The unique protein kinase Cη: Implications for breast cancer (Review)

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Abstract. Deregulation of key signal transduction pathways that govern important cellular processes leads to cancer. The development of effective therapeutics for cancer warrants a comprehensive understanding of the signaling pathways that are deregulated in cancer. The protein kinase C (PKC) family has served as an attractive target for cancer therapy for decades owing to its crucial roles in several cellular processes. PKCη is a novel member of the PKC family that plays critical roles in various cellular processes such as growth, proliferation, differentiation and cell death. The regulation of PKCη appears to be unique compared to other PKC isozymes, and there are conflicting reports regarding its role in cancer. This review focuses on the unique aspects of PKCη in terms of its structure, regulation and subcellular distribution and speculates on how these features could account for its distinct functions. We have also discussed the functional implications of PKCη in cancer with particular emphasis on breast cancer.

Contents

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1. Introduction

The protein kinase C (PKC) family is a family of serine/threonine kinases that play diverse roles in fundamental cellular processes including cell proliferation, cell death and differentiation (1,2). PKCs respond to extracellular signals that promote phospholipid hydrolysis and facilitate the generation of diacylglycerol (DAG) and release of Ca^{2+} from intracellular stores. These two second messengers activate PKCs in the presence of acidic phospholipids, such as phosphatidylserine (2). The PKC family garnered considerable attention by the discovery that PKCs could serve as receptors for tumor-promoting phorbol esters which was the first evidence to establish a link between PKCs and cancer (3,4). These phorbol esters are potent activators of PKCs and can substitute for the physiological stimulator DAG. Based on the structural features and cofactor requirements, the PKC family consists of 10 isozymes categorized as the conventional or the classical (c) PKCs (α, βI, βII, γ), the novel (n) PKCs (δ, ε, η, θ) and the atypical (a) PKCs (ζ, λ/ι) (1). While the conventional PKCs are sensitive to Ca^{2+} and DAG/phorbol ester, novel PKCs are insensitive to Ca^{2+} but respond to DAG/phorbol ester and atypical PKCs are insensitive to both Ca^{2+} and DAG/phorbol esters. The distinct structural and biochemical features of the PKC isozymes pave the way for the distinctive cellular responses attributed to the PKC family. Owing to the central role of PKCs in cellular regulation and signal transduction, significant research efforts have been devoted to the PKC family. However, much less is known about PKCη, which is a unique member of the novel PKC family. This review focuses on the biology of PKCη and its implications in breast cancer.

2. Protein kinase Cη, a unique PKC

Protein kinase Cη (PKCη) is a novel member of the PKC family. It is classified as a calcium-independent but DAG/phorbol ester-dependent PKC (5). It was first isolated from a cDNA library of mouse epidermis (5). PKCη is assigned to human chromosome 14 (14q22-23) and mouse chromosome 12 (12C3-D2) (6,7) and contains an open reading frame encoding 683 amino acid residues (8). Contrary to other PKCs which are primarily enriched in the brain tissue, PKCη is mainly expressed in lung, skin and heart tissues (9). PKCη participates in various cellular processes including

proliferation, differentiation, secretion and apoptosis (10-16). Recent reports have revealed the role of PKCη in immune function (17,18). PKCη was shown to be important for T-cell proliferation and homeostasis (19), and was also implicated in the regulation of toll-like receptor-2 (TLR-2) responses in macrophages (20).

3. Structure

All PKC isozymes contain a common structural backbone comprised of a highly conserved catalytic domain at the C-terminal and a regulatory domain at the N-terminal (Fig. 1). PKCs possess 4 conserved modules (C1-4): C1 and C2 are the membrane targeting modules that along with the pseudosubstrate region form the regulatory domain; C3 and C4 comprise the catalytic domain (21). A proteolytically labile hinge region connects the regulatory domain to the catalytic domain (22). The catalytic domain consists of motifs that are required for ATP/substrate binding and catalysis. The N-terminal contains the autoinhibitory pseudosubstrate sequence that contains an alanine in place of the serine/threonine phosphoacceptor site, but otherwise resembles a PKC substrate. The pseudosubstrate thus holds the enzyme in an inactive conformation by occupying the catalytic site (21). The pseudosubstrate sequence of PKCη is the most divergent amongst the PKC isozymes (9).

The structure of PKCη comprises of a highly conserved catalytic domain at the C-terminal and the regulatory domain at the N-terminal similar to other PKCs (21). A characteristic cysteine-rich region is present in the C1 domain of PKCη which allows binding to physiological stimulator DAG and pharmacological activators such as tumor-promoting phorbol esters (23). In addition, C1 domain confers selectivity for phosphatidylserine that acts as the activator for PKCs (24). The C2 domain of PKCη lacks the key aspartic acid residues to bind Ca^{2+} and consequently renders PKCη insensitive to Ca^{2+} (23). PKCη shares greatest homology with PKCε, another novel PKC (9).

Similar to other PKC isozymes, PKCη has three conserved phosphorylation sites - activation loop (Thr-513), turn motif (Thr-655) and hydrophobic domain (Ser-674) (21). Although the order of priming phosphorylations of PKCη is not well established, phosphoinositide-dependent kinase 1 (PDK1) is believed to phosphorylate PKCη at the activation loop *in vitro* (25). In mouse A9 fibroblasts infected with parovirus, Lachmann *et al* demonstrated that PKCλ phosphorylates PKCη at the hydrophobic site thus allowing PDK1 access to the activation loop (26). The C2 domain of PKCη was found to be similar to PKCε with significant differences at the putative lipid binding site. Mass spectrometric analysis of the C2 domain of PKCη revealed two autophosphorylation sites at Ser-28 and Ser-32 (27). The autophosphorylation site at Ser-28 but not Ser-32 is conserved in PKCε (27). It has been speculated that autophosphorylation at these sites could affect the lipid-binding of PKCη (27).

4. Regulation

The PKC isozymes are under tight structural and spatial regulation that underlies their biochemical functions, intracellular localization and tissue distribution (21). PKCs can be regulated by phosphorylation, cofactor binding and membrane targeting through interaction with scaffold proteins (28).

Anionic phospholipids such as phosphatidylserine and DAG/phorbol esters regulate PKCη (5,9). However, in contrast to other phorbol-ester sensitive PKC isozymes, PKCη resists downregulation by prolonged treatment with phorbol esters, suggesting its unique regulation (11,29,30). We have shown that PKCη not only resists downregulation by phorbol esters but is in fact upregulated by several structurally and functionally distinct PKC activators (31). We further reported that transphosphorylation by novel PKCε is responsible for activator-induced upregulation of PKCη (31).

PKCη is specifically activated by cholesterol sulfate and sulfatide (32). It was reported that cholesterol sulfate-mediated activation of PKCη involved casein kinase I (33). In addition, PKCη was shown to be activated by treatment with type I interferons (IFNs), IFNα or IFNβ in chronic myeloid leukemia cells (34). Interestingly, other novel PKC isozymes such as PKCδ, -ε and -θ are also activated by type I and type II IFNs and participate in type I and/or type II IFN-induced responses (35-38). However, contrary to these isozymes, IFN-inducible transcription of IFN-stimulated genes or generation of antiviral responses is independent of PKCη. PKCη is also elevated in response to estradiol treatment in estrogen-sensitive breast cancer cells in a time- and concentration-dependent manner (39).

PKCη is subject to translational regulation under both normal and stressed conditions caused by amino acid starvation (40). Raveh-Amit and colleagues reported that the 5'-UTR of PKCη is unusually long (659 nucleotides) and rich in GC content and identified two upstream open reading frames (uORF) in the 5'-UTR which function as repressive elements under normal growth conditions. However, under amino acid starvation, the repression is removed by leaky scanning leading to the translational upregulation of PKCη (40). PKCε is the only other PKC isozyme for which the presence of a regulatory uORF has been reported (41).

5. Signal termination and downregulation of PKCη

Termination of PKC signaling can occur via different mechanisms such as release of PKC isozymes from the membrane, metabolism of DAG by DAG kinases (DGKs) (42,43), agonist-induced degradation or the removal of priming phosphorylation which leads to downregulation and rapid degradation (42,44,45). Several mechanisms of degradation have been proposed for the PKC isozymes. Conventional PKCs are believed to be downregulated by calcium-activated proteases, such as calpains (46,47) whereas PKCα, -δ and -ε were shown to be degraded via proteasome-mediated pathway (48-50). Our studies have demonstrated that PKCη can be downregulated by both proteasomal-dependent and -independent pathways. While inhibition/knockdown of PDK1 caused PKCη downregulation via the proteasomal pathway, the downregulation of PKCη caused by the depletion of PKCε or by PKC inhibitors was independent of the proteasome-mediated pathway (51). Another study reported that dephosphorylation of PKCη was mediated by integrin-associated serine threonine phosphatase PP1γ in human platelets which was shown to be independent of the ubiquitin-mediated degradation (52). In addition, differential expression analysis in the neoplastic cell

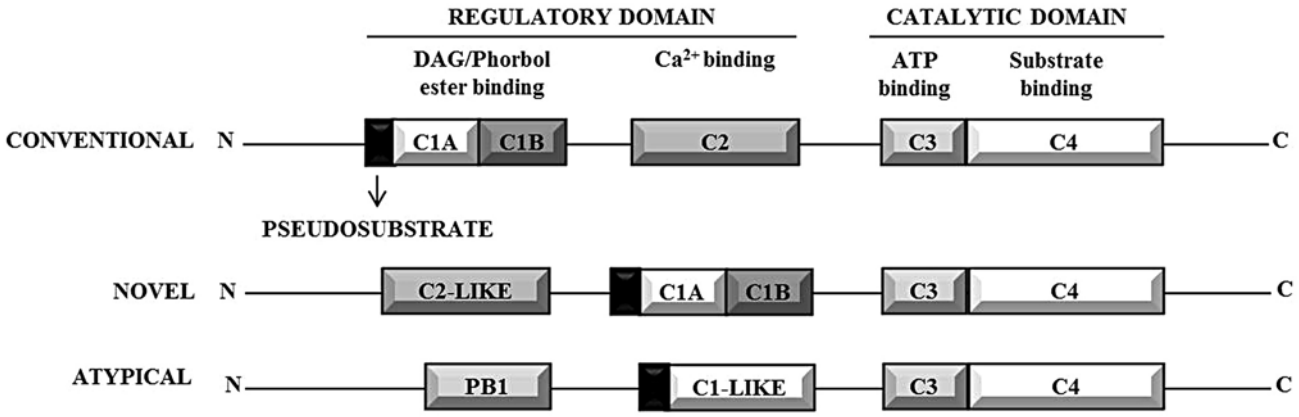

Figure 1. Domain structure of PKC isozymes. The PKC family comprises of three classes, conventional, novel and atypical. Each isozyme has a regulatory domain (C1, C2) and a catalytic domain (C3, C4). The C1 domain binds phosphatidylserine for all PKCs and consists of motifs that form the DAG/phorbol ester binding site for the conventional and novel PKCs while C2 domain binds anionic lipids and Ca^{2+} for conventional PKCs. Atypical PKCs possess a Phox and Bem 1 (PB1) module for protein-protein interactions. C3 and C4 form the ATP and the substrate binding domains of PKCs, respectively.

line 8701-BC demonstrated that PKCη downregulation can be induced by type V collagen (53).

6. Localization of PKCη

PKCη is localized in the Golgi, endoplasmic reticulum (ER) and the nuclear envelope (54). Although the C1A domain of PKCη lacks a Golgi localization signal similar to the other members of the novel PKC family, the C1B domain of PKCη facilitates its translocation to the Golgi complex (54). The localization of PKCη in the Golgi could account for the involvement of PKCη in Golgi vesicular transport. It has been previously reported that Golgi-cell surface transport requires protein kinase D (PKD) which is specifically activated by G protein subunits β1γ2 and β3γ2 via the Golgi-associated PKCη (55). In response to serum starvation and PMA, PKCη translocates to the nuclear envelope. While C1B domain is sufficient to drive Golgi translocation of PKCη, both the C1 and the pseudosubstrate region are required for the localization at the nuclear envelope and ER (54). PKCη is localized in the plasma membrane and the nuclear envelope upon stimulation with phorbol esters, while serum starvation leads to distribution only in the nuclear envelope (54). Furthermore, a recent study reported that in hepatocellular carcinoma cells, PKCη is targeted to lipid droplets where it limited the formation of larger lipid droplets (56).

7. Role of PKCη in breast cancer

PKC isozymes have been extensively researched as potent targets for cancer therapeutics since their discovery as receptors for tumor promoters (3,57). The role of PKCη in cancer is controversial owing to its divergent responses in different cancers. Although, PKCη-deficient mice were more susceptible to tumor promotion in two-stage skin carcinogenesis model (58), PKCη mediates chemotherapeutic resistance in breast cancer (10,59), glioblastoma (60), lung cancer (61) and several other cancers (62,63). It has been reported that PKCη is downregulated in hepatocellular carcinoma (64) but is associated with the progression of renal cell carcinoma (65). Thus, PKCη may promote or inhibit malignant growth depending on the cellular context.

PKCη is a regulator of mammary gland development (66). It is upregulated in the rat mammary gland during the transition from the resting to the pregnant state (66). Furthermore, a marked decrease in PKCη levels was observed during gland regression which is typically characterized by the onset of apoptotic processes leading to involution (66). Qualitative and quantitative alterations in PKCη have been reported in human breast cancer tissues (67). PKCη expression was increased in locally invasive breast tumor tissues and high levels of PKCη were detected in invasive tumors associated with significant lymph node metastases which suggests a role for PKCη in cancer progression (67). This is consistent with a report which demonstrated the importance of PKCη in maintaining tight junction integrity via interaction and subsequent phosphorylation of occludin on its C-terminal domain (68). Since key changes in the barrier function of tight junctions have been shown to be critical in cancer progression (69), it is likely that PKCη may have potential roles in survival and progression of cancer cells. We also observed that the levels of PKCη progressively increase with breast cancer aggressiveness in the MCF10 breast cancer series (51). It is also noteworthy that the promoter region of PKCη contains multiple sites for the transcription factors Ets1 and AP-1 (6), both of which have been implicated in breast cancer growth and progression (70,71). However, contrary to these reports, decreased PKCη expression was observed in invasive breast tumor tissues compared to the surrounding normal epithelium, suggesting that PKCη is decreased during breast cancer progression (67). Thus the role of PKCη in cancer progression remains controversial.

PKCη mRNA is elevated in multidrug-resistant breast tumors (72), and overexpression of PKCη has been shown to protect against apoptosis (10,11,15). We have previously reported that overexpression of PKCη attenuated caspase activation and TNF-induced cell death in breast cancer cells (10). PKCη also protects against camptothecin-induced DNA

damage by activating NF-κB and promoting nuclear localization of RelA/p65 in breast cancer (15). Upon etoposide-induced stress, PKCη is tethered to the nuclear membrane and confers protection against cell death (73). Moreover, PKCη was effective in blocking apoptosis via the suppression of c-Jun N-terminal kinase (JNK) activity upon UV irradiation (59). PKCη is also critical for cell cycle control. Although PKCη induced growth arrest in NIH3T3 fibroblasts and keratinocytes (74,75), it enhanced cell cycle progression in breast cancer cells (12). Induced expression of PKCη led to an increase in the levels of cyclin E and cyclin D (12). Although the levels of the cell cycle inhibitor p27 (kip1) were unaltered by PKCη overexpression, it facilitated the removal of the cell cycle inhibitor p27 (kip1) from the cyclin E/cdk2 complex, thereby activating the cyclin E/cdk2 complex (12). Consistent with these findings, we observed that PKCη promotes breast cancer cell growth and proliferation, similar to its role in glioblastoma (14,51,76). On the other hand, PKCη was shown to negatively regulate Akt leading to decrease in insulin-like growth factor I (IGF-I)-induced cell proliferation in MCF-7 breast cancer cells (77). There are thus, contrasting functional responses of PKCη not only in different cancers, but also within the same cancer type.

8. Conclusions

PKCη is a unique member of the PKC family. Its distinct regulation in response to tumor promoters compared to the other PKCs has potential implications in cancer. Although several studies have established the role of PKCη in cell growth, proliferation and chemoresistance, conflicting reports have added ambiguity to the functional role of PKCη. Moreover, PKCη interacts with several signaling pathways, such as the PI3K/Akt, NF-κB and ERK/Elk-1 (15,76,78). In addition, most cells express multiple PKC isozymes which display redundant as well as opposing functions. The distinct biochemical properties, tissue distribution and subcellular localization of different PKC isozymes have been reported to result in divergent responses in cancer (79-81). Thus, the crosstalk between these proteins will eventually influence the final outcome.

While the published reports have helped discern the regulation and function of PKCη, many questions remain regarding the paradoxical actions of PKCη. It would be worthwhile to understand the specific interactions of PKCη with other signaling pathways and the subsequent consequences on cellular regulation. Studies focused on the interaction of transcription factors such as AP-1 or Ets1 with PKCη in breast cancer could also yield interesting results. Thus, future studies should help determine the molecular cues which govern the dynamic role of PKCη.

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