Review

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Protein kinase C-α and the regulation of diverse cell responses

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Abstract: Protein kinase C (PKC) comprises a family of lipid-sensitive enzymes that have been involved in a broad range of cellular functions. PKC-α is a member of classical PKC with ubiquitous expression and different cellular localization. This unique PKC isoform is activated by various signals which evoke lipid hydrolysis, after activation it interacts with various adapter proteins and is localized to specific cellular compartments where it is devised to work. The universal expression and activation by various stimuli make it a perfect player in uncountable cellular functions including differentiation, proliferation, apoptosis, cellular transformation, motility, adhesion and so on. However, these functions are not intrinsic properties of PKC-α, but depend on cell types and conditions. The activities of PKC-α are managed by the various pharmacological activators/inhibitors and antisense oligonucleotides. The aim of this review is to elaborate the structural feature, and provide an insight into the mechanism of PKC-α activation and regulation of its key biological functions in different cellular compartments to develop an effective pharmacological approach to regulate the PKC-α signal array.

Keywords: apoptosis; cancer; cell proliferation; cell signaling; signal transduction.

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Introduction

Protein kinase C (PKC) is a family of lipid-sensitive serine/threonine protein kinases that regulate various cellular functions including proliferation, differentiation, migration, adhesion and apoptosis. The members of the PKC isoform belong to the AGC family of protein kinases with certain basic structural features. Based on structural differences that dictate their ability to bind with cofactor at the regulatory domain for optimal activation, the PKC family with 10 members can be divided into three subfamilies. Conventional or classical PKC isoform (cPKCs: α, βI, βII and γ) comprises a C1 domain (C1A and C1B) with two cysteine-rich zinc fingers that function as the binding site for diacylglycerol (DAG) and phorbol 12-myristate 13-acetate (PMA) and a C2 domain which functions as Ca^{2+}-dependent membrane-binding modules (1, 2). Novel PKCs (nPKCs: δ, θ, ϵ, η) also have twin C1 and C2 domains (but the orientation of these domains is inverted with respect to cPKC). It is important to note that the C2 domain lacks the Ca^{2+}-coordinating residues in the nPKC isoform. The differences in the C2 domain underlie the distinct pharmacology of the cPKC and the nPKC isoforms. Atypical PKC (aPKCs: ζ, λ/ι) contains an atypical C1 domain and a PB1 (Phox and Bem1) domain. The aPKC's C1 domain is responsible for binding with phosphatidylinositol 3,4,5-trisphosphate (PIP_3) or ceramide for activation while the PB1 mediates protein-protein interaction with other PB1-containing scaffold proteins including p62, MEK5 and PAR6 (3, 4). Recently, it has been found that PKC-α plays an important role in the development of several major diseases including cancer (5, 6), and there are multiple approaches to target PKC-α pharmacologically (7), therefore, it is essential to gain an elementary knowledge of how PKC-α contributes to implication in biological events. The present article will focus on the structural features, activation, translocation and biological functions of PKC-α.

Structure of PKC-α

PKC-α is a member of cPKC, which consists of 672 amino acids, ubiquitously expressed in all tissues, in contrast to other PKC isoforms whose expression is restricted in particular tissues (8). Similar to the other cPKC isoforms, PKC-α shares the same architecture: a highly conserved kinase domain at the C-terminal linked by a flexible hinge segment to a variable regulatory domain at the N-terminal (Figure 1). A regulatory module consists of a C1 domain and a C2 domain, which confer sensitivity to second messengers DAG and Ca^{2+}, respectively, leading to kinase activation. The globular C1 domain stereospecifically binds with phosphatidylserine and also acts as a binding site for phorbol-esters while the C2 domain has the ability to bind with anionic lipids in the presence of Ca^{2+} (9). The highly conserved kinase domain comprises a smaller N-terminal ATP-binding loop (C3) made up of β-sheets and contains the consensus GXGXXG sequence (a structural hallmark of protein kinases and nucleotide binding proteins) and an invariant lysine residue which remodels the enzyme for phosphoryl transfer. The C-terminal lobe of the kinase domain comprises mainly α-helical sheets and contains the activation loop segment that positions magnesium and peptide substrate for catalysis. A highly conserved hydrophobic amino acid ‘gatekeeper residue’ established in the sequence, which links the two lobes of the kinase domain, controls the preexisting cavity in the ATP-binding pocket. A genetic approach to creating mutations in gatekeeper residues has been used to modify kinases that are uniquely sensitive to certain unnatural inhibitor or activator ATP analogs and provide a powerful approach to compromise the substrate of individual kinases in cells (10). As the hinge region has been considered as a caspase dependent cleavage target site in response to a range of apoptogenic stimuli and is also involved in protein-protein interaction, cleavage of hinge region results in the release of catalytic domain fragment (1, 11).

Activation and translocation of PKC-α

PKC-α activation is mediated by a series of ordered, tightly coupled phosphorylation events, cofactor recruitment and binding with docking proteins. PKC-α first phosphorylated at threonine-497 residue in the highly conserved activation loop of the kinase domain catalyzed by 3-phosphoinositide-dependent protein kinase-1 (PDPK-1), an essential event to generate a catalytically competent enzyme. Phosphorylation at this activation loop triggers additional autophosphorylation at Thr638 and Ser657 at the hydrophobic C-terminal site (12, 13). Phosphorylation at Thr497 in the activation loop is necessary for the complete activation of the PKC-α. In the absence of phosphorylation at this site, PKC-α is unable to signal and accumulates as in the detergent-insoluble fraction of the cell. Mutational analysis revealed that phosphorylation at Thr-638 works to stabilize PKC-α, provide thermal stability and make PKC-α phosphatase resistant (14). Furthermore, recent studies introduce two new factors: heat shock protein 90 (Hsp90) and the mammalian target of rapamycin complex 2 (mTORC2), which are essential for priming phosphorylation of cPKC (Figure 2) (15, 16). Previous studies suggested that maturation of cPKC required integrity of chaperone Hsp90 and co-chaperone Cdc37. This complex binds to a conserved PXXP motif in the kinase domain of PKC; a molecular clamp to facilitate phosphorylation at hydrophobic motif. Disorganization in the

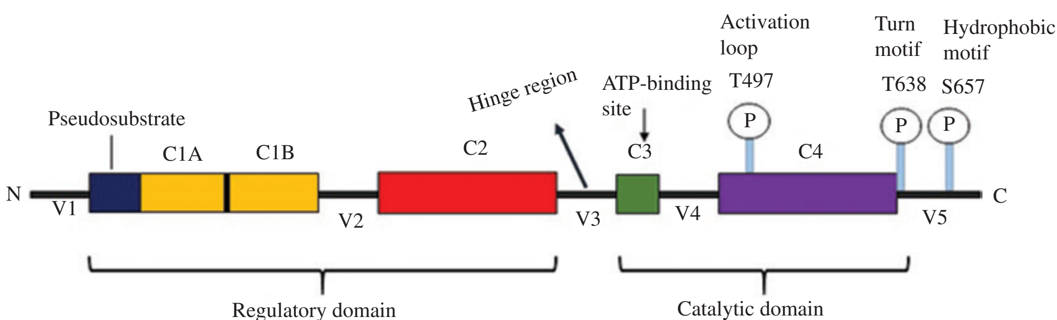

Figure 1: Diagrammatic representation of the domain composition of PKC-α showing four conserved domains and five variable domains. Conserved domains are: C1 (yellow) responsible for DAG binding, Ca^{2+}-coordinating domain C2 (red). PKC-α comprises conserved catalytic domains C3 (green) and C4 (purple) recognized as ATP and the substrate-binding site, respectively, and pseudosubstrate (blue) which maintains the PKC-α in an inactive conformation. V3 acts as a hinge region which connects the regulatory domain to the catalytic domain and proteolytically hydrolyzes during apoptosis by caspases.

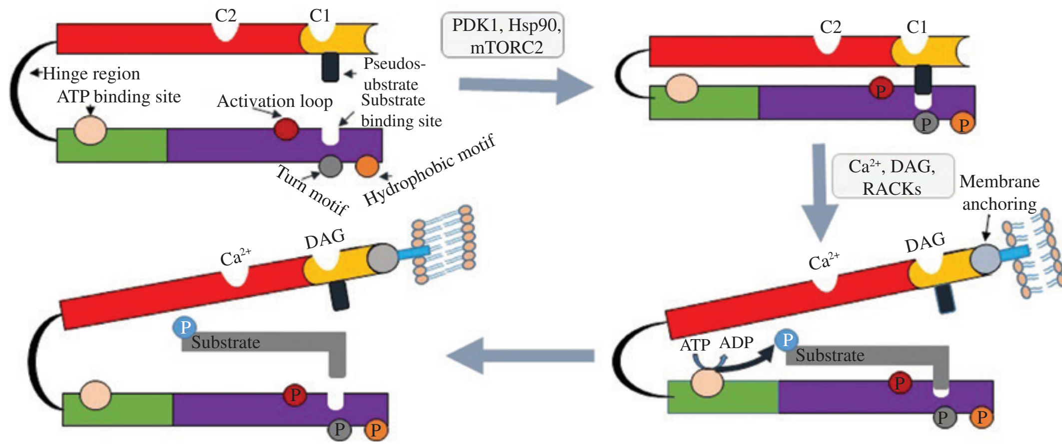

Figure 2: Newly synthesized PKC-α shows an open confirmation in which the substrate-binding cavity is unmasked and allows phosphorylation by PDPK-1 at the activation loop.
Activation loop phosphorylation is essential to generate catalytically competent enzyme. This phosphorylation triggers the autophosphorylation at the turn motif and the hydrophobic motif assisted by Hsp90 and mTORC2. Now phosphorylated PKC-α localizes to the cytosol in an inactive condition due to the binding of the pseudosubstrate to the substrate-binding site. The signals that evoke lipid hydrolysis recruit PKC-α to membrane via PIP2 interaction where C2 domain binds with Ca^{2+} and C1 domain binds with DAG. This binding assists in unmasking the substrate-binding cavity and allows to phosphorylate the appropriate substrate for further downstream processing. The localization of active PKC-α is assisted by adapter protein (RACKs) to particular cellular compartments.

integrity of Hsp90 and Cdc37 likely plays a role in pathophysiological states. A recent finding that the mutation in the first proline residue of conserved PXXP motif in the kinase domain leads to the formation of glioblastoma supports this notion. Phosphorylation of turn motif is regulated by mTORC2. Cells lacking mTORC2 have significantly low levels of PKC because unphosphorylated PKC becomes unstable and proteolytically degraded. Regulation of turn motif phosphorylation by mTORC2 is complicated to define. Possibly mTORC2 may activate other kinases or positioning nascent PKC-α for phosphorylation (17).

The phosphorylated PKC-α resides in the cytoplasm of a cell in an inactive form due to the presence of an N-terminal autoinhibitory pseudosubstrate sequence that occupies the active site within the catalytic domain rendering it unable to bind and phosphorylate the substrate (Figure 2). Upon activation of a specific receptor within the cell, phospholipase C cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into DAG and inositol 1,4,5-trisphosphate (IP_3), a Ca^{2+} mobiliser. Ca^{2+} binds to the C2 domain and increases its affinity toward the membrane. However, once PKC-α is recruited to the membrane, it diffuses within the plane of lipid bilayer and mediates secondary C1A domain interaction with DAG which serves as an anchor to recruit PKC-α to the plasma membrane with high affinity, where it binds with phosphatidylserine, found exclusively at the cytoplasmic surface (18). Thus, binding of the cofactor enables PKC-α to bind to the plasma membrane and introduces a conformational change that facilitates the separation of an autoinhibitory substrate domain from the substrate binding site, leading to PKC-α activation (19). Recently, PKC-α spatial distribution is being studied by fluorescence resonance energy transfer (FRET) and confocal microscopy. Several researchers observed their movement by the application of fusion proteins consisting of PKC-α and the jelly fish green fluorescent protein in living cells (20). A number of anchoring or docking proteins are available which interact with PKC isoforms and regulate their intracellular localization at specialized cell compartments (21). Almost 30 years ago, Mochly-Rosen and colleagues (7) worked on an intramolecular interaction of PKC and characterized a number of scaffold proteins termed as receptors for activated C kinase (RACKs). It acts as a molecular scaffold which has the ability to bind with the PKC isozyme and relieves autoinhibition of the enzyme and localizes individual PKCs to their downstream substrates. RACK1 was the first anchoring protein discovered which binds with various PKC isoforms including PKC-α, PKC-ϵ and PKC-βII. Vinculin and talin are identified as docking proteins, which recruit PKC-α to focal contacts in REF52 cells. Caveolin binds with PKC-α and recruits PKC-α to caveolae. PKC-α co-localizes with β-1 integrin in MCF-7 cells (11). Calponin, an actin filament-associated protein, binds to the C2 domain of PKC-α and enhances autophosphorylation to increase activity in the smooth muscle cells (22). Interestingly, studies on PKC-β suggested that Hsp-70 acts as a chaperone, binds with unphosphorylated turn motif, prolonging its half-life (in the same manner as

with all cPKC isoforms) (23). Therefore, a more promising approach to target Hsp-70 will be an emerging strategy to regulate cPKC activity. The information that the PKC isoform contains a RACK-binding sequence and that these sequences play a vital role in the intramolecular interaction to maintain the enzyme in an inactive state is also a target to regulate PKC activity without altering the natural stoichiometry of the given PKC isoform. The peptide-based modulation of PKC activity is not much known and requires further studies.

Furthermore, the basal level of PKC-α and, thus, signal amplitude is maintained by an E3 ligase termed RINCK, a ring-finger domain-containing protein that mediates ubiquitination and degradation of PKC-α in breast cancer cell lines (24). Moreover, the PKC-α activity is also regulated by interaction with the DAG kinase ζ (DGK-ζ) by metabolizing locally available DAG, which would activate the PKC enzyme (25). In addition, the downregulation of cPKC-mediated signal is accomplished by the docking of peptide-prolyl-isomerase Pin1 onto the hydrophobic motif which controls the isomerization of the Phospho-Thr-Pro peptide bond from a *cis* to a *trans* confirmation of the turn motif (26).

The biological function of PKC-α

PKC-α sits at the crossroads of many signal transduction pathways and is implicated in a wide range of cellular responses (Figure 3). Explanation of the biological function of PKC-α is possible by using specific pharmacological inhibitors and activators. The implication of PKC-α in specific cellular responses is determined in a culture cell system by using wild-type and mutant-type PKC-α via the transfection method. Defining the exact role of PKC-α is more difficult due to its ubiquitous expression and involvement in myriad cellular functions. There is consistent literature available, albeit few, which reveal that PKC-α is activated by a variety of stimulators and controls major cellular functions like proliferation, differentiation, apoptosis, motility and adhesion (Figure 3) (27). It has been found that the biological responses obtained by PKC-α manipulation are based on cell types. The literature reveals that overexpression of PKC-α led to proliferation in a few cell types while it became a cause of death in others. Therefore, biological output of PKC-α depends on the place and time of activation and the nature of the substrate it acts on.

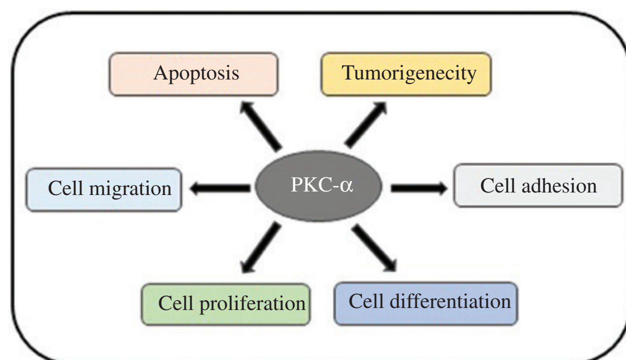

Figure 3: After activation by various stimuli, PKC-α is implicated in various cellular functions.

Cell proliferation and differentiation

The role of PKC-α in cell proliferation is very contradictory. PKC-α exhibits an anti-proliferative function in particular cell types and may function as growth-stimulatory factor in others, causing more difficulty to specify a role for PKC-α in cell proliferation (28). The first demonstration of PKC in cell growth was established in quiescent Swiss 3T3 cells. PKC-α overexpression shows a more aggressive phenotype and enhanced cell proliferation in MCF-7 human breast cancer cells (29). Studies indicate that overexpression of PKC-α is sufficient to stimulate proliferation in several cell lines like human glioma U87 cell, chick embryo hepatocytes, osteoblasts and hepatocellular carcinoma cells, among others. The proliferative effect of PKC-α includes an enhanced level of cyclin-D1 and cdk4, and an induced level of cyclin/cdk2 complex activity. PKC-α also upregulates p21cip1 and enhances cell proliferation in human glioma cells (30). Another possible target of PKC-α can also be RAf-1 which is phosphorylated and activated by PKC-α leading to the activation of extracellular signal-regulated kinase-mitogen-activated protein kinase cascade (ERK-MAPK) and resulting in enhanced proliferation of different cell types. This notion has also been supported by the finding that PKC-α mediates differentiation and proliferation of pre-T cell via ERK-MAPK pathways during T cell development. Additionally, PKC-α can also independently activate AP-1 through an interaction with GTPase and Rho of the ERK-MAPK signaling cascade at the plasma membrane. Moreover, PKC-α is involved in T cell activation in Jurkat cells (31). Importantly, $PKC^{-/-}$ mice show the defect in Th1-dependent IgG2a/b class switching and represent the value of PKC-α in Th1 cells (32). The kinase action of PKC-α has been recently identified in the phosphorylation of Akt on serine 473 in T cells (33). This phosphorylation action is further supported by studies in Th1 cells that Akt links mTORC2 to Th2 differentiation. PKC-α also affects

the cell cycle progression and enhanced proliferation in human RPE cells through the downregulation of $p27^{KIP1}$ activity (34). Recent experiments in T24 cell demonstrate that the function of a defective G1 checkpoint due to retinoblastoma (Rb) hyperphosphorylation can be activated using a pharmacological inhibitor of PKC-α. An emerging statement has been given by Ways and colleagues (29) that increased α isoform of PKC in MCF-7 breast cancer cells display an enhanced proliferative rate and confer anchorage-independent growth and unexpected alteration in cellular morphology by loss of an epithelioid appearance. They have also shown that MCF-7 cells with increased PKC-α exhibited a reduction in estrogen receptor (ER) levels and thus a decrease in the expression of ER-dependent genes. This notion is further supported by Tonetti and his colleagues (35) who observed that stable overexpression of PKC-α in T47-D breast cancer cells resulted in a significantly reduced level of ER function.

In contrast, PKC-α is identified as an anti-proliferative factor in several cell types, such as mammary epithelial cells, melanoma cells, keratinocytes and intestinal epithelial cells (28). The anti-proliferative effect of PKC-α on the cell cycle machinery includes downregulation of cyclin-D1 as well as $p27^{KIP1}$ to affect G1→S transient during the cell cycle. A recent research in intestinal epithelial cells has shown that PKC-α signaling downregulates the expression of CDK1. In other cell lines, PKC-α downregulates the expression of cdk1 via the ERK-MAPK pathway, and ROR-α-mediated mitigation of Wnt/β-catenine signaling. PKC-α overexpression results in increased doubling time in the MCF-10 human mammary epithelial cell line in comparison to the parental cell. PKC-α is closely related to the cell cycle and accumulates the hyper-phosphorylated growth inhibitory form of Rb and the induction of $p21^{cip1}$ to delay S-phase transient and induce G2/M arrest (30).

PKC-α is closely involved in the differentiation of specific types of cells, such as lens epithelial cells (36), hematopoietic progenitor cells (37), melanoma cells (38) and F9 embryonal carcinoma cells (39). PKC-α plays a vital role in macrophage development. PKC-α translocates to the nucleus in response to the stimulation of hematopoietic granulocyte macrophage-colony forming cells (GM-CFC) with macrophage colony-stimulating factor (M-CSF) during macrophage differentiation. PKC-α is also involved in the chondrification of mesenchymal cells (40). It has been postulated that the translocation of PKC-α to the nucleus during differentiation may be involved in cell cycle control or in mediating the expression of genes required for differentiation. Studies reveal that PKC-α regulates retinoic acid-mediated differentiation of F-9 embryonic carcinoma cells (41).

Apoptosis

The preparatory studies to set up the role of PKC-α in apoptotic signaling pathways is very antithetical. Overexpression of PKC-α is capable of inducing apoptosis in some cell types while preventing it in others. Therefore, it is very confusing to predict a role for PKC-α in apoptosis signaling. This statement is based on the finding that PKC-α is often proteolytically hydrolyzed by caspases, but it is activated in several cell types during apoptosis, e.g. in human prostate cancer (41). According to the currently available literature PKC-α negatively regulates apoptosis. Several cell lines, including glioma cells (42) and endothelial cells (43), undergo apoptosis as a result of cellular PKC-α depletion by using pharmacological inhibitors or antisense oligonucleotides. The expression pattern of PKC-α also reveals its role as an anti-apoptotic factor. Due to wild-type PKC-α transfection, cells became resistant to apoptosis (44). Anti-apoptotic activity of PKC-α is also observed in salivary epithelial cells where loss of PKC-α results in the activation of the PKC-δ-dependent apoptotic pathway, while in COS cells the loss of PKC-α activity leads to the downregulation of expression of Bcl-2 (31). The procedure by which PKC-α prevents apoptosis is not clearly understood. One of the possible targets that has been considered is the anti-apoptotic Bcl-2 proteins. PKC-α co-localized with Bcl-2 in the mitochondrial membrane in the HL-60 cell line; this demonstrates that PKC-α is associated with Bcl-2. Several studies have demonstrated that PKC-α phosphorylates Bcl-2 on Ser70 and stabilizes its function to inhibit apoptosis (Figure 4) (45). Another possible target that has been recognized for PKC-α is ser/thr protein kinase Raf-1. Raf-1 has been known to implicate in the cell survival pathway PKB/Akt through a PKC-α-dependent mechanism (46). PKC-α phosphorylates and activates Raf-1, and after phosphorylation Raf-1 is believed to phosphorylate and inactivate the pro-apoptotic protein BAD in the mitochondrial membrane and prevent apoptosis (Figure 4). A recent work has detected the involvement of PKC-α in mitochondrial-dependent apoptosis of breast cancer cells. Deka and his colleagues (47) have shown that the alkyl cinnmates caused the loss of mitochondrial membrane potential, release of cytochrome c, activation of caspases, genomic DNA fragmentation and induced oxidative stress via interfering the PKC-α translocation in MDAMB-231 cells. The activity of PKC-α is inhibited through phosphatase PP2A (48) in Jurkat cells during apoptosis. This finding is further supported by evidence that overexpression of PKC-α suppresses mitochondrial PP2A activity. Moreover, antisense oligonucleotide-mediated downregulation of

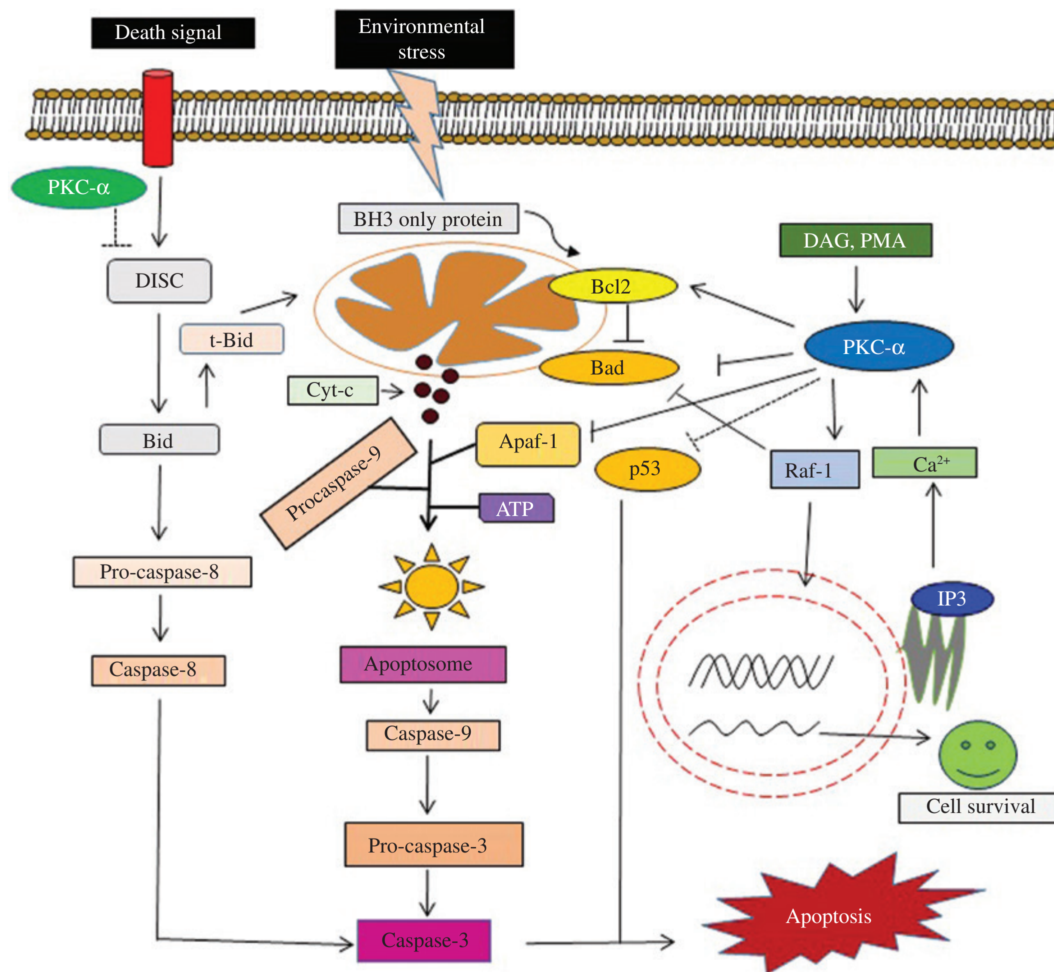

Figure 4: Possible involvement of PKC-α in the intrinsic and extrinsic apoptotic pathways.
PKC-α-mediated regulation of apoptosis is partly known. During apoptosis, PKC-α phosphorylates and enhances the activity of anti-apoptotic proteins while downregulating the activity of pro-apoptotic proteins. Further, PKC-α regulates the formation of apoptosome by inhibiting Apaf-1 activity. Another possible target of PKC-α is Raf-1, which promotes cell survival by activating the ERK-MAPK pathway. PKC-α may also prevent apoptosis by modulating the activity of death receptor components and death-inducing signaling complex (DISC) formation, but the mechanism is not determined. However, PKC-α-regulated p53-dependent apoptosis is poorly understood and needs further investigation.

PKC-α expression results in the induction of p53 and apoptosis. The above study reveals that PKC-α prevents cells from apoptosis by blockage of p53 induction, but the actual relationship between PKC-α and p53 is not understood and needs further investigation to decipher the underlying mechanism.

On the other hand, there is scant literature which could introduce the PKC-α as a pro-apoptotic factor in few cell types. PMA-induced activation of PKC-α promoted the death of human gastric cancer cell lines. In p53-null HL-60 human leukemia cells, PKC-α induces apoptosis in response to the treatment of camptothecin. PKC-α is also involved in the phosphorylation of nuclear Lamin-b and causes abrogation of the nucleus. PKC-α acts as a pro-apoptotic factor in LNCaP prostate cancer cells (11).

Tumorigenicity

PKC-α has been implicated in neoplastic changes by regulating the expression of factors of key biological processes such as cell division, apoptosis, proliferation, invasion, migration and anticancer drug resistance through their direct or indirect interaction with various cellular signaling pathways. PKC-α suppresses the activity of anti-cancer-associated signals such as the Bax subfamily and cascades of caspase and stimulates survival-associated pathways like ERK, phosphatidylinositol-3-kinase (PI3K)/Akt or mTOR. However, the carcinogenic action of PKC-α largely depends on the type of cells because few literatures are also available which explain that overexpression of PKC-α reduces cancer in several cancer cells e.g. colon

cancer (49). However, overexpression of PKC-α is directly related to the progression of cancer, and therefore, it can be used as a diagnostic or therapeutic tool in various cell lines (50, 51). PKC-α is involved in proliferation of bladder cancer cell lines 5637 and T24. This notion has been further proved by inhibition of PKC-α by Go6976 resulting in cell cycle arrest in the Go/G1 phase and reducing cell migration and invasion (52). The elevated level of PKC-α in human breast cancer cells shows a more aggressive metastatic phenotype and tumorigenicity in nude mice (29). In breast cancer cells, PKC-α controls cell proliferation by activating ERK and telomerase (53, 54). Furthermore, PKC-α plays a role in metastasis of breast cancer cells by upregulating the activity of matrix metalloproteinase (MMP-9) (55). A recent research has suggested that PKC-α activates Notch-4, in particular, in both *in vitro* as well as *in vivo* conditions through activator protein 1 (AP1). In this way, PKC-α promotes estrogen-independent growth and activates signaling pathways, making cells endocrine-resistant and chemoresistant. Therefore, Notch-4 inhibitors should be an emerging therapeutic regime to control endocrine-resistant breast cancer (56). In addition, overexpression of PKC-α in breast cancer cells inhibits heregulin-induced apoptosis by upregulation of Bcl-2 and the downregulation of caspase-7. PKC-α also takes part in the development of multidrug resistance in various cell lines through phosphorylation and activation of p-glycoprotein, a membrane-bound efflux pump (57). A point mutation Asp294Gly found in the V3 region of PKC-α was identified in human thyroid and pituitary tumors (58). In glioma cells, PKC-α is required for the activation of an ERK1/2 pathway for cell proliferation. Moreover, it also takes part as a signaling intermediate between mTOR and EGFR and leads to an Akt-independent proliferation of glioma cells (59). Apart from this PKC-α is also involved in proliferation, differentiation, survival and metastasis and acts as an anti-apoptotic agent in melanoma cells.

In context, PKC-α acts as a vital factor which directly or indirectly implicates in the progression of tumors of various cell types, e.g. head and neck cancer, hepatocellular carcinoma, lung cancer, myeloid and lymphocytic leukemia, ovarian cancer, pancreatic cancer, renal cell carcinoma and thyroid cancer (60). PKC-α has generally been considered a positive regulator of angiogenesis (61).

In the light of the facts and finding discussed in the present review, it can be concluded that inhibition of PKC-α activity by using specific pharmacological inhibitors and antisense oligonucleotide could be a possible approach to suppress the tumor growth. In fact, some studies reported that antisense oligonucleotide blocking of PKC-α activity reverses the transformed phenotype in cancer cells, e.g. human lung carcinoma cells (62) and glioma cells (63). Therefore, PKC-α can act as a possible target to reverse the malignant cells to the normal phenotype.

Cell adhesion and migration

Cancer cells are metastatic in nature and have an ability to spread in the body from one place to another. The metastasis occurs in a series of steps that include breaking from the originating site to move through the blood or lymph system or by invading or growing into nearby normal tissue until a tiny tumor forms. Cells with increased levels of PKC-α activity show anchorage-independent growth and morphological alteration. A large number of proteins like vinculin, fascine, syndecan-4, β-1 integrin are involved in cell migration and adhesion and are co-localized and phosphorylated by PKC-α (Figure 5). PKC-α also regulates the migration of mammary epithelial cells by phosphorylating and blocking the activity of β-1 integrin, a protein responsible for actin assembly. It has been hypothesized that PKC-α regulates β-1 integrin in the Rho-dependent signaling pathway during cytoskeleton rearrangement. β-1 Integrin binds with the V3 region of PKC-α and mediates its cellular localization in pituitary cells. This statement is further supported by a finding that a naturally occurring mutation in the V3 domain (D294G) causes lack of cell-cell contact (31). PKC-α also allies with an actin-bundling protein, fascin, at the plasma membrane where association occurs through the C1B domain of PKC-α rather than the hinge region. Wallis and colleagues (64) proved that PKC-α is the only isoform that is involved in Ca^{2+}-dependent desmosome formation in Madin-Darby Canine Kidney (MDCK) epithelial cells. A previous study indicates that PKC-α interacts and phosphorylates at the C-terminal of F-actin-binding proteins such as ezrin, radixin and moesin, which are involved in the extension of lamellipodia and facilitate cell shape. This is further proved by

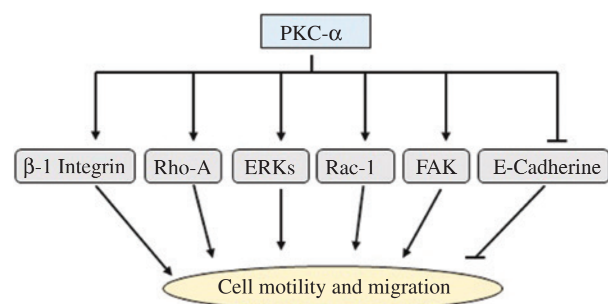

Figure 5: Possible interaction of PKC-α with various factors to control cell motility and migration.

the finding that the mutation at the C-terminal in ezrin protein inhibits PKC-α-mediated cell migration. In MCF-10 cells, overexpression of PKC-α regulates Rac-1 (a small GTPase protein) and enhances cell motility; this motility is blocked by pharmacological inhibitors and dominant negative RAC1 transfection (Figure 5). PKC-α also participates in the migration of vascular endothelial cells and smooth muscle cells. During migration, there is pervasive interaction between these cells and extra-cellular matrix take place. Another link between the α isoform and breast cancer cell migration was established by Larsson et al. whose findings demonstrated that PKC-α is associated with poor prognosis, ER and progesterone receptor negativity and support proliferation rates under *in vitro* conditions (65).

Strong evidence for the involvement of PKC-α in colon carcinoma cell migration was provided by Masur and colleagues (66) by using pharmacological inhibitors and antisense oligonucleotides. They demonstrated that PKC-α was involved in colon carcinoma cell migration when the expression of E-cadherin was lowered. They also showed that after activation PKC-α translocates toward the plasma membrane and co-localizes with surrounding collagen fiber within the contact areas of the cells (66). Increasing evidence suggests that PKC-α directly binds and phosphorylates filamin, and is responsible for cell adhesion, cell migration and actin arrangement in the cells. Filamin phosphorylation mediates recruitment of filamin/actin at the plasma membrane essential for the prevention of force-induced apoptosis (67). The cell migratory effect of PKC-α includes activation and localization of ERKs to focal adhesion in glioma cells, but the exact role remains unclear (68).

Expert opinion and outlook

The exploration of the PKC field, in particular PKC-α, has attracted a great deal of interest over the last decade because of its implication in major cellular functions in terms of both normal as well as pathophysiological states. At the basal level, PKC-α activity operates as a lipid-dependent ser/thr protein kinase that stimulates or represses the gene expression of distinct cellular processes, including cell survival, proliferation, differentiation, migration, adhesion and so on. However, biological outcomes of PKC-α vary from cell to cell and depend on cell types and substrates which it acts on. Therefore, precise control of PKC-α signaling amplitude is necessary for normal physiological condition and this is accompanied by coupling molecular events such as phosphorylation, cofactor binding and cellular translocation. The versatile role of this kinase, which ranges from control of normal cellular functions to regulation of complex events, makes it a tentative and elusive therapeutic target to reverse the pathophysiological status of the cells. In this article, we have emphasized the structure and activation of PKC-α and gained an insight into the implication in various cellular signaling pathways and regulation of key biological functions. Recent research has established that PKC-α is associated with poor prognosis, chemoresistance and anchorage-independent growth in various cancer cell lines. However, the exact role of PKC-α in cellular transformation is not clearly defined, and will be an important point for future study. Some pharmacological activators/inhibitors and antisense oligonucleotides that could inhibit the activity of PKC-α are available in the market and give some hope to develop an effective approach to cancer treatment in the near future. Unfortunately, both the academic and pharmacological industries have failed to develop a single agent which specifically regulates PKC-α activity. The multifaceted role of PKC-α makes it a tentative therapeutic target, and requires more extensive and consistent research to explore the networks of the signal transduction pathways mediated by PKC-α.

Highlights

- PKC-α is a ser/thr protein kinase activated by various signals which evoke lipid hydrolysis; after activation, it interacts with multiple scaffold proteins and is localized to specific cellular compartments where it is presumed to work.
- The ubiquitous expression and activation by various stimuli make it a perfect a player in distinct cellular contexts, including cell survival, proliferation, differentiation, motility, adhesion and so on. However, these functions are not intrinsic properties of PKC-α, but depend on cell types and conditions.
- The PKC-α amplitude is mediated by a series of ordered, tightly coupled phosphorylation events, cofactor recruitment and binding with scaffold proteins.
- The role of PKC-α in cell proliferation is very contradictory. PKC-α exhibits an anti-proliferative function in particular cell types and may function as a growth stimulatory factor in others.
- The preparatory studies to set up the role of PKC-α in cell survival is very antithetical. Overexpression of

PKC-α is capable of inducing apoptosis in some cell types while preventing it in others.
- PKC-α suppresses the activity of anticancer associated signals such as the Bax subfamily and cascades of caspase and stimulates survival-associated pathways like ERK, PI3K/Akt or mTOR.
- PKC-α has generally been considered as a positive regulator of angiogenesis.
- A large number of proteins like vinculin, fascin, syndecan-4 and β-1 integrin are involved in cell migration and adhesion and are co-localized and phosphorylated by PKC-α.
- PKC-α has been shown to interact with F-actin-binding proteins such as ezrin, radixin and moesin, which are involved in the extension of lamellipodia and thus facilitate cell shape.
- It has been established that PKC-α is the only isoform which is involved in Ca^{2+}-dependent desmosome formation in MDCK cells.
- Targeting PKC-α will be an emerging strategy to reverse pathophysiological states.

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List of abbreviations

DAG	diacylglycerol
ERK	extracellular signal-regulated kinase
ERK-MAPK	extracellular-signal regulated kinase-mitogen activated protein kinase
IP3	inositol-1,4,5-triphosphate
PDPK1	3-phosphoinositide-dependent protein kinase-1
PI3K	phosphatidylinositol-3-kinase
PIP_3	phosphatidylinositol (3,4,5)-trisphosphate
PKC	protein kinase C
PLC	phospholipase C
PMA	phorbol 12-myristate 13-acetate

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