

PKC α : regulation and implication for cellular transformation

Abstract

PKC- α is a serine/threonine protein kinase regulate many signaling pathways in response to various extracellular stimuli. It has implicated in the regulation of myriad cellular functions including proliferation, differentiation, survival, and migration. Therefore, precise control of PKC- α mediated signal transduction is necessary for normal cellular physiology. For PKC- α , this signal transduction is regulated by coupling molecular events such as phosphorylation, cofactor requirement and translocation towards specific cellular compartments. PKC- α functionally interacts with various proto-oncogenes, therefore it is plausible to assume that perturbation in PKC- α mediated signaling pathways is associated with pathophysiological states. Therefore, it is necessary to put some light on how PKC- α get activated and regulate multiple cellular functions. The present review will provide better knowledge about the regulation of PKC- α activity and its participation in various neoplastic changes, and better understanding for the development of better therapeutic approach to combat cancer development associated with PKC- α dysfunction.

Keywords: PKC, Cancer, PKC Regulation, PKC Inhibition

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Abbreviations: PKC, Protein Kinase C; AP, Activation loop; HM, Hydrophobic motif; TM, Turn Motif; DAG, Diacylglycerol; mTORC2, mammalian target of rapamycin complex 2; HSP90, Heat shock protein 90.

PKC- α

The protein kinase C (PKC) represents a family of closely related ser/thr protein kinase that participate in numerous signaling pathways. These signal control multiple cellular processes such as proliferation, differentiation, migration, apoptosis invasion and so on. Therefore, deregulation of PKC activity has been linked to numerous pathophysiological status, including cancer, Alzheimer, Parkinson disease, diabetes etc.¹ Subsequently, based on the regulatory domain architecture, 10 different isoform of PKC can be divided into 3 subfamilies; the conventional PKC (cPKC: α , β I, β II and γ), novel PKC (nPKC: δ , θ , ϵ & η) and atypical PKC (aPKC: ζ , λ 1).

PKC- α is a member of cPKC, containing 672 amino acid and ubiquitously expressed Ser/Thr protein kinase.² Similar to other members of cPKC subfamily, PKC- α have the same architecture with a variable N-terminal regulatory moiety, and a highly conserved kinase domain at the C-terminal. The conserved catalytic domain contains a C3 (ATP binding) and a C4 (substrate binding) domain, and the regulatory domain comprises a C1 (DAG binding) and C2 (Ca²⁺ coordinating) domain and a pseudosubstrate sequence in Figure 1.

Regulation of PKC- α activation

PKC- α has been implicated in both normal physiology as well as the pathophysiological status of a cell, therefore, spatial regulation of PKC- α transmitted signal amplitude is necessary for cellular homeostasis. PKC- α is matured by a series of tightly regulated coupled molecular mechanism such as phosphorylation, cofactor binding and intracellular localization, these events are essential for stability and catalytically competence of enzyme.³ Therefore, regulation of PKC- α activity depends on three mechanisms:

- Phosphorylation
- Cofactor binding
- Intracellular localization

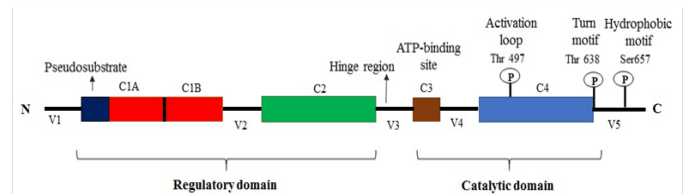


Figure 1 Schematic representation of domain composition of the PKC- α . A regulatory domain at N-terminal comprises two basic modules, C1 and C2 domain. This domain mediate DAG binding and contains autoinhibitory pseudosubstrate sequence. The C2 domain mediates Ca²⁺-regulated phospholipid binding. The highly conserved catalytic domain comprises C3 domain act as ATP-binding domain and C4 domain is the substrate-binding kinase core.

Phosphorylation

Phosphorylation is absolutely critical for catalytically competence confirmation and protect PKC- α from degradation.⁴ Studies involving mass spectrophotometry and mutational analysis demonstrated three conserved key phosphorylation sites termed as an activation loop (AP) turn motif (TM) and hydrophobic motif (HM).⁵ Newly synthesized nascent PKC undergoes phosphorylation at activation loop (Thr497) catalyzed by 3-phosphoinositide dependent protein kinase-1 (PDK-1). Phosphorylation at AP induces two tightly coupled and ordered phosphorylation at TM (Thr-638) and intramolecular autophosphorylation at the HM (S-657).⁶ In particular, TM phosphorylation is responsible for stabilization of conformation of active kinase leads to make PKC- α phosphatase resistant.⁷ Recently, it has been found that two new factors implicated in prime phosphorylation of PKC- α ; HSP90 and mTORC2. Binding of HSP90 and co-chaperon cdc37 with a specific molecular clamp in kinase core is an essential event for processing and stabilization of an enzyme.⁸ mTORC2 is required to allow the phosphorylation at HM.⁹

Cofactor binding

Canonically, to attain fully functional structure, PKC α is required to bind with second messenger DAG and Ca²⁺. The Primed phosphorylated PKC- α resides in the cytoplasm in inactive form due to the presence of N-terminal autoinhibitory pseudosubstrate sequence that masks the active site within the catalytic core rendering it unable to bind and phosphorylate the substrate. The appropriate extracellular stimuli act on the cell surface receptor result in activation of phospholipase C (PLC), thus hydrolysis of Phosphatidylinositol 3-phosphate to generate DAG and IP₃; a Ca²⁺ mobilizer. Ca²⁺ binds to the C2 domain to facilitate translocation of PKC- α towards plasma membrane. DAG recruited PKC- α at the plasma membrane and increases its affinity for phosphatidylserine. Ultimately, these cofactor binding with C1 and C2 domain, generate enough energy to expel the autoinhibitory pseudosubstrate domain from sequence binding site.¹⁰ However, recent studies have demonstrated that PKC- α undergoes activation in the absence of second messenger.¹¹

Intracellular localization

It is surprising that multiple PKC isoform co-exist in the same tissue with similar substrate specificity and activators, but performs distinct cellular functions. These functions depend on the differential subcellular localization of PKC- α after activation. This phenomenon is regulated by binding with scaffold proteins commonly known as Receptors for Activated C Kinase (RACKs) and Receptor for Inactive C Kinase (RICKs). The anchoring proteins bind with regulatory domain as well as variable domain and activate PKC by relieving auto-inhibition. In this way scaffold proteins mediate both localization as well as duration of PKC activity in Figure 2.¹²

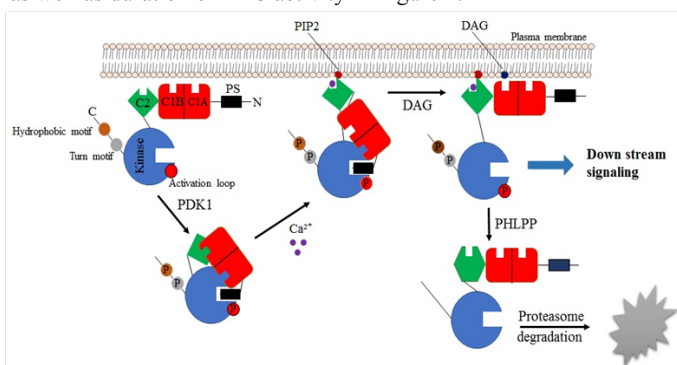


Figure 2 Diagrammatic representation of the life cycle of PKC- α . Unprimed PKC- α resides in open confirmation in membrane-associated form, priming phosphorylation at its activation loop catalyzed by PDK-1, followed by phosphorylation at the turn motif and the hydrophobic motif. PKC α localizes to cytosol where it remain in inactive state due to binding of pseudosubstrate region to substrate recognition site. Agonists promote PIP₂ hydrolysis, and PKC α is recruited to the plasma membrane in a Ca²⁺ dependent manner. Dephosphorylation of activated PKC α make it susceptible to ubiquitination and leads to its proteasome-mediated degradation, thus terminating signal relay.

Down regulation of PKC- α signaling

The PKC- α mediate various cellular functions, therefore, basal levels of PKC- α signal amplitude is critical for cellular homeostasis. In normal physiological condition, E3 ligase, a RING finger domain containing protein, interacts with C1A region of PKC and mediate ubiquitination in both *in vitro* and *in vivo* condition. This notion further supported by the fact that genetic knockdown of RINCK is

associated with increased level of PKC, while overexpression of RINCK results in decreased level of PKC expression. One interesting point is that this degradation mechanism is independent of PKC phosphorylation.¹³ Further, it has also been found that PKC- α activity regulated by interaction with DAG kinase- ζ . Moreover, the down regulation of cPKC accompanied by a PH domain leucine rich repeat phosphatase (PHLPP), which directly involved in dephosphorylation at HM.¹⁴ It has been found that primed PKC- α undergo lipid raft mediated endocytic pathway, and ultimately degraded by lysosome.¹⁵ The basal level of PKC- α is also regulated by mTORC2 and PDK1 which is essential for priming phosphorylation.

PKC- α : biological functions and role in cellular transformation

PKC- α has long been demonstrated as a regulator of multiple functions during tumor growth, such as survival, proliferation, differentiation and migration. There are several studies that recognize PKC- α as anti-apoptotic factor^{16,17} in glioma cells, endothelial cells and salivary epithelial cells.¹⁸ The exact mechanism by which PKC- α prevents apoptosis is partially known. One of possible target that has been identified as anti-apoptosis is BCL-2 protein in HL 60 cells. PKC- α co-localized with BCL-2 protein in the mitochondria,¹⁹ while other convincing experiments with murine growth factor dependent cell lines, established that PKC- α phosphorylated BCL-2 at ser-70 and stabilizes its function to inhibit apoptosis.²⁰ Another possible target of PKC- α is Raf-1 which has been known to mediate cell survival pathway led by PKB/AKT in hematopoietic cell. Other recent research has demonstrated that PKC- α inhibition results in mitochondrial dependent apoptosis in breast cancer cells.²¹ Moreover, it has been suggested that knockdown of PKC- α expression reduced the activation of Akt/Erk, demonstrated that PKC- α is an upstream regulator of these survival signaling pathways.²² Convening results from various studies indicate that up-regulation of PKC- α activity is sufficient to stimulate proliferation of several cell lines including human glioma U87 cell, the osteoblast, chick-embryo hepatocyte and hepatocellular carcinoma cells. The proliferatory effect of PKC- α also enhances activity of cdk-4 and cyclin D1, and cyclin/cdk2 complex activity.²³ Further, PKC- α also phosphorylate Raf-1, leading to the activation of ERK/MAPK signaling pathway. Recently, it has been found that in null mice, PKC- α effectively suppress tumor development,²⁴ and support the assumption that PKC- α control apoptosis and cell survival during tumor progression.²⁵ PKC- α regulates the activation of NF- κ B, which results in pro-survival phenotypes in U1242 glioblastoma cells.²⁶ Another interesting link between PKC- α and cancer progression is the ectopic overexpression of PKC- α in MCF-7 cells results in enhanced proliferation, alteration in morphology, and anchorage independent growth.²⁷ Stable overexpression of PKC- α in T47-D breast cancer cells is accompanied by down-regulated ER function.²⁸ Moreover, PKC- α also inhibited the Wnt/ β -catenin pathway in colon cancer cells to inhibit the expression of cMyc.²⁹ Activation of PKC- α in LNCaP cells leads to a rapid and reversible dephosphorylation of Akt, possibly via activation of a PP2A phosphatase.³⁰

Furthermore, it is reported that PKC- α actively participates in K-Ras mediated lung Tumorigenesis.³¹ This notion is further supported by finding that PKC- α knockout mice show enhanced K-Ras lung Tumorigenesis.³² PKC- α has been implicated in adhesion and migration of cancer cells. It has been hypothesized that PKC- α regulate β -1 integration in the rho dependent signaling pathway during cytoskeleton rearrangement. It is also associated with fascin,

an actin binding protein present at the plasma membrane. Years ago, it has been founded that PKC- α interact and phosphorylate F-actin binding protein, including radixin and moesin which are involved in the extension of lamellipodia and acquisition of well-defined shape and size of a cell.³³Therefore, more insight study is required to underlying mechanism to understood how PKC α mediated signal transduction govern to tumorigenesis.

Conclusion

The exploration of PKC α field has attracted a great deal of interest over the last three decades due to its implication in myriad cellular functions. At basal level, PKC- α activity managed as a lipid dependent ser/thr protein kinase which stimulates or represses gene expression of multiple cellular aspects including cell survival, cell proliferation, cell differentiation, cell migration and so on. The basal level of PKC- α is regulated under intricate control. This spatial and temporal regulation comes from tightly regulated coupled molecular mechanism such as phosphorylation, cofactor binding and binding to scaffold proteins. So for, perturbation in any of these mechanism leads to pathophysiological states including cancer. It has long been established that PKC- α functionally interacts with various proto-oncogenes. However, the exact mechanism by which PKC α mediate cellular transformation is not clearly understood and it will become an elusive question in PKC field. Therefore, get an insight how PKC- α activity regulated and implicated in cellular transformation is key to design novel therapeutic to manage PKC α activity at physiological level.

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Conflict of interest

The authors declare no conflict of interest.

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