, these are by-
382 stander events or contributors to disease and/or disease progression. The
383 penetrance of cancer-associated mutations for *PRKCA* (encoding PKCα) is similar to
384 non-synonymous mutations seen in correspondingly sized genes from the clotting
385 cascade (for example, genes encoding Protein S and Protein C, based on data from
386 cBioPortal). Aggregating data from fifteen tumour groups, there is no significantly
387 greater frequency of non-synonymous mutations in *PRKCA*, that would reflect a
388 selective advantage, nor is there any pattern of mutational change that indicates a
389 tissue specific behaviour, rather a higher incidence for one gene in a particular
390 tumour type reflects a higher incidence for all genes. So, is there mutation selection
391 or are these bystander events? This remains to be resolved and will require further
392 analysis alongside a wider assessment of the dominance or recessive behaviour of
393 these heterozygous mutations that are predicted to confer a loss-of-function.
394

395 *[H2] cPKC mutations in rare cancers*

396 High penetrance somatic variants provide robust evidence for their role in diseases.
397 For PKC this is a small collection of smoking guns with just two relatively rare tumour
398 types where cPKC gene mutations are highly penetrant, ATLL and chordoid gliomas.
399 The issue here is how we interpret the functionality of these somatic variants.

400

401 *[H3] PRKCB* mutation in ATLL

402 Adult T-Cell Leukemia Lymphoma (ATLL) is associated with HTLV-1 infection, a
403 retrovirus endemic in certain areas of the world. The virus establishes lifelong
404 latency in T-cells leading to an ATLL lifetime risk of 4-7%[115]. In a comprehensive
405 survey of the ATLL mutational landscape, somatic changes were documented along
406 the T-cell receptor (TCR)-NFκB pathway, including frequent mutations in genes
407 encoding phospholipase Cγ (PLCγ; 36%) and PKCβ (33%)[116]. Mutations along this
408 pathway have been predicted as gain-of-function mutations including those found in
409 *PRKCB*; in the case of inhibitory inputs to this pathway, somatic changes have been
410 assigned as loss-of-function providing a consistent view of pathway activation[116].

411

412 The most penetrant ATLL mutation in *PRKCB* results in an amino acid substitution at
413 D427 in the kinase domain (Figure 2B), typically D427N. Both the pattern of
414 mutations in genes of the TCR pathway and the limited functional data available
415 suggest that this D427 mutation is an activating mutation. Based upon homology
416 modelling informed by a substrate peptide bound kinase domain structure of
417 PKCι[117], it is inferred that the D427 residue lies proximal to the substrate binding
418 pocket of PKCβ, such that substitution may compromise binding of the autoinhibitory
419 pseudosubstrate. While these interactions are not the totality of the regulatory
420 domain-catalytic domain interface[35], it is the case that point mutations and deletions
421 in the inhibitory pseudosubstrate sequence lead to a more active and open
422 conformer **[G]** in cells[34]. The implication is that as an open conformer, the mutated
423 PKCβ is activated and/or downregulated (see above). This has yet to be resolved
424 directly, although it has been reported for B-cells that PKCβ is required to support
425 the NFκB pathway through CARD11 and IKK[118] consistent with the gain-of-function
426 analysis predicted in T-cells[116]. If PKCβ activation is causative in driving tumour

427 growth, might current PKCβ directed drugs work? Not necessarily for this D427
428 mutation, as manipulation of the homologous region of PKCι has been shown to
429 influence substrate interactions[119] and pharmacology[120].
430
431 The specific nature of these effects in PKCβ will require further analysis. It will also
432 be of interest to understand whether this hotspot mutation is associated with a
433 particular clinical course, segregating with one of the four ATLL subtypes originally
434 defined[121]. Might D427 mutations generate unique actions distinct from that
435 consequent to PLCγ gene mutation, or other ATLL-associated *PRKCB* mutations?
436
437 *[H3] PRKCA* mutation in chordoid glioma
438 Chordoid gliomas, are rare, slow growing, low grade tumours originating in the third
439 ventricle of the brain[122]. Although well circumscribed, access and precise location
440 mean surgical intervention can be associated with a high risk of morbidity[123].
441 Notably, in two recent publications, it was found that there was an essentially fully
442 penetrant, heterozygous mutation in *PRKCA* associated with these tumours[124,125].
443 This consistent D463H mutation is at the highly conserved aspartate residue that is
444 responsible for positioning the incoming substrate sidechain hydroxyl and is a
445 residue essential for catalytic activity as originally defined for the analogous
446 aspartate 166 residue in PKA[126] (Figure 2B).
447
448 At face value, the chordoid glioma-associated mutation in *PRKCA* is a simple,
449 dominant loss-of-function mutant. This is supported by the predicted loss of catalytic
450 potential, reduced half-life and altered subcellular distribution of the D463H
451 mutant[124]. There are four considerations that suggest this is an over-simplistic
452 interpretation. Firstly, there are many routes to a loss-of-function in these proteins
453 and the singular mutation identified in these chordoid tumours (always histidine to
454 date) clearly does not reflect an entirely random process. Secondly, it is known that
455 mutations at this aspartate residue of PKCα and the equivalent in other family
456 members, whilst blocking catalytic activity, serves to maintain kinase domain
457 conformation, as judged by priming phosphorylations; this contrasts with the
458 experimentally more commonly used kinase inactivating mutation at the conserved
459 lysine 368 residue[22]. The implication is that the D463H mutation will specifically (but

possibly not uniquely) permit a retention of conformation and priming site phosphorylations in the absence of activity. Thirdly, whilst acknowledging the limitations of mouse models for slow growing tumours, tumour formation in the central nervous system (CNS) of *Prkca* knock-out mice has not been reported[127]. Evidently simple loss-of-function is not a tumour driver or mice are poor surrogates of humans in this context. Finally, there is an interesting precedent set for distinctive scaffolding behaviour of PKCα in another CNS tumour. In glioblastoma cell models, PKCα expression is associated with protection from apoptosis, with survival compromised on inhibiting expression below a threshold level[128]. This behaviour is not phenocopied by catalytic site inhibitors, but is blocked by the C1 domain directed inhibitor Calphostin C. These observations suggest that PKCα plays some scaffold role in a survival pathway independent of catalytic activity[128].

It appears that in chordoid glioma, one allele of *PRKCA* encodes a catalytically incompetent enzyme, but one which may retain partner interaction capabilities. This may be a dominant effect on the wildtype protein encoded by the second allele or related to pathway operation through scaffolding functions. A definitive view on gain or loss-of-function and their effect on tumour growth will be derived from unravelling mechanism(s), which in turn should inform on interventions in this difficult to treat disease – either way, the potential drug candidate is unlikely to be a catalytic inhibitor of PKCα.

[H1] Emergent dependencies and their origins

[H2] PKC and cell cycle controls

Echoing what is described above regarding the landscape of PKC action in transformation, there is a related complexity to the reported actions of PKC isoforms across the breadth of cell cycle controls (reviewed in [129,130]). This complexity is particularly well illustrated in the review from Black and Black where the positive and negative proliferative impacts of PKC family members and their cell-type specific behaviours are clearly illustrated[129].

493 With respect to cell cycle entry (G0>G1 transition) of arrested cells in culture, a great
494 deal of evidence exists for the engagement of PKC isoforms in response to growth
495 factor and hormone action (Figure 3A). However, excepting some haematopoietic
496 cell types[129], there is little clarity over whether these specific responses play out as
497 critical to cell cycle progression *in vivo*, reflected in the generally normal
498 development of individual PKC gene knock-out mice (see above). Belying this
499 developmental normality of murine knockouts, there is published evidence for the
500 involvement of specific PKC isoforms in aspects of cell cycle progression including
501 both positive effects on CDKs via the inhibitor $p27^{kip1}$ [131] and negative effects on
502 CDKs as observed for PKCη association with the CyclinE-Cdk2-p21 complex acting
503 via p21[132] (Figure 3B). There are also observations relating to the organisational
504 requirements associated with cell cycle progression as reported for the DAG-
505 dependent disassembly of nuclear lamin B1 during the cell cycle[133], consistent with
506 the observation that lamins are targets for PKC [134,135], although it is noted that DAG
507 also modifies intrinsic membrane behaviour associated with nuclear envelope
508 formation[136]. The extent to which these influences of PKC on cell cycle progression
509 reflect the nutrient rich, over-indulged, stressed and/or transformed state of the cell
510 culture models remains to be determined. However, it would provide a rationalisation
511 of observations if for example the controls exerted on CDKs reflected responses to
512 covert stress inherent in cell culture models. This brings us to the third class of
513 controls where stress is definitively involved.
514
515 *[H2] PKCε dependency in transformed cells*
516 There is an emergent property associated with PKCε that is linked to a distinct
517 subset of transformed cells[137]. This manifests as a requirement for PKCε to alleviate
518 the threat of sister chromatid non-disjunction in these particular cells (Figure 3C).
519 The subset of transformed cells where PKCε is engaged has been defined
520 experimentally as those cell types that do not arrest in G2 in response to the
521 Topoisomerase 2α **[G]** (Topo2α) catalytic inhibitor, ICRF193[138]. This arrest pathway
522 has long been known, but until recently there was a somewhat limited description of
523 its requirements[139,140].
524

When prompted, the failure of this G2 arrest leads to engagement of PKCε where it influences prometaphase-metaphase transition[141], the metaphase-anaphase transition[137,142] and finally the abscission checkpoint[143]. During transit through M-Phase, PKCε exerts control on centrosome separation[141] as also reported for PKCβII[144]. At the metaphase-anaphase transition and at cytokinesis, PKCε has been shown to act via phosphorylation of Aurora B[142,143]. In both contexts, the PKCε phosphorylation of Aurora B at S227 switches its specificity towards critical sites on Topo2α[142] and Borealin **[G]** [143]. The engagement of PKCε in these cancer genome protective processes suggests that PKCε offers an interventional opportunity in the context of a subset of tumours – defining which tumours should be tractable through mechanism-specified biomarkers.

[H1] The PKC pharmacopoeia and cancer trials

There is a long history of small molecule inhibitors of PKC dating back to the mid-80's and the work of Hidaka and colleagues who recognised the drugability of kinases[145]. Many chemotypes followed over the years including the notoriously non-specific indolocarbazole, staurosporine[146] and the somewhat more selective bisindolylmaleimides[147], alongside many other inhibitors as reviewed elsewhere[148,149]. There are also multiple pharmacological activators of cPKCs and nPKCs as noted above, including the tiglianes[150] and bryostatin 1[151]; these agents as well as a number of catalytic site inhibitors have been used clinically with broadly but not exclusively disappointing outcomes.

[H2] Drugs, trials and tribulations

The extent to which there is a need for exquisite drug specificity is moot, however for targeted therapeutics the line of sight into the clinic is inevitably focused through the lens of the target. For PKC there have been some significant specificity challenges, clouding interpretation of many preclinical and clinical studies exploiting the PKC inhibitor inventory. This is reflected in the wealth of literature around the effects of 'PKC inhibitors' such as rottlerin and chelerythrine which actually target other cellular functions (see recent examples[152,153]). For the staurosporine derivative midostaurin

558 (PKC412) originally developed as a more selective PKC inhibitor[154], the evolving
559 clinical history has led to US Federal drug administration (FDA) approval for its use
560 in AML, albeit through its action on FLT3 (reviewed[155]). There are a variety of trials
561 investigating PKC412 in AML and MDS. A second staurosporine derivative, potent
562 against PKC isoforms, UCN-01 (7-hydroxystauropsorine) was subsequently
563 identified as a potent CHK1 inhibitor[156], but unlike midostaurin has not fared well in
564 clinical trials.
565
566 Enzastaurin is a PKCβ preferential inhibitor and has been employed in many clinical
567 trials (reviewed[157]). Its ineffectiveness to date is hard to interpret with the lack of
568 molecular data from these clinical studies. Even the original dose escalation Phase I
569 trial failed to report any pharmacodynamic data, did not reach dose-limiting toxicity
570 and settled on pharmacokinetic behaviour to define the 525mg daily dose for the
571 expansion cohort[158]. There is no data to indicate whether PKCβ or other targeted
572 PKCs in the tumour (or stroma) are blocked at this dose.
573
574 In respect of cPKC and nPKC activators there is limited specificity for cPKC or nPKC
575 isoforms and other binding-competent C1 domain proteins[75]. With no
576 pharmacodynamic data it is hard to assess actions in terms of targeting PKC
577 clinically. Nevertheless, the protection from phorbol ester-induced tumour
578 promotion[110] led to early phase oncology trials of bryostatins (reviewed[159]). FDA
579 orphan status was designated to bryostatin in combination with paclitaxel for
580 esophageal cancer in 2001, however subsequent trials did not support further
581 development[160]. A Phase I trial has been completed for tigilanol tiglate, a second
582 class of activator[161]. As noted above defining the target(s) of action for these agents
583 introduced intratumourally is complex, nevertheless following the recent approval of
584 this PKC activator in a veterinary setting, evidence from efficacy studies in patients is
585 eagerly awaited.
586
587 *[H2] 6.2 Uveal melanoma*
588 Intraocular melanoma (uveal melanoma) is associated with penetrant driver
589 mutations in the *GNAQ*, *GNA11*, *BAP1*, *EIF1AX* and *SF3B1* genes[162]. *GNAQ* and
590 *GNA11* encode the heterotrimeric G-protein α-subunits that trigger activation of the

β-class of phosphoinositide-specific phospholipase C proteins[163], elevating DAG levels and hence recruiting and activating cPKC and nPKC (and other DAG-responsive targets). In this context and with the to date intractability of phosphoinostide-specific phospholipase C inhibitors, there has been interest in targeting PKC isoforms. Currently, among the 33 active trials in uveal melanoma there are three targeting PKC. The first employs the orally available drug sotrastaurin (AEB071)[164] a maleimide derivative with potent PKC inhibitory activity[165]. This agent was well tolerated in Phase I studies and showed modest activity, principally stable disease[166]. The other active uveal melanoma trials (Phase I/II) involve another orally available drug, IDE196 also known as LXS196[167,168]. IDE196 was well tolerated and showed modest activity in a reported Phase I trial[169]. The outcomes of further efficacy trials and combination studies are awaited.

[H2] PKC inhibitors in other cancers

There have been a number of trials for other PKC inhibitors in a variety of cancers. PKCβ up-regulation in diffuse large B-cell lymphoma (DLBCL) has prompted a series of trials. The PKCβ discriminating drug enzastaurin has shown some limited single agent efficacy in DLBCL[170], and is currently in a trial in combination with the standard of care treatment R-CHOP[171]. A second PKCβ selective drug, MS-533, is in trials in chronic lymphocytic leukaemia and small lymphocytic leukaemia[172]. There is no published information on the specificity of this agent. There is an active trial for auranofin in combination with sirolimus[173]; a related cysteine-alkylating gold compound has been reported to specifically target aPKCι[174].

[H1] Concluding remarks

Preclinical investigation has yielded a complex landscape of PKC family actions in experimental cancers the translation of which into the clinical setting is generally hampered by a lack of mechanistic insights that afford robust biomarkers for pathological settings. Reciprocally, the unbiased ‘omics data derived from patient tumour biopsies has yielded limited insights to distinguish driver from by-stander

events and where providing clear direction, leave open significant issues in relation to interpretation of gain-, change- or loss-of-function.

The lack of straightforward correlations reflects the ambiguity of isoform steady state concentration as a marker for anything other than perhaps a complete absence. Similarly, the priming phosphorylation state of isoforms is not simply reflective of their action, at most this reveals latent potential. There is need for insight into the non-redundant pathological mechanisms at play and for this to be understood both in a tumour cell context as well as in the TME. Mechanisms will afford the biomarkers required to address the (in)action of isoforms clinically and importantly resolve where gain, change or loss of function operates guiding the nature of any intervention.

Decades on from PKC's linkage to the action of tumour promoters[7], the drugging of these kinases still offers much promise, but when and how remains moot and there is much to be done to resolve this.

References

1 Manning, G., Whyte, D. B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase complement of the human genome. *Science* **298**, 1912-1934, doi:10.1126/science.1075762 (2002).

2 Mellor, H. & Parker, P. J. The extended protein kinase C superfamily. *The Biochemical journal* **332 (Pt 2)**, 281-292 (1998).

3 Levin, D. E., Fields, F. O., Kunisawa, R., Bishop, J. M. & Thorner, J. A candidate protein kinase C gene, PKC1, is required for the S. cerevisiae cell cycle. *Cell* **62**, 213-224, doi:10.1016/0092-8674(90)90360-q (1990).

4 Suh, P. G. *et al.* Multiple roles of phosphoinositide-specific phospholipase C isozymes. *BMB Rep* **41**, 415-434, doi:10.5483/bmbrep.2008.41.6.415 (2008).

5 Bunney, T. D. & Katan, M. PLC regulation: emerging pictures for molecular mechanisms. *Trends Biochem Sci* **36**, 88-96, doi:10.1016/j.tibs.2010.08.003 (2011).

6 Haga, R. B. & Ridley, A. J. Rho GTPases: Regulation and roles in cancer cell biology. *Small GTPases* **7**, 207-221, doi:10.1080/21541248.2016.1232583 (2016).

7 Castagna, M. *et al.* Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257**, 7847-7851 (1982).
This paper was the first to define PKC as a target for tumour promoters.

8 Gallegos, L. L. & Newton, A. C. Spatiotemporal dynamics of lipid signaling: protein kinase C as a paradigm. *IUBMB Life* **60**, 782-789, doi:10.1002/iub.122 (2008).

663 9 Tobias, I. S. & Newton, A. C. Protein Scaffolds Control Localized Protein Kinase
664 Czeta Activity. *J Biol Chem* **291**, 13809-13822, doi:10.1074/jbc.M116.729483
665 (2016).
666 10 Hong, Y. aPKC: the Kinase that Phosphorylates Cell Polarity. *F1000Res* **7**,
667 doi:10.12688/f1000research.14427.1 (2018).
668 11 Amano, M. *et al.* Identification of a putative target for Rho as the serine-threonine
669 kinase protein kinase N. *Science* **271**, 648-650, doi:10.1126/science.271.5249.648
670 (1996).
671 12 Bauer, A. F. *et al.* Regulation of protein kinase C-related protein kinase 2 (PRK2) by
672 an intermolecular PRK2-PRK2 interaction mediated by Its N-terminal domain. *J Biol*
673 *Chem* **287**, 20590-20602, doi:10.1074/jbc.M111.327437 (2012).
674 13 Jaken, S. & Parker, P. J. Protein kinase C binding partners. *Bioessays* **22**, 245-254,
675 doi:10.1002/(SICI)1521-1878(200003)22:3<245::AID-BIES6>3.0.CO;2-X (2000).
676 14 Schechtman, D. & Mochly-Rosen, D. Adaptor proteins in protein kinase C-mediated
677 signal transduction. *Oncogene* **20**, 6339-6347, doi:10.1038/sj.onc.1204778 (2001).
678 15 Saurin, A. T. *et al.* The regulated assembly of a PKCepsilon complex controls the
679 completion of cytokinesis. *Nat Cell Biol* **10**, 891-901, doi:ncb1749 [pii]
680 10.1038/ncb1749 (2008).
681 16 Schonwasser, D. C., Marais, R. M., Marshall, C. J. & Parker, P. J. Activation of the
682 mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by
683 conventional, novel, and atypical protein kinase C isotypes. *Mol Cell Biol* **18**, 790-
684 798, doi:10.1128/mcb.18.2.790 (1998).
685 17 Le Good, J. A. *et al.* Protein kinase C isotypes controlled by phosphoinositide 3-
686 kinase through the protein kinase PDK1. *Science* **281**, 2042-2045 (1998).
687 18 Chou, M. M. *et al.* Regulation of protein kinase C zeta by PI 3-kinase and PDK-1.
688 *Curr Biol* **8**, 1069-1077, doi:10.1016/s0960-9822(98)70444-0 (1998).
689 19 Dutil, E. M., Toker, A. & Newton, A. C. Regulation of conventional protein kinase C
690 isozymes by phosphoinositide-dependent kinase 1 (PDK-1). *Curr Biol* **8**, 1366-1375,
691 doi:10.1016/s0960-9822(98)00017-7 (1998).
692 20 Cameron, A. J., Linch, M. D., Saurin, A. T., Escribano, C. & Parker, P. J. mTORC2
693 targets AGC kinases through Sin1-dependent recruitment. *The Biochemical journal*
694 **439**, 287-297, doi:10.1042/bj20110678 (2011).
695 21 Newton, A. C. Protein kinase C as a tumor suppressor. *Semin Cancer Biol* **48**, 18-26,
696 doi:10.1016/j.semcancer.2017.04.017 (2018).
697 22 Cameron, A. J., Escribano, C., Saurin, A. T., Kostelecky, B. & Parker, P. J. PKC
698 maturation is promoted by nucleotide pocket occupation independently of intrinsic
699 kinase activity. *Nature structural & molecular biology* **16**, 624-630,
700 doi:10.1038/nsmb.1606 (2009).
701 **This paper demonstrates that in cells, the occupation of the nucleotide binding**
702 **pocket of PKC with nucleotides or inhibitors has a profound impact on its**
703 **priming phosphorylation state.**
704 23 Gould, C. M. *et al.* Active site inhibitors protect protein kinase C from
705 dephosphorylation and stabilize its mature form. *J Biol Chem* **286**, 28922-28930,
706 doi:10.1074/jbc.M111.272526 (2011).
707 24 Srivastava, J., Goris, J., Dilworth, S. M. & Parker, P. J. Dephosphorylation of
708 PKCdelta by protein phosphatase 2Ac and its inhibition by nucleotides. *FEBS Lett*
709 **516**, 265-269 (2002).
710 25 Torbett, N. E., Casamassima, A. & Parker, P. J. Hyperosmotic-induced protein kinase
711 N 1 activation in a vesicular compartment is dependent upon Rac1 and 3-

712 phosphoinositide-dependent kinase 1. *J Biol Chem* **278**, 32344-32351,
713 doi:10.1074/jbc.M303532200 (2003).
714 26 Newton, A. C. Protein kinase C: poised to signal. *Am J Physiol Endocrinol Metab*
715 **298**, E395-402, doi:10.1152/ajpendo.00477.2009 (2010).
716 27 Balendran, A., Hare, G. R., Kieloch, A., Williams, M. R. & Alessi, D. R. Further
717 evidence that 3-phosphoinositide-dependent protein kinase-1 (PDK1) is required for
718 the stability and phosphorylation of protein kinase C (PKC) isoforms. *FEBS Lett* **484**,
719 217-223, doi:10.1016/s0014-5793(00)02162-1 (2000).
720 28 Cameron, A. J. *et al.* Protein kinases, from B to C. *Biochemical Society transactions*
721 **35**, 1013-1017, doi:10.1042/BST0351013 (2007).
722 29 Nishikawa, K., Toker, A., Johannes, F. J., Songyang, Z. & Cantley, L. C.
723 Determination of the specific substrate sequence motifs of protein kinase C isozymes.
724 *J Biol Chem* **272**, 952-960, doi:10.1074/jbc.272.2.952 (1997).
725 30 Betson, M. & Settleman, J. A rho-binding protein kinase C-like activity is required for
726 the function of protein kinase N in Drosophila development. *Genetics* **176**, 2201-
727 2212, doi:10.1534/genetics.107.072967 (2007).
728 31 Lachmann, S. *et al.* Regulatory domain selectivity in the cell-type specific PKN-
729 dependence of cell migration. *PLoS One* **6**, e21732,
730 doi:10.1371/journal.pone.0021732 (2011).
731 32 Carracedo, S., Sacher, F., Brandes, G., Braun, U. & Leitges, M. Redundant role of
732 protein kinase C delta and epsilon during mouse embryonic development. *PLoS One*
733 **9**, e103686, doi:10.1371/journal.pone.0103686 (2014).
734 33 Linch, M. *et al.* A cancer-associated mutation in atypical protein kinase Ciota occurs
735 in a substrate-specific recruitment motif. *Sci Signal* **6**, ra82,
736 doi:10.1126/scisignal.2004068 (2013).
737 34 Pears, C. J., Kour, G., House, C., Kemp, B. E. & Parker, P. J. Mutagenesis of the
738 pseudosubstrate site of protein kinase C leads to activation. *Eur J Biochem* **194**, 89-94
739 (1990).
740 35 Antal, C. E., Callender, J. A., Kornev, A. P., Taylor, S. S. & Newton, A. C.
741 Intramolecular C2 Domain-Mediated Autoinhibition of Protein Kinase C βII. *Cell*
742 *reports* **12**, 1252-1260, doi:10.1016/j.celrep.2015.07.039 (2015).
743 36 Stabel, S., Rodriguez-Pena, A., Young, S., Rozengurt, E. & Parker, P. J. Quantitation
744 of protein kinase C by immunoblot--expression in different cell lines and response to
745 phorbol esters. *J Cell Physiol* **130**, 111-117, doi:10.1002/jcp.1041300116 (1987).
746 37 Lee, H. W., Smith, L., Pettit, G. R. & Smith, J. B. Bryostatin 1 and phorbol ester
747 down-modulate protein kinase C-alpha and -epsilon via the ubiquitin/proteasome
748 pathway in human fibroblasts. *Mol Pharmacol* **51**, 439-447 (1997).
749 38 Lee, H. W., Smith, L., Pettit, G. R., Vinitsky, A. & Smith, J. B. Ubiquitination of
750 protein kinase C-alpha and degradation by the proteasome. *J Biol Chem* **271**, 20973-
751 20976 (1996).
752 39 Lu, Z. *et al.* Activation of protein kinase C triggers its ubiquitination and degradation.
753 *Mol Cell Biol* **18**, 839-845, doi:10.1128/mcb.18.2.839 (1998).
754 40 Hansra, G. *et al.* Multisite dephosphorylation and desensitization of conventional
755 protein kinase C isotypes. *The Biochemical journal* **342 (Pt 2)**, 337-344 (1999).
756 41 Prevostel, C., Alice, V., Joubert, D. & Parker, P. J. Protein kinase C(alpha) actively
757 downregulates through caveolae-dependent traffic to an endosomal compartment. *J*
758 *Cell Sci* **113 (Pt 14)**, 2575-2584 (2000).
759 42 Leontieva, O. V. & Black, J. D. Identification of two distinct pathways of protein
760 kinase Calpha down-regulation in intestinal epithelial cells. *J Biol Chem* **279**, 5788-
761 5801, doi:10.1074/jbc.M308375200 (2004).

762 43 Lum, M. A., Pundt, K. E., Paluch, B. E., Black, A. R. & Black, J. D. Agonist-induced
763 down-regulation of endogenous protein kinase c alpha through an endolysosomal
764 mechanism. *J Biol Chem* **288**, 13093-13109, doi:10.1074/jbc.M112.437061 (2013).
765 44 Melnikov, S. & Sagi-Eisenberg, R. Down-regulating protein kinase C alpha:
766 functional cooperation between the proteasome and the endocytic system. *Cell Signal*
767 **21**, 1607-1619, doi:10.1016/j.cellsig.2009.06.007 (2009).
768 45 Chen, D. *et al.* Amplitude control of protein kinase C by RINCK, a novel E3 ubiquitin
769 ligase. *J Biol Chem* **282**, 33776-33787, doi:10.1074/jbc.M703320200 (2007).
770 46 Min, X., Zhang, X., Sun, N., Acharya, S. & Kim, K. M. Mdm2-mediated
771 ubiquitination of PKCβII in the nucleus mediates clathrin-mediated endocytic
772 activity. *Biochemical pharmacology* **170**, 113675, doi:10.1016/j.bcp.2019.113675
773 (2019).
774 47 Nakamura, M., Tokunaga, F., Sakata, S. & Iwai, K. Mutual regulation of conventional
775 protein kinase C and a ubiquitin ligase complex. *Biochem Biophys Res Commun* **351**,
776 340-347, doi:10.1016/j.bbrc.2006.09.163 (2006).
777 48 Baffi, T. R., Van, A. N., Zhao, W., Mills, G. B. & Newton, A. C. Protein Kinase C
778 Quality Control by Phosphatase PHLPP1 Unveils Loss-of-Function Mechanism in
779 Cancer. *Mol Cell* **74**, 378-392.e375, doi:10.1016/j.molcel.2019.02.018 (2019).
780 49 Abrahamsen, H. *et al.* Peptidyl-prolyl isomerase Pin1 controls down-regulation of
781 conventional protein kinase C isozymes. *J Biol Chem* **287**, 13262-13278,
782 doi:10.1074/jbc.M112.349753 (2012).
783 50 Perander, M., Bjorkoy, G. & Johansen, T. Nuclear import and export signals enable
784 rapid nucleocytoplasmic shuttling of the atypical protein kinase C lambda. *J Biol*
785 *Chem* **276**, 13015-13024, doi:10.1074/jbc.M010356200 (2001).
786 51 Ivaska, J., Bosca, L. & Parker, P. J. PKCepsilon is a permissive link in integrin-
787 dependent IFN-gamma signalling that facilitates JAK phosphorylation of STAT1. *Nat*
788 *Cell Biol* **5**, 363-369, doi:10.1038/ncb957
789 ncb957 [pii] (2003).
790 52 Pelech, S. L., Meier, K. E. & Krebs, E. G. Rapid microassay for protein kinase C
791 translocation in Swiss 3T3 cells. *Biochemistry* **25**, 8348-8353,
792 doi:10.1021/bi00374a002 (1986).
793 53 Sakai, N. *et al.* Direct visualization of the translocation of the gamma-subspecies of
794 protein kinase C in living cells using fusion proteins with green fluorescent protein. *J*
795 *Cell Biol* **139**, 1465-1476, doi:10.1083/jcb.139.6.1465 (1997).
796 54 Gao, X. *et al.* Single cell analysis of PKC activation during proliferation and
797 apoptosis induced by laser irradiation. *J Cell Physiol* **206**, 441-448,
798 doi:10.1002/jcp.20484 (2006).
799 55 Flint, A. J., Paladini, R. D. & Koshland, D. E., Jr. Autophosphorylation of protein
800 kinase C at three separated regions of its primary sequence. *Science* **249**, 408-411,
801 doi:10.1126/science.2377895 (1990).
802 56 Ng, T. *et al.* Imaging protein kinase Calpha activation in cells. *Science* **283**, 2085-
803 2089, doi:10.1126/science.283.5410.2085 (1999).
804 57 Durgan, J. *et al.* The identification and characterization of novel PKCepsilon
805 phosphorylation sites provide evidence for functional cross-talk within the PKC
806 superfamily. *The Biochemical journal* **411**, 319-331, doi:10.1042/bj20071348 (2008).
807 58 Durgan, J., Michael, N., Totty, N. & Parker, P. J. Novel phosphorylation site markers
808 of protein kinase C delta activation. *FEBS Lett* **581**, 3377-3381,
809 doi:10.1016/j.febslet.2007.06.035 (2007).

810 59 Rodriguez, J. *et al.* aPKC Cycles between Functionally Distinct PAR Protein Assemblies to Drive Cell Polarity. *Dev Cell* **42**, 400-415.e409, doi:10.1016/j.devcel.2017.07.007 (2017).
813 60 Wodarz, A. & Näthke, I. Cell polarity in development and cancer. *Nat Cell Biol* **9**, 1016-1024, doi:10.1038/ncb433 (2007).
815 61 Linch, M. *et al.* Regulation of polarized morphogenesis by protein kinase C iota in oncogenic epithelial spheroids. *Carcinogenesis* **35**, 396-406, doi:10.1093/carcin/bgt313 (2014).
818 62 Slaga, T. J. Overview of tumor promotion in animals. *Environ Health Perspect* **50**, 3-14, doi:10.1289/ehp.83503 (1983).
820 63 Hecker, E. Three stage carcinogenesis in mouse skin--recent results and present status of an advanced model system of chemical carcinogenesis. *Toxicol Pathol* **15**, 245-258, doi:10.1177/019262338701500221 (1987).
823 64 Balmain, A. Transforming ras oncogenes and multistage carcinogenesis. *Br J Cancer* **51**, 1-7, doi:10.1038/bjc.1985.1 (1985).
825 65 Fujiki, H., Sueoka, E. & Suganuma, M. Tumor promoters: from chemicals to inflammatory proteins. *J Cancer Res Clin Oncol* **139**, 1603-1614, doi:10.1007/s00432-013-1455-8 (2013).
828 66 Nishizuka, Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* **308**, 693-698, doi:10.1038/308693a0 (1984).
830 67 Ono, Y. *et al.* Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. *Proc Natl Acad Sci U S A* **86**, 4868-4871, doi:10.1073/pnas.86.13.4868 (1989).
833 **The first demonstration that the cysteine-rich, C1 domains were bound by phorbol esters, impacting our definition of this entire class of responsive proteins.**
836 68 Fujiki, H. *et al.* Activation of calcium-activated, phospholipid-dependent protein kinase (protein kinase C) by new classes of tumor promoters: teleocidin and debromoaplysiatoxin. *Biochem Biophys Res Commun* **120**, 339-343, doi:10.1016/0006-291x(84)91259-2 (1984).
840 69 Miyake, R. *et al.* Activation of protein kinase C by non-phorbol tumor promoter, mezerein. *Biochem Biophys Res Commun* **121**, 649-656, doi:10.1016/0006-291x(84)90231-6 (1984).
843 70 Arcoleo, J. P. & Weinstein, I. B. Activation of protein kinase C by tumor promoting phorbol esters, teleocidin and aplysiatoxin in the absence of added calcium. *Carcinogenesis* **6**, 213-217, doi:10.1093/carcin/6.2.213 (1985).
846 71 Thastrup, O., Cullen, P. J., Drobak, B. K., Hanley, M. R. & Dawson, A. P. Thapsigargin, a tumor promoter, discharges intracellular Ca2+ stores by specific inhibition of the endoplasmic reticulum Ca2(+)-ATPase. *Proc Natl Acad Sci U S A* **87**, 2466-2470, doi:10.1073/pnas.87.7.2466 (1990).
850 72 Haystead, T. A. *et al.* Effects of the tumour promoter okadaic acid on intracellular protein phosphorylation and metabolism. *Nature* **337**, 78-81, doi:10.1038/337078a0 (1989).
853 73 Manzow, S., Richter, K. H., Stempka, L., Fürstenberger, G. & Marks, F. Evidence against a role of general protein kinase C downregulation in skin tumor promotion. *Int J Cancer* **85**, 503-507, doi:10.1002/(sici)1097-0215(20000215)85:4<503::aid-ijc10>3.0.co;2-l (2000).
857 74 Arnott, C. H. *et al.* Tumour necrosis factor-alpha mediates tumour promotion via a PKC alpha- and AP-1-dependent pathway. *Oncogene* **21**, 4728-4738, doi:10.1038/sj.onc.1205588 (2002).

860 75 Kazanietz, M. G. Novel "nonkinase" phorbol ester receptors: the C1 domain
861 connection. *Mol Pharmacol* **61**, 759-767, doi:10.1124/mol.61.4.759 (2002).
862 76 Soloff, R. S., Katayama, C., Lin, M. Y., Feramisco, J. R. & Hedrick, S. M. Targeted
863 deletion of protein kinase C lambda reveals a distribution of functions between the
864 two atypical protein kinase C isoforms. *J Immunol* **173**, 3250-3260,
865 doi:10.4049/jimmunol.173.5.3250 (2004).
866 77 Quetier, I. *et al.* Knockout of the PKN Family of Rho Effector Kinases Reveals a
867 Non-redundant Role for PKN2 in Developmental Mesoderm Expansion. *Cell reports*
868 **14**, 440-448, doi:10.1016/j.celrep.2015.12.049 (2016).
869 78 Oster, H. & Leitges, M. Protein kinase C alpha but not PKCzeta suppresses intestinal
870 tumor formation in ApcMin/+ mice. *Cancer Res* **66**, 6955-6963, doi:10.1158/0008-
871 5472.Can-06-0268 (2006).
872 **Direct evidence that in the ApcMin/+ mouse model of colorectal cancer PKCα**
873 **suppresses tumour progression.**
874 79 Cataisson, C. *et al.* Activation of cutaneous protein kinase C alpha induces
875 keratinocyte apoptosis and intraepidermal inflammation by independent signaling
876 pathways. *J Immunol* **171**, 2703-2713, doi:10.4049/jimmunol.171.5.2703 (2003).
877 80 Wang, H. Q. & Smart, R. C. Overexpression of protein kinase C-alpha in the
878 epidermis of transgenic mice results in striking alterations in phorbol ester-induced
879 inflammation and COX-2, MIP-2 and TNF-alpha expression but not tumor
880 promotion. *J Cell Sci* **112 (Pt 20)**, 3497-3506 (1999).
881 81 Hara, T. *et al.* Deficiency of protein kinase Calpha in mice results in impairment of
882 epidermal hyperplasia and enhancement of tumor formation in two-stage skin
883 carcinogenesis. *Cancer Res* **65**, 7356-7362, doi:10.1158/0008-5472.Can-04-4241
884 (2005).
885 82 Reddig, P. J. *et al.* Transgenic mice overexpressing protein kinase Cdelta in the
886 epidermis are resistant to skin tumor promotion by 12-O-tetradecanoylphorbol-13-
887 acetate. *Cancer Res* **59**, 5710-5718 (1999).
888 83 Aziz, M. H., Wheeler, D. L., Bhamb, B. & Verma, A. K. Protein kinase C delta
889 overexpressing transgenic mice are resistant to chemically but not to UV radiation-
890 induced development of squamous cell carcinomas: a possible link to specific
891 cytokines and cyclooxygenase-2. *Cancer Res* **66**, 713-722, doi:10.1158/0008-
892 5472.Can-05-2684 (2006).
893 84 Miyamoto, A. *et al.* Increased proliferation of B cells and auto-immunity in mice
894 lacking protein kinase Cdelta. *Nature* **416**, 865-869, doi:10.1038/416865a (2002).
895 **Description of the B-lymphoproliferative disorder in the PKCδ knock-out**
896 **mouse.**
897 85 Mecklenbrauker, I., Saijo, K., Zheng, N. Y., Leitges, M. & Tarakhovsky, A. Protein
898 kinase Cdelta controls self-antigen-induced B-cell tolerance. *Nature* **416**, 860-865,
899 doi:10.1038/416860a (2002).
900 86 Kuehn, H. S. *et al.* Loss-of-function of the protein kinase C δ (PKCδ) causes a B-cell
901 lymphoproliferative syndrome in humans. *Blood* **121**, 3117-3125, doi:10.1182/blood-
902 2012-12-469544 (2013).
903 87 Allen-Petersen, B. L., Carter, C. J., Ohm, A. M. & Reyland, M. E. Protein kinase
904 Cdelta is required for ErbB2-driven mammary gland tumorigenesis and negatively
905 correlates with prognosis in human breast cancer. *Oncogene* **33**, 1306-1315,
906 doi:10.1038/onc.2013.59 (2014).
907 88 Symonds, J. M. *et al.* Protein kinase C delta is a downstream effector of oncogenic K-
908 ras in lung tumors. *Cancer Res* **71**, 2087-2097, doi:10.1158/0008-5472.Can-10-1511
909 (2011).

910 89 Leitges, M. *et al.* Immunodeficiency in protein kinase cbeta-deficient mice. *Science* **273**, 788-791, doi:10.1126/science.273.5276.788 (1996).
912 90 Tsui, C. *et al.* Protein Kinase C-beta Dictates B Cell Fate by Regulating Mitochondrial Remodeling, Metabolic Reprogramming, and Heme Biosynthesis. *Immunity* **48**, 1144-1159.e1145, doi:10.1016/j.immuni.2018.04.031 (2018).
915 91 Pfeifhofer, C. *et al.* Defective IgG2a/2b class switching in PKC alpha-/- mice. *J Immunol* **176**, 6004-6011, doi:10.4049/jimmunol.176.10.6004 (2006).
917 92 Martini, S. *et al.* PKCepsilon promotes human Th17 differentiation: Implications in the pathophysiology of psoriasis. *Eur J Immunol* **48**, 644-654, doi:10.1002/eji.201747102 (2018).
920 93 Castrillo, A. *et al.* Protein kinase Cepsilon is required for macrophage activation and defense against bacterial infection. *J Exp Med* **194**, 1231-1242 (2001).
922 94 Pfeifhofer, C. *et al.* Protein kinase C theta affects Ca2+ mobilization and NFAT cell activation in primary mouse T cells. *J Exp Med* **197**, 1525-1535, doi:10.1084/jem.20020234 (2003).
925 95 Thuille, N. *et al.* Loss-of-function phenotype of a PKCtheta(T219A) knockin mouse strain. *Cell Commun Signal* **17**, 141, doi:10.1186/s12964-019-0466-8 (2019).
927 96 He, X. *et al.* Targeting PKC in human T cells using sotrastaurin (AEB071) preserves regulatory T cells and prevents IL-17 production. *J Invest Dermatol* **134**, 975-983, doi:10.1038/jid.2013.459 (2014).
930 97 Kwon, M. J., Ma, J., Ding, Y., Wang, R. & Sun, Z. Protein kinase C-theta promotes Th17 differentiation via upregulation of Stat3. *J Immunol* **188**, 5887-5897, doi:10.4049/jimmunol.1102941 (2012).
933 98 Kong, K. F. *et al.* Protein kinase C-eta controls CTLA-4-mediated regulatory T cell function. *Nat Immunol* **15**, 465-472, doi:10.1038/ni.2866 (2014).
935 99 Chida, K. *et al.* Disruption of protein kinase Ceta results in impairment of wound healing and enhancement of tumor formation in mouse skin carcinogenesis. *Cancer Res* **63**, 2404-2408 (2003).
938 100 Park, D. W. *et al.* TLR2 stimulates ABCA1 expression via PKC-eta and PLD2 pathway. *Biochem Biophys Res Commun* **430**, 933-937, doi:10.1016/j.bbrc.2012.11.135 (2013).
941 101 Fu, G. *et al.* Protein kinase C eta is required for T cell activation and homeostatic proliferation. *Sci Signal* **4**, ra84, doi:10.1126/scisignal.2002058 (2011).
943 102 Wallace, J. A. *et al.* Protein kinase C Beta in the tumor microenvironment promotes mammary tumorigenesis. *Front Oncol* **4**, 87, doi:10.3389/fonc.2014.00087 (2014).
945 103 Park, E. *et al.* Stromal cell protein kinase C-beta inhibition enhances chemosensitivity in B cell malignancies and overcomes drug resistance. *Sci Transl Med* **12**, doi:10.1126/scitranslmed.aax9340 (2020).
948 104 Mukai, H. *et al.* PKN3 is the major regulator of angiogenesis and tumor metastasis in mice. *Sci Rep* **6**, 18979, doi:10.1038/srep18979 (2016).
950 105 Hattori, Y., Kikuchi, T., Nakamura, M., Ozaki, K. I. & Onishi, H. Therapeutic effects of protein kinase N3 small interfering RNA and doxorubicin combination therapy on liver and lung metastases. *Oncol Lett* **14**, 5157-5166, doi:10.3892/ol.2017.6830 (2017).
954 106 Leenders, F. *et al.* PKN3 is required for malignant prostate cell growth downstream of activated PI 3-kinase. *Embo j* **23**, 3303-3313, doi:10.1038/sj.emboj.7600345 (2004).
956 107 Kraft, A. S., Smith, J. B. & Berkow, R. L. Bryostatin, an activator of the calcium phospholipid-dependent protein kinase, blocks phorbol ester-induced differentiation of human promyelocytic leukemia cells HL-60. *Proc Natl Acad Sci U S A* **83**, 1334-1338, doi:10.1073/pnas.83.5.1334 (1986).

960 108 Mackanos, E. A., Pettit, G. R. & Ramsdell, J. S. Bryostatins selectively regulate
961 protein kinase C-mediated effects on GH4 cell proliferation. *J Biol Chem* **266**, 11205-
962 11212 (1991).
963 109 Szallasi, Z., Smith, C. B., Pettit, G. R. & Blumberg, P. M. Differential regulation of
964 protein kinase C isozymes by bryostatin 1 and phorbol 12-myristate 13-acetate in NIH
965 3T3 fibroblasts. *J Biol Chem* **269**, 2118-2124 (1994).
966 110 Hennings, H. *et al.* Bryostatin 1, an activator of protein kinase C, inhibits tumor
967 promotion by phorbol esters in SENCAR mouse skin. *Carcinogenesis* **8**, 1343-1346,
968 doi:10.1093/carcin/8.9.1343 (1987).
969 **Demonstration that despite its shared ability to activate PKC, Bryostatin 1**
970 **inhibits phorbol ester promoted tumour formation**.
971 111 Boyle, G. M. *et al.* Intra-lesional injection of the novel PKC activator EBC-46 rapidly
972 ablates tumors in mouse models. *PLoS One* **9**, e108887,
973 doi:10.1371/journal.pone.0108887 (2014).
974 **Evidence that the PKC-activating, epoxytigliane EBC-46 can trigger tumour**
975 **regression on intratumoural injection.**
976 112 Miller, J. *et al.* Dose Characterization of the Investigational Anticancer Drug
977 Tigilanol Tiglate (EBC-46) in the Local Treatment of Canine Mast Cell Tumors.
978 *Front Vet Sci* **6**, 106, doi:10.3389/fvets.2019.00106 (2019).
979 113 Davies, H. *et al.* Mutations of the BRAF gene in human cancer. *Nature* **417**, 949-954,
980 doi:10.1038/nature00766 (2002).
981 114 Antal, C. E. *et al.* Cancer-associated protein kinase C mutations reveal kinase's role as
982 tumor suppressor. *Cell* **160**, 489-502, doi:10.1016/j.cell.2015.01.001 (2015).
983 115 Bangham, C. R. & Ratner, L. How does HTLV-1 cause adult T-cell
984 leukaemia/lymphoma (ATL)? *Curr Opin Virol* **14**, 93-100,
985 doi:10.1016/j.coviro.2015.09.004 (2015).
986 116 Kataoka, K. *et al.* Integrated molecular analysis of adult T cell leukemia/lymphoma.
987 *Nature genetics* **47**, 1304-1315, doi:10.1038/ng.3415 (2015).
988 **A comprehensive description of the mutational landscape of ATLL and the**
989 **identification of PKCβ as a frequent mutation target.**
990 117 Wang, C., Shang, Y., Yu, J. & Zhang, M. Substrate recognition mechanism of
991 atypical protein kinase Cs revealed by the structure of PKCiota in complex with a
992 substrate peptide from Par-3. *Structure* **20**, 791-801, doi:10.1016/j.str.2012.02.022
993 (2012).
994 118 Shinohara, H. *et al.* PKC beta regulates BCR-mediated IKK activation by facilitating
995 the interaction between TAK1 and CARMA1. *J Exp Med* **202**, 1423-1431,
996 doi:10.1084/jem.20051591 (2005).
997 119 Soriano, E. V. *et al.* aPKC Inhibition by Par3 CR3 Flanking Regions Controls
998 Substrate Access and Underpins Apical-Junctional Polarization. *Dev Cell* **38**, 384-
999 398, doi:10.1016/j.devcel.2016.07.018 (2016).
1000 120 Linch, M. *Protein kinase C iota in mammalian cell polarity and cancer* PhD thesis,
1001 University College London, (2012).
1002 121 Shimoyama, M. Diagnostic criteria and classification of clinical subtypes of adult T-
1003 cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984-87). *Br*
1004 *J Haematol* **79**, 428-437, doi:10.1111/j.1365-2141.1991.tb08051.x (1991).
1005 122 Brat, D. J. *et al.* Third ventricular chordoid glioma: a distinct clinicopathologic entity.
1006 *J Neuropathol Exp Neurol* **57**, 283-290, doi:10.1097/00005072-199803000-00009
1007 (1998).

123 Morais, B. A., Menendez, D. F., Medeiros, R. S., Teixeira, M. J. & Lepski, G. A. Chordoid glioma: Case report and review of the literature. *Int J Surg Case Rep* **7c**, 168-171, doi:10.1016/j.ijscr.2015.01.027 (2015).

124 Rosenberg, S. *et al.* A recurrent point mutation in PRKCA is a hallmark of chordoid gliomas. *Nature communications* **9**, 2371, doi:10.1038/s41467-018-04622-w (2018).
One of the two papers demonstrating a fully penetrant mutation in PKCα in chordoid gliomas.

125 Goode, B. *et al.* A recurrent kinase domain mutation in PRKCA defines chordoid glioma of the third ventricle. *Nature communications* **9**, 810, doi:10.1038/s41467-018-02826-8 (2018).
One of the two papers demonstrating a fully penetrant mutation in PKCα in chordoid gliomas.

126 Madhusudan *et al.* cAMP-dependent protein kinase: crystallographic insights into substrate recognition and phosphotransfer. *Protein Sci* **3**, 176-187, doi:10.1002/pro.5560030203 (1994).

127 Leitges, M. *et al.* Knockout of PKC alpha enhances insulin signaling through PI3K. *Mol Endocrinol* **16**, 847-858, doi:10.1210/mend.16.4.0809 (2002).

128 Cameron, A. J., Procyk, K. J., Leitges, M. & Parker, P. J. PKC alpha protein but not kinase activity is critical for glioma cell proliferation and survival. *Int J Cancer* **123**, 769-779, doi:10.1002/ijc.23560 (2008).

129 Black, A. R. & Black, J. D. Protein kinase C signaling and cell cycle regulation. *Front Immunol* **3**, 423, doi:10.3389/fimmu.2012.00423 (2012).

130 Poli, A., Mongiorgi, S., Cocco, L. & Follo, M. Y. Protein kinase C involvement in cell cycle modulation. *Biochemical Society transactions* **42**, 1471-1476, doi:10.1042/bst20140128 (2014).

131 Gao, Q. *et al.* PKC alpha affects cell cycle progression and proliferation in human RPE cells through the downregulation of p27kip1. *Mol Vis* **15**, 2683-2695 (2009).

132 Kashiwagi, M. *et al.* PKCeta associates with cyclin E/cdk2/p21 complex, phosphorylates p21 and inhibits cdk2 kinase in keratinocytes. *Oncogene* **19**, 6334-6341, doi:10.1038/sj.onc.1204028 (2000).

133 Mall, M. *et al.* Mitotic lamin disassembly is triggered by lipid-mediated signaling. *J Cell Biol* **198**, 981-990, doi:10.1083/jcb.201205103 (2012).

134 Edens, L. J., Dilsaver, M. R. & Levy, D. L. PKC-mediated phosphorylation of nuclear lamins at a single serine residue regulates interphase nuclear size in Xenopus and mammalian cells. *Mol Biol Cell* **28**, 1389-1399, doi:10.1091/mbc.E16-11-0786 (2017).

135 Goss, V. L. *et al.* Identification of nuclear beta II protein kinase C as a mitotic lamin kinase. *J Biol Chem* **269**, 19074-19080 (1994).

136 Larijani, B. *et al.* Principle of duality in phospholipids: regulators of membrane morphology and dynamics. *Biochemical Society transactions* **42**, 1335-1342, doi:10.1042/bst20140224 (2014).

137 Brownlow, N., Pike, T., Zicha, D., Collinson, L. & Parker, P. J. Mitotic catenation is monitored and resolved by a PKCepsilon-regulated pathway. *Nature communications* **5**, 5685, doi:10.1038/ncomms6685 (2014).
The paper defines for the first time, the cell cycle dependence on PKCε in cells with a dysfunctional Topo2-dependent G2 arrest.

138 Downes, C. S. *et al.* A topoisomerase II-dependent G2 cycle checkpoint in mammalian cells. *Nature* **372**, 467-470, doi:10.1038/372467a0 (1994).

139 Pandey, N. *et al.* Topoisomerase II SUMOylation activates a metaphase checkpoint via Haspin and Aurora B kinases. *J Cell Biol*, doi:10.1083/jcb.201807189 (2019).

1058 140 Deiss, K. *et al.* A genome-wide RNAi screen identifies the SMC5/6 complex as a
1059 non-redundant regulator of a Topo2a-dependent G2 arrest. *Nucleic acids research* **47**,
1060 2906-2921, doi:10.1093/nar/gky1295 (2019).
1061 **A genome-wide screen for genes engaged in the Topo2-dependent G2 arrest**
1062 **provide molecular insight into the context of PKCε dependence.**
1063 141 Martini, S. *et al.* PKCepsilon Controls Mitotic Progression by Regulating Centrosome
1064 Migration and Mitotic Spindle Assembly. *Mol Cancer Res* **16**, 3-15,
1065 doi:10.1158/1541-7786.MCR-17-0244 (2018).
1066 142 Kelly, J. R. *et al.* The Aurora B specificity switch is required to protect from non-
1067 disjunction at the metaphase/anaphase transition. *Nature communications* **11**, 1396,
1068 doi:10.1038/s41467-020-15163-6 (2020).
1069 143 Pike, T., Brownlow, N., Kjaer, S., Carlton, J. & Parker, P. J. PKCepsilon switches
1070 Aurora B specificity to exit the abscission checkpoint. *Nature communications* **7**,
1071 13853, doi:10.1038/ncomms13853 (2016).
1072 144 Chen, D., Purohit, A., Halilovic, E., Doxsey, S. J. & Newton, A. C. Centrosomal
1073 anchoring of protein kinase C betaII by pericentrin controls microtubule organization,
1074 spindle function, and cytokinesis. *J Biol Chem* **279**, 4829-4839,
1075 doi:10.1074/jbc.M311196200 (2004).
1076 145 Kawamoto, S. & Hidaka, H. 1-(5-Isoquinolinesulfonyl)-2-methylpiperazine (H-7) is a
1077 selective inhibitor of protein kinase C in rabbit platelets. *Biochem Biophys Res*
1078 *Commun* **125**, 258-264, doi:10.1016/s0006-291x(84)80362-9 (1984).
1079 146 Tamaoki, T. *et al.* Staurosporine, a potent inhibitor of phospholipid/Ca++dependent
1080 protein kinase. *Biochem Biophys Res Commun* **135**, 397-402, doi:10.1016/0006-
1081 291x(86)90008-2 (1986).
1082 147 Toullec, D. *et al.* The bisindolylmaleimide GF 109203X is a potent and selective
1083 inhibitor of protein kinase C. *J Biol Chem* **266**, 15771-15781 (1991).
1084 148 Mackay, H. J. & Twelves, C. J. Targeting the protein kinase C family: are we there
1085 yet? *Nat Rev Cancer* **7**, 554-562, doi:10.1038/nrc2168 (2007).
1086 149 Roffey, J. *et al.* Protein kinase C intervention: the state of play. *Curr Opin Cell Biol*
1087 **21**, 268-279, doi:10.1016/j.ceb.2009.01.019 (2009).
1088 150 Wang, H. B., Wang, X. Y., Liu, L. P., Qin, G. W. & Kang, T. G. Tigliane
1089 diterpenoids from the Euphorbiaceae and Thymelaeaceae families. *Chem Rev* **115**,
1090 2975-3011, doi:10.1021/cr200397n (2015).
1091 151 Raghuvanshi, R. & Bharate, S. B. Preclinical and Clinical Studies on Bryostatins, A
1092 Class of Marine-Derived Protein Kinase C Modulators: A Mini-Review. *Curr Top*
1093 *Med Chem* **20**, 1124-1135, doi:10.2174/1568026620666200325110444 (2020).
1094 152 Saavedra, A. *et al.* Chelerythrine promotes Ca(2+)-dependent calpain activation in
1095 neuronal cells in a PKC-independent manner. *Biochim Biophys Acta Gen Subj* **1861**,
1096 922-935, doi:10.1016/j.bbagen.2017.01.021 (2017).
1097 153 Dar, M. I. *et al.* Rottlerin is a pan phosphodiesterase inhibitor and can induce
1098 neurodifferentiation in IMR-32 human neuroblastoma cells. *Eur J Pharmacol* **857**,
1099 172448, doi:10.1016/j.ejphar.2019.172448 (2019).
1100 154 Meyer, T. *et al.* A derivative of staurosporine (CGP 41 251) shows selectivity for
1101 protein kinase C inhibition and in vitro anti-proliferative as well as in vivo anti-tumor
1102 activity. *Int J Cancer* **43**, 851-856, doi:10.1002/ijc.2910430519 (1989).
1103 155 Kayser, S., Levis, M. J. & Schlenk, R. F. Midostaurin treatment in FLT3-mutated
1104 acute myeloid leukemia and systemic mastocytosis. *Expert Rev Clin Pharmacol* **10**,
1105 1177-1189, doi:10.1080/17512433.2017.1387051 (2017).

1106 156 Graves, P. R. *et al.* The Chk1 protein kinase and the Cdc25C regulatory pathways are
1107 targets of the anticancer agent UCN-01. *J Biol Chem* **275**, 5600-5605,
1108 doi:10.1074/jbc.275.8.5600 (2000).
1109 157 Bourhill, T., Narendran, A. & Johnston, R. N. Enzastaurin: A lesson in drug
1110 development. *Crit Rev Oncol Hematol* **112**, 72-79,
1111 doi:10.1016/j.critrevonc.2017.02.003 (2017).
1112 158 Carducci, M. A. *et al.* Phase I dose escalation and pharmacokinetic study of
1113 enzastaurin, an oral protein kinase C beta inhibitor, in patients with advanced cancer.
1114 *J Clin Oncol* **24**, 4092-4099, doi:10.1200/jco.2005.05.3447 (2006).
1115 159 Clamp, A. & Jayson, G. C. The clinical development of the bryostatins. *Anticancer*
1116 *Drugs* **13**, 673-683, doi:10.1097/00001813-200208000-00001 (2002).
1117 160 Ku, G. Y. *et al.* Phase II trial of sequential paclitaxel and 1 h infusion of bryostatin-1
1118 in patients with advanced esophageal cancer. *Cancer Chemother Pharmacol* **62**, 875-
1119 880, doi:10.1007/s00280-008-0677-y (2008).
1120 161 Panizza, B. J. *et al.* Phase I dose-escalation study to determine the safety, tolerability,
1121 preliminary efficacy and pharmacokinetics of an intratumoral injection of tigilanol
1122 tiglate (EBC-46). *EBioMedicine* **50**, 433-441, doi:10.1016/j.ebiom.2019.11.037
1123 (2019).
1124 162 Decatur, C. L. *et al.* Driver Mutations in Uveal Melanoma: Associations With Gene
1125 Expression Profile and Patient Outcomes. *JAMA Ophthalmol* **134**, 728-733,
1126 doi:10.1001/jamaophthalmol.2016.0903 (2016).
1127 163 Gresset, A., Sondek, J. & Harden, T. K. The phospholipase C isozymes and their
1128 regulation. *Subcell Biochem* **58**, 61-94, doi:10.1007/978-94-007-3012-0_3 (2012).
1129 164 US National Library of Medicine. ClinicalTrials.gov.
1130 https://clinicaltrials.gov/ct2/show/NCT02273219?term=aeb071&draw=2&rank=1.
1131 (2018).
1132 165 Skvara, H. *et al.* The PKC inhibitor AEB071 may be a therapeutic option for
1133 psoriasis. *J Clin Invest* **118**, 3151-3159, doi:10.1172/jci35636 (2008).
1134 166 Piperno-Neumann, S. *et al.* Genomic Profiling of Metastatic Uveal Melanoma and
1135 Clinical Results of a Phase I Study of the Protein Kinase C Inhibitor AEB071. *Mol*
1136 *Cancer Ther*, doi:10.1158/1535-7163.Mct-19-0098 (2020).
1137 167 US National Library of Medicine. ClinicalTrials.gov.
1138 https://clinicaltrials.gov/ct2/show/NCT03947385. (2020).
1139 168 US National Library of Medicine. ClinicalTrials.gov.
1140 https://clinicaltrials.gov/ct2/show/NCT02601378. (2020).
1141 169 Kapiteijn, E. *et al.* Abstract CT068: A Phase I trial of LXS196, a novel PKC inhibitor
1142 for metastatic uveal melanoma. *Cancer Research* **79**, CT068-CT068,
1143 doi:10.1158/1538-7445.Am2019-ct068 (2019).
1144 170 Robertson, M. J. *et al.* Phase II study of enzastaurin, a protein kinase C beta inhibitor,
1145 in patients with relapsed or refractory diffuse large B-cell lymphoma. *J Clin Oncol*
1146 **25**, 1741-1746, doi:10.1200/jco.2006.09.3146 (2007).
1147 171 US National Library of Medicine. ClinicalTrials.gov.
1148 https://clinicaltrials.gov/ct2/show/NCT03263026. (2020).
1149 172 US National Library of Medicine. ClinicalTrials.gov.
1150 https://clinicaltrials.gov/ct2/show/NCT03492125. (2019).
1151 173 US National Library of Medicine. ClinicalTrials.gov.
1152 https://clinicaltrials.gov/ct2/show/NCT01737502. (2020).
1153 174 Erdogan, E. *et al.* Aurothiomalate inhibits transformed growth by targeting the PB1
1154 domain of protein kinase Ciota. *J Biol Chem* **281**, 28450-28459,
1155 doi:10.1074/jbc.M606054200 (2006).

175 Suzuki, A. & Ohno, S. The PAR-aPKC system: lessons in polarity. *J Cell Sci* **119**, 979-987, doi:10.1242/jcs.02898 (2006).

176 Drummond, M. L. & Prehoda, K. E. Molecular Control of Atypical Protein Kinase C: Tipping the Balance between Self-Renewal and Differentiation. *J Mol Biol* **428**, 1455-1464, doi:10.1016/j.jmb.2016.03.003 (2016).

177 Etienne-Manneville, S. From signaling pathways to microtubule dynamics: the key players. *Curr Opin Cell Biol* **22**, 104-111, doi:10.1016/j.ceb.2009.11.008 (2010).

178 Murray, N. R. & Fields, A. P. Atypical protein kinase C iota protects human leukemia cells against drug-induced apoptosis. *J Biol Chem* **272**, 27521-27524, doi:10.1074/jbc.272.44.27521 (1997).

179 Weichert, W., Gekeler, V., Denkert, C., Dietel, M. & Hauptmann, S. Protein kinase C isoform expression in ovarian carcinoma correlates with indicators of poor prognosis. *Int J Oncol* **23**, 633-639 (2003).

180 Zhang, L. *et al.* Integrative genomic analysis of protein kinase C (PKC) family identifies PKCiota as a biomarker and potential oncogene in ovarian carcinoma. *Cancer Res* **66**, 4627-4635, doi:10.1158/0008-5472.Can-05-4527 (2006).

181 Eder, A. M. *et al.* Atypical PKCiota contributes to poor prognosis through loss of apical-basal polarity and cyclin E overexpression in ovarian cancer. *Proc Natl Acad Sci U S A* **102**, 12519-12524, doi:10.1073/pnas.0505641102 (2005).

182 Regala, R. P. *et al.* Atypical protein kinase C iota is an oncogene in human non-small cell lung cancer. *Cancer Res* **65**, 8905-8911, doi:10.1158/0008-5472.Can-05-2372 (2005).

183 Li, Q. *et al.* Correlation of aPKC-iota and E-cadherin expression with invasion and prognosis of cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int* **7**, 70-75 (2008).

184 Yang, Y. L. *et al.* Amplification of PRKCI, located in 3q26, is associated with lymph node metastasis in esophageal squamous cell carcinoma. *Genes Chromosomes Cancer* **47**, 127-136, doi:10.1002/gcc.20514 (2008).

185 Scotti, M. L., Bamlet, W. R., Smyrk, T. C., Fields, A. P. & Murray, N. R. Protein kinase Ciota is required for pancreatic cancer cell transformed growth and tumorigenesis. *Cancer Res* **70**, 2064-2074, doi:10.1158/0008-5472.Can-09-2684 (2010).

186 Ishiguro, H. *et al.* aPKClambda/iota promotes growth of prostate cancer cells in an autocrine manner through transcriptional activation of interleukin-6. *Proc Natl Acad Sci U S A* **106**, 16369-16374, doi:10.1073/pnas.0907044106 (2009).

187 Ma, L. *et al.* Control of nutrient stress-induced metabolic reprogramming by PKCzeta in tumorigenesis. *Cell* **152**, 599-611, doi:10.1016/j.cell.2012.12.028 (2013).

188 Reina-Campos, M., Diaz-Meco, M. T. & Moscat, J. The Dual Roles of the Atypical Protein Kinase Cs in Cancer. *Cancer Cell* **36**, 218-235, doi:10.1016/j.ccell.2019.07.010 (2019).
A detailed commentary on aPKC in cancer models.

189 Reina-Campos, M. *et al.* Increased Serine and One-Carbon Pathway Metabolism by PKCλ/ι Deficiency Promotes Neuroendocrine Prostate Cancer. *Cancer Cell* **35**, 385-400.e389, doi:10.1016/j.ccell.2019.01.018 (2019).

190 Nakanishi, Y. *et al.* Simultaneous Loss of Both Atypical Protein Kinase C Genes in the Intestinal Epithelium Drives Serrated Intestinal Cancer by Impairing Immunosurveillance. *Immunity* **49**, 1132-1147.e1137, doi:10.1016/j.immuni.2018.09.013 (2018).

191 Huang, X. *et al.* An atypical protein kinase C (PKC zeta) plays a critical role in lipopolysaccharide-activated NF-kappa B in human peripheral blood monocytes and macrophages. *J Immunol* **182**, 5810-5815, doi:10.4049/jimmunol.0804073 (2009).

192 Murray, N. R. *et al.* Protein kinase Ciota is required for Ras transformation and colon carcinogenesis in vivo. *J Cell Biol* **164**, 797-802, doi:10.1083/jcb.200311011 (2004).

193 Regala, R. P. *et al.* Atypical protein kinase C{iota} is required for bronchioalveolar stem cell expansion and lung tumorigenesis. *Cancer Res* **69**, 7603-7611, doi:0008-5472.CAN-09-2066 [pii] 10.1158/0008-5472.CAN-09-2066 (2009).
Demonstration that knockout of PKCι suppresses lung tumour formation on switching on G12D mutant K-Ras expression.

Acknowledgements. We are grateful to Alan Fields and Mary Reyland for commenting on the manuscript. We also acknowledge support from the Francis Crick Institute, which receives its core funding from Cancer Research UK [FC001130]; UK Medical Research Council [FC001130]; Wellcome Trust [FC001130]. ML is supported by the National Institute for Health Research, the University College London Hospitals Biomedical Research Centre (no grant numbers apply)

Author contributions PJP contributed to all aspects of the article. VC, ML and PC contributed to researching data for the article and reviewing and/or editing the manuscript before submission. SB, MC, JJTM, SM, NM, TNS, and LW contributed to writing the article and reviewing and/or editing it before submission.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Peer review information

Nature Reviews Cancer thanks M. G. Kazanietz, M. Leitges and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Competing interests

The authors declare no competing interests.

Supplementary information

Supplementary information is available for this paper at https://doi.org/10.1038/s415XX-XXX-XXXX-X

Related links

Tigilanol tiglate: https://www.ema.europa.eu/en/medicines/veterinary/EPAR/stelfonta

cBioPortal: https://www.cbioportal.org

FDA orphan status was designated to bryostatin: https://www.accessdata.fda.gov/scripts/opdlisting/oopd/listResult.cfm

1244 Trials investigating PKC412 in AML and MDS:
1245 https://clinicaltrials.gov/ct2/results?cond=AML&term=PKC412
1246 https://clinicaltrials.gov/ct2/results?cond=MDS&term=PKC412
1247
1248 **Figure Legends**
1249
1250 Figure 1. *Domain Organisation, activation and downregulation pathways for the PKC*
1251 *Family.* A. Domain organisation and activation. For cPKCs, calcium increases
1252 membrane association through C2 domains, promoting C1A/B sensing and
1253 engagement of diacylglycerol (DAG) at the membraneThis leads to dissociation of
1254 the pseudosubstrate site from the catalytic domain permitting substrate engagement.
1255 For the nPKCs, C2 domain interactions with partner proteins recruit isoforms to the
1256 membrane. Membrane occupancy enables efficient C1A/B-mediated DAG
1257 monitoring and binding, pseudosubstrate release, enablingcatalysis. Some nPKCs
1258 are subject to caspase-dependent V3 domain cleavage, leading to kinase activation.
1259 For aPKC isoforms, Par6 interacts with the N-terminal PB1 domain enabling
1260 membrane recruitment through Par6-Cdc42 binding. The single C1 domains of
1261 aPKCs do not bind DAG but have non-specific membrane binding activity, and
1262 possibly enabling release of the pseudosubstrate site and activation of kinase
1263 function. aPKCs are held in membrane compartments by other proteins in addition to
1264 these core functions. For the PKNs, extrapolating from the PKN2 behaviour, the
1265 cytosolic autoinhibited dimer is activated by recruitment to the membrane through its
1266 HR1a/b domains at the N-terminus. These make a bivalent contact with
1267 isoprenylated, GTP bound (active), Rac or Rho family proteins at the membrane
1268 leading to dissociation of the dimer And activation The additional input from the C2
1269 domain is likely to be through supplementary membrane/partner interactions.
1270 B. Activation-induced degradation pathways for PKC. In some cell types,
1271 degradation proceeds through the loss of nucleotide pocket occupation through ATP
1272 or ADP, altered conformation of the kinase domain and efficient dephosphorylation.
1273 This is followed by ubiquitination and proteasomal degradation. Alternatively,
1274 activation induced endocytosis leads to degradation in lysosomes (can facilitate
1275 ubiquitination-dependent degradation, possibly involves dephosphorylation).
1276
1277 Figure 2. *Biomarkers of PKC action and inaction*

Panel A illustrates the PKC isoform attributes that have been considered biomarkers to inform on roles in pathological settings. These are: genomic alterations, transcriptional and translational changes, the extent of priming phosphorylation, subcellular localisation, complex formation, conformation, self phosphorylation (autophosphorylation) and substrate phosphorylation (transphosphorylation). There is richness in the concentration data (mRNA in particular) but paucity of functional data (eg substrate phosphorylation) and development of this latter, functional information would be of significant value. The value of priming phosphorylation data as a PKC functional readout is doubtful.

In panel B, the sites of penetrant mutations in the kinase domains of PKCα and PKCβ are indicated in the context of their solved kinase domain structures, alongside the hotspot but infrequent kinase domain mutation in the PKCι substrate docking motif.

Figure 3. *Cell Cycle controls and PKC.* A. A variety of growth promoting stimuli acting through their cell surface receptors (GPCRs, tyrosine kinase associated/linked receptors), can act on different members of the PLC gene family to trigger signalling cascades through PKC family members. These events are circumstantially linked to entry into cell cycle, i.e. a G0 to G1 transition and early G1 progression. Ligands engaging GPCRs (7-transmembrane receptors) act through activated heterotrimeric G-protein subunits ($G\alpha_q$.GTP, $G\alpha_{11}$.GTP, βγ) to activate members of the phospholipase Cβ class of phosphodiesterases, responsible for the hydrolysis of $PI4,5P_2$ and the generation of IP_3 and DAG; the latter activating PKC isoforms. For ligands acting on receptor tyrosine kinases (RTK) or receptor linked tyrosine kinases (RLTK), SH2 domain-dependent recruitment and phosphorylation of PLCγ proteins will also lead to IP_3 and DAG production and consequent PKC activation. B. During G1 progression, and entry into and progression through S-phase, there are a series of interconnected events that sequentially cause activation of Cyclin/CDK complexes. These events have been reported to be influenced by PKC isoforms in various cellular settings, including: Cyclin D expression, PKCα,β,δ,ε,η,ζ; CDK4,6 activity, PKCα; Cyclin E expression, PKCδ,ε,η,ι; CDK2 activity, PKCα,δ,η; CDK inhibitor (CIP/KIP) expression, PKCα,β,δ,ε,η,θ,ζ; Cyclin A expression, PKCδ; cdc25

activity, PKCβ. C. Progression through M-Phase is impacted by PKCβ and PKCε as indicated.

BOX 1 The DAG non-responsive, atypical PKC isoforms in cancer.

aPKC isoforms, which include PKCζ and PKCι, are involved in a wide range of cellular functions including the maintenance of polarity, proliferation, cytoskeletal functions, apoptosis and growth factor signalling[175-178]. Unsurprisingly, there are numerous reports associating aPKC deregulation to cancer.

Patient tumour profiling, while of uncertain interpretation for PKC (see text), has generally implicated PKCι as pro-oncogenic. Chromosome 3q26, where the *PRKCI* gene is located, is commonly amplified in human cancer and both the transcript and protein have been inversely correlated with patient outcomes[179-186]. Infrequent, hot-spot mutation of the gene region encoding the polarity-required substrate docking site in PKCι has also been observed[33] (Figure 2B). By contrast PKCζ has been implicated as a tumour suppressor in colon cancer, correlating with reduced expression[187].

In several cancer models, a body of literature has accumulated from the Moscat laboratory indicating that PKCι has a suppressive role in tumourigenesis (recently reviewed[188]). In prostate cancer cell lines PKCι knockout induced a neuroendocrine phenotype, increased proliferation and tumour growth, an effect mediated by increased serine biosynthesis[189]. Combined knockout of *Prkcz* (encoding PKCζ) and *Prkci* in the mouse intestine led to the formation of serrated colon tumours with impaired IFNγ expression and decreased CD8+ infiltration suggestive of deficient immune surveillance[190]. In studies of human peripheral blood mononuclear cells, PKCζ was shown to modulate the activation of NFκB in monocytes and macrophages[191] with an anticipated impact on the behaviour of the tumour niche. Juxtaposed to these experimental observations is the requirement for PKCι in mutant-RAS induced lung, colon and pancreatic tumours[185,192,193] and the *ex vivo* reversal of RAS-transformed phenotype with PKCι-selective inhibition[61].

For aPKC isoforms the contrasting literature prescribes the need for direct insight into the roles of aPKC in tumour growth in patients through application of biomarkers informing on aPKC action/inaction.

Glossary

Borealin is one of the components of the Chromosome Passenger Complex (CPC), alongside INCENP and survivin, regulating the localisation and activity of the co-associated Aurora B which completes the CPC.

Bryostatins are trace bioactive cyclic polyketides first identified in marine bryozoan bugula neritina; they likely originate from the symbiont B. neritina.

Conformer is used in a generic manner to indicate a particular protein conformation.

Diacylglycerol. (DAG). DAG is a neutral lipid component of membranes, serving in the biosynthesis of more complex lipids and as a signalling lipid.

Epoxytiglianes are bioactive compounds originally identified in the kernels of *F. picrosperma* fruits and are related to phorbol esters (tigliane family of diterpenes).

MMTV-ErbB2 transformation model is a transgenic mouse model with expression of the receptor tyrosine kinase ErbB2 under the control of the mammary gland selective MMTV promoter.

Private mutations refer to those rare mutations that appear only once in cancer genomes, i.e. are private to that patient.

Topoisomerase 2α is one of two genes in mammals that catalyse the resolution of intertwined, catenated DNA, through double strand cutting, strand passage and religation reactions.

Supplementary video 1. **An animated life cycle of PKCε.**

A scaled structural model of PKCε is shown and the domains noted. As a primary translation product, the kinase lacks modification and the initial step in its life cycle is phosphorylation by the upstream kinases TORC2 and PDK1. This is illustrated as taking place at the plasma membrane (no details of upstream co-recruitment are shown). Stable phosphorylation is seen to proceed under conditions of nucleotide pocket occupation (green, ATP molecule) since it has been shown that pocket occupation is key to prevent dephosphorylation (as originally shown for PKCδ (REF 1) and recently confirmed with inhibitors *in vitro* and in cells[2,3]). Fully phosphorylated PKCε is active and can cycle through substrate and product binding and release;

1389 here illustrated by ATP/ADP and a scale model of the AuroraB kinase a PKCε
1390 substrate (see text). The loss of membrane interaction leads to a switch in
1391 conformation, where the pseudosubstrate site occupies the substrate binding pocket
1392 of the kinase domain yielding an autoinhibited, closed conformer. In this
1393 conformation the phosphorylations remain stable but the protein is inactive. Diffusion
1394 and sensing of DAG in the membrane allows the kinase to re-establish its open
1395 active conformation. In this state, the turnover of ATP can lead to the apo form
1396 (nucleotide unbound form) being dephosphorylated and this form of the protein is
1397 susceptible to degradation; see Figure 1B. Animation by Phospho;
1398 https://www.phospho.co.uk/.
1399 References:
1400 1 Gould, C. M. *et al.* Active site inhibitors protect protein kinase C from
1401 dephosphorylation and stabilize its mature form. *J Biol Chem* **286**, 28922-
1402 28930, doi:10.1074/jbc.M111.272526 (2011).
1403 2 Srivastava, J., Goris, J., Dilworth, S. M. & Parker, P. J. Dephosphorylation of
1404 PKCdelta by protein phosphatase 2Ac and its inhibition by nucleotides. *FEBS*
1405 *Lett* **516**, 265-269 (2002).
1406 3 Cameron, A. J., Escribano, C., Saurin, A. T., Kostelecky, B. & Parker, P. J. PKC
1407 maturation is promoted by nucleotide pocket occupation independently of
1408 intrinsic kinase activity. *Nature structural & molecular biology* **16**, 624-630,
1409 doi:10.1038/nsmb.1606 (2009).
1410
1411
1412
1413 Toc blurb
1414 This Review discusses protein kinase C (PKC) isoforms in cancer, in particular
1415 focusing on their functional properties in the context of tumour suppression or
1416 promotion, target validation, PKC pharmacology and therapeutic exploitation.
1417
1418