

Protein Kinase C α (PKC α): Regulation and Biological Function

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Protein kinase C α (PKC α) is a serine/threonine kinase and a member of the conventional (classical) PKCs (cPKCs), which have four conserved (C1 to C4) regions. This ubiquitously expressed PKC isotype is activated in response to many different kinds of stimuli and translocates from cytosol to the specialized cellular compartments (nucleus, focal adhesion, caveolae, *etc.*) where it is presumed to work. Therefore, PKC α has been implicated in a variety of cellular functions including proliferation, apoptosis, differentiation, motility, and inflammation. However, the responses induced by activation or overexpression of PKC α vary depending on the types, and sometimes conditions, of cells. For example, in some types of cells, PKC α is implicated in cell growth. In contrast, it may play a role in cell cycle arrest and differentiation in other types of cells. Therefore, alterations of cell responses induced by PKC α are not an intrinsic property of this isoform. The responses are modulated by dynamic interactions with cell-type specific factors: substrates, modulators and anchoring proteins.

Key words: apoptosis, differentiation, migration, proliferation, translocation.

1. Introduction

Mammalian PKC α consists of 672 amino acids and is distributed in all tissues, in contrast to other PKC isotypes whose expression is restricted in the particular tissues (1, 2). PKC α is activated by a variety of stimuli, including signals binding to guanine-nucleotide-binding protein-coupled receptors and to tyrosine kinase receptors (1–5), and also physical stresses like hypoxia (6) and mechanical strain (7). Therefore, this isotype plays important roles in the control of major cellular functions: proliferation, apoptosis, differentiation, motility and so on. However, the distinct cellular responses are not due to a multipotential property of this isotype but are regulated through its dynamic interactions with special factors. In other words, the output after activation absolutely depends on where and when it is activated, and what substrates it acts on. However, no complete list of these cell-specific factors is yet available. This review discusses the current understanding of activation mechanisms and possible biological functions of PKC α .

2. Activation and translocation

PKC α is activated by a variety of stimuli originating from receptor activation, cell contact and physical stresses. Diacylglycerol (DG) and Ca²⁺ increased in the cell upon stimulation synergistically drive the release of a pseudo-substrate region from the active site, leading to activation (8). Also, kinase activity of PKC α is regulated by phosphorylation of three conserved residues in its kinase domain: the activation-loop site Thr-497, the autophosphorylation site Thr-638, and the hydrophobic C-terminal site Ser-657 (9, 10). PKC α exhibits almost no activity without phosphorylation at these sites. Phosphorylation of Ser-657 is cur-

rently used as a marker for PKC α activation (7, 9). The general mechanisms of PKC activation are discussed in the other article in this minireview series. Here, the possible regulatory mechanisms for stimulus-induced translocation to the particular cellular compartments and signal relays between other important signaling pathways are briefly introduced.

2.1. Translocation. Upon stimulation, PKC α translocates from cytosol to a so-called particulate fraction. This movement was first identified by Western blotting using a specific antibody against PKC α after fractionation of cells and later by immunofluorescent study. Recently, new approaches to analyze the spatial distribution of the protein in cells, such as fluorescence resonance energy transfer (FRET) and confocal microscopy, provide a method for identifying the exact location of PKC α . The application of the fusion protein consisting of PKC α and jellyfish green fluorescent protein (GFP) allows resolution of its distribution in living cells (11). These new approaches (11–13) revealed that PKC α redistributed from cytosol to the entire membrane in response to non-specific activator for PKCs such as phorbol myristate acetate (PMA). However, the specific stimuli cause translocation of PKC α to the plasma membrane, where DG is presumed to be produced, and also to the specialized cell compartments such as nuclei, focal adhesions, regions of cell–cell contact and so on.

One of the compartments is the nucleus (14). In fibroblasts, growth factors like PDGF (platelet-derived growth factor), EGF (epidermal growth factor), and IGF-1 (insulin-like growth factor-1) cause redistribution of PKC α to the nucleus. PKC α , like other PKC isotypes, does not contain the nuclear localization signals (NLS). Although the exact mechanism of nuclear translocation is not yet known, the components for NLS-dependent transport, importin and Ran/GTPase, are not necessary (15).

Focal adhesions and regions of cell–cell contact are other common compartments (13, 16, 17). Accumulation of PKC α

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in focal adhesions and lamellipodia is often observed when cells spread and migrate. Upon wounding, epithelial cells migrate from the edge to reepithelialize the wound. In the scratch wounding model of cultured Madin-Darby canine kidney (MDCK) epithelial cells, PKC α translocates to the cell periphery at the wound edge and becomes concentrated in lamellipodia during migration (18).

2.2. Anchoring proteins. The proteins which interact with specific PKC isoforms for localization at the specialized cell compartments are called anchoring (or docking) proteins (19). In the absence of these isoform-specific (and probably tissue-specific) anchoring proteins, translocation may not be strictly defined and may depend on variations of DG and Ca²⁺ concentrations. RACK1 (receptor for activated protein kinase C-1), which was the first anchoring protein to be isolated for PKC β II, was shown to bind several PKC isoforms including PKC ϵ and PKC α (20). The proteins responsible for localization of PKC α at focal contacts have been identified by overlay assay and density gradient cell fractionation. Vinculin and talin, two focal contact proteins were isolated by overlay assay in REF52 cells (21). β 1-integrin co-sedimented with PKC α in velocity sucrose gradient centrifugation in MCF-7 cells (13). Caveolin was identified as an anchoring protein that recruits PKC α to caveolae (22). However, the proteins implicated in localization of PKC α at the nucleus are still unknown.

2.3. Signal relay. Upon activation, PKC α is presumed to phosphorylate a variety of substrates depending on the types of stimuli and cells. The identification of substrates in distinct cellular responses will provide a clue to understanding the roles of PKC α in biological functions. However, no complete list of substrates is available. Here, the important signaling pathways that interact with PKC α are introduced (Fig. 1).

The extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade, which involves the kinases Raf, MAPK/ERK kinase (MEK), and ERK/MAPK, is ubiquitously expressed and serves to alter cell functions. The activated ERK finally moves into the nucleus, where it phosphorylates target molecules such as transcription factors, Elk-1 and SAP1. PKC α functions as a potent activator of c-Raf-1 and turns on the ERK/MAPK

cascade (23, 24).

The low molecular weight GTP-binding proteins of the Rho family, Rho, Rac, and cdc42, have been assigned pivotal roles in actin stress fiber formation, membrane ruffling, cell movement and transcription factor activation. Rac1 acts downstream of PKC α in lamellipodia formation and migration (25). Moreover, PKC α is shown to closely interact with RhoA in transcription factor AP-1 activation in T cells (26) and in caveolae formation in carbachol-stimulated smooth muscle cells (27).

PKC α is implicated in cell growth and differentiation, and thus control of cell cycle machinery. Although the exact signaling cascade is not known, PKC α has been shown to induce cyclin-dependent kinase inhibitors, p21^{Waf1/Cip1} and p27^{Kip1} in several types of cells (28–30).

Several biological responses, such as proliferation and apoptosis, are inversely regulated by PKC α and PKC δ (2, 31–34). It appears that these two isoforms mutually regulate the expression and activity of other isoforms (31, 32).

As mentioned above, PKC α kinase activity is regulated by phosphorylation. Since PKC α activity can be inhibited through its dephosphorylation by protein phosphatase 2A (PP2A), an okadaic acid-sensitive phosphatase, the termination of PKC α activity is assumed to be partly mediated by PP2A (5). During activation and translocation of PKC α by PMA, PP2A also translocates to the membrane and is coimmunoprecipitated with PKC α (35). Therefore, it is likely that PP2A is physically associated with PKC α and plays a role in the termination of its activation through dephosphorylation.

3. Biological functions

PKC α is implicated in a variety of biological responses. Selective activators and inhibitors of PKC, and downregulation by the prolonged incubation of cells with phorbol esters, have provided useful information to analyze the roles of PKCs in biological functions. However, such approaches do not discriminate the specific roles of individual isoforms. In PKC isoforms that are expressed only in particular tissues, such as PKC γ , PKC δ , PKC ϵ , PKC ζ , and PKC θ , functional analysis is performed in the null mice by homologous recombination (this will be discussed in other articles in this minireview series). However, PKC α is expressed ubiquitously in tissues and is implicated in oocyte development and fertilization (36). The implication of PKC α in specific cell responses is analyzed in culture cell systems by introduction of wild-type PKC α or its dominant negative (kinase-dead) mutant *via* transfection or adenoviral infection. Specific targeting of PKC α by antisense oligonucleotides or by ribozymes also provides similar specificity for *in vitro* and *in situ* studies. The biological responses obtained by the manipulation of PKC α are cell-type specific. For example, overexpression of PKC α promoted proliferation in some types of cells, but caused cell cycle arrest and differentiation in other types. These observations indicate that alterations of cell responses induced by PKC α overexpression are not an intrinsic property of this isoform. The responses are modulated by dynamic interactions with cell-type specific factors: substrates, modulators, and anchoring proteins.

3.1. Proliferation. The implication of PKC in cell growth was first demonstrated in quiescent Swiss 3T3 cells stimulated by PMA. Several growth factors, such as PDGF,

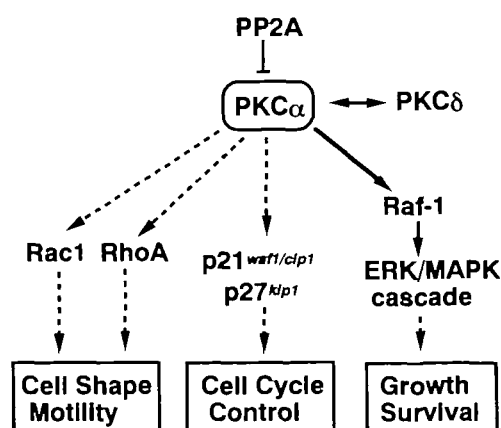


Fig. 1. Possible interactions of PKC α with other signaling pathways. \rightarrow , signal transduction pathways, direct (solid lines), and indirect (broken lines); \rightarrow , inhibition; \leftrightarrow antagonistic regulation.

activate and cause nuclear translocation of PKC. Transfection studies indicate that overexpression of PKC α is sufficient to promote proliferation in some types of cells. For example, overexpression of PKC α in the human glioma U87 cell line resulted in enhanced cell proliferation and decreased expression of glial fibrillary acidic protein (GFAP), a glial differentiation marker (34). Overexpression of PKC α in MCF-7 human breast cancer cells leads to a more aggressive phenotype, and cells exhibit an enhanced proliferation rate, anchorage-independent growth in soft agar, and increased tumorigenicity in nude mice (37). The proliferative effect of PKC α appears to be mediated via activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade initiated by Raf-1 (23, 24), and/or upregulation of p21^{WAF1/Cip1} (28), which facilitate formation of complex between cyclin and cyclin-dependent kinase (CDK).

In contrast, overexpression of PKC α results in cell cycle arrest and growth inhibition in several types of cells. In contrast to MCF-7 cells, transfection of PKC α in MCF-10 human mammary epithelial cell line resulted in slower growth with extended doubling time compared to parent cells (25). Murine fibroblast cell lines R6, BALB/C, and Ψ 2 expressing PKC α grew slower, as assessed by growth rate and saturation densities, than their parent cells (38). Another study (39) confirmed the inhibition of proliferation by PKC α in R6 cells, although overexpression of PKC α enhanced PMA-induced expression of growth-regulatory genes *c-jun*, *c-myc*, and collagenase. In the IEC-18 intestinal crypt cell line (30), PKC α activation appeared to lead to cell cycle arrest by accumulation of the hypophosphorylated growth-suppressive form of the retinoblastoma protein (Rb) and induction of the cyclin-dependent kinase inhibitors, p21^{WAF1/Cip1} and p27^{Kip1}. Therefore, PKC α is closely involved in the regulation of the cell cycle.

3.2. Apoptosis. Apoptosis is a genetically programmed form of cell death, which is important in morphogenesis and development, and for the removal of damaged cells as well as tumor cells. The role of PKC α in apoptosis also depends on cell types (Fig. 2).

PKC α exhibits an anti-apoptotic function and may function as a survival factor in some types of cells (2). This notion is based on the observations that PKC α is often blocked during apoptotic processes. In some cases PKC α

undergoes proteolytic hydrolysis by calpain and/or caspases. Ceramide, a known inducer of apoptosis, causes inactivation of PKC α , probably through dephosphorylation by phosphatases (40). During induction of apoptosis by Fas ligation in Jurkat human lymphoblastoid T cells (41), the activity of PKC α is inhibited through activation of okadaic acid-sensitive phosphatase, PP2A. Induction of apoptosis of Jurkat cells by α -tocophenyl succinate, a vitamin E analog, is accompanied by the inhibition of PKC α through increased activity of PP2A (42). Upregulation and downregulation of PKC α also confirmed its anti-apoptotic role. Cells transfected with wild-type PKC α became resistant to apoptosis (43). In contrast, inhibition of PKC α by antisense oligonucleotides increased the sensitivity to apoptotic inducers (42, 43). In COS-1 cells, expression of dominant negative PKC α is sufficient to induce apoptosis (44). Jurkat cell line was sensitive to apoptosis induced by α -tocophenyl succinate. Jurkat cells overexpressing PKC α became less susceptible to α -tocophenyl succinate-induced apoptosis (42). In contrast, antisense oligonucleotide to PKC α increased apoptosis after exposure to α -tocophenyl succinate. In glioblastoma cells (45), down-regulation of PKC α by antisense oligonucleotide was accompanied by the induction of p53 prior to the appearance of apoptotic cells. p53 is known to regulate the expression of proapoptotic proteins, Bax, Apaf-1, caspases, and insulin-like growth factor binding protein-3 (IGFBP-3). Expression of IGFBP-3 induced apoptosis of glioma and breast cancer cells. These observations suggest that PKC α protects cells from apoptosis, in part through blockage of p53 induction. The anti-apoptotic action of PKC α is also shown to be mediated via activation of the Raf-1/ERK/MAPK cascade (23, 24) and/or phosphorylation of anti-apoptotic protein Bcl2 (46).

On the other hand, PKC α may also play a proapoptotic role. PMA promoted death of human gastric cancer cell lines MKN45 and MKN47 with increase of activity of PKC α , but not PKC β , when they lost anchorage (47). Overexpression of PKC α via vaccinia viruses augmented apoptosis of detached MKN45 and MKN47 cells, leaving the viability of the attached cells unaffected. These observations indicate that integrin-mediated signals inhibit the apoptotic action of PKC α . PKC α may play a role in the induction of apoptosis of p53-null HL60 human leukemia cells induced by camptothecin, a DNA topoisomerase I

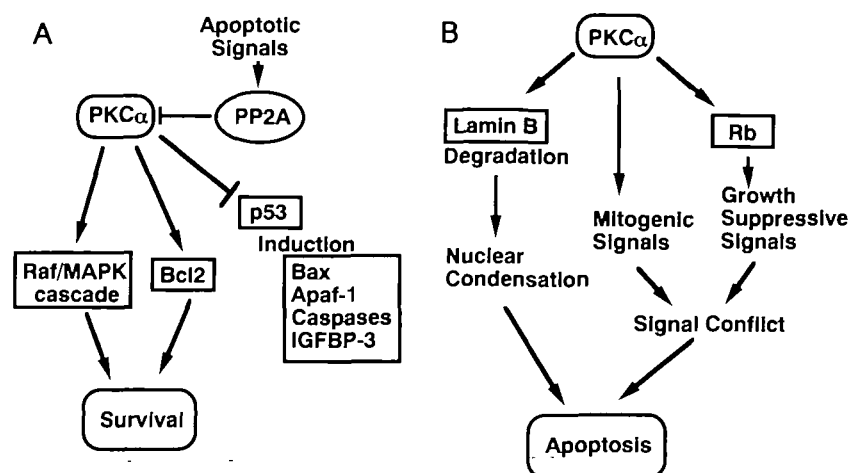


Fig. 2. Possible involvement of PKC α in both antiapoptotic (A) and proapoptotic (B) signaling.

inhibitor (48). PKC α is implicated in phosphorylation of nuclear lamin B, which promotes disassembly of nuclear lamina. Therefore, PKC α -mediated phosphorylation and subsequent solubilization (degradation) of lamin B is likely to affect nuclear and chromatin structure. The proapoptotic role of PKC α in LNCaP human prostate cancer cells is complicated (49, 50). Apoptosis of this cell line by PMA is initiated by a conflict between growth-suppressive signals from RB and growth-promoting mitogenic signals. The involvement of PKC α in both growth-suppressive and mitogenic signals is confirmed by its inducible expression. This signal conflict was blocked by cell aggregation through E-cadherin-mediated signals and the aggregated cells survived.

3.3. Differentiation. Cell differentiation is accompanied by the inhibition of cell cycle progression and expression of cell-specific functions. In some types of cells, such as hematopoietic progenitor cells (51, 52), lens epithelial cells (53), F9 embryonal carcinoma cells (54), and melanoma cells (55), PKC α is closely involved in differentiation. In the development of hematopoietic cells, the progression of erythroid progenitor cells (51) and development of macrophages are controlled by PKC α (52). Stimulation of hematopoietic granulocyte macrophage colony forming cells (GM-CFC) with macrophage colony stimulating factor (M-CSF) leads to macrophage formation with translocation of PKC α to the nucleus. When GM-CFC are transfected with a constitutively activated form of PKC α that is devoid of the N-terminal regulatory domain, the expressed protein is located primarily in the nucleus. These transfected cells are committed to development toward macrophage lineage even in the presence of factors that normally promote only neutrophilic development. Overexpression of PKC α in melanoma cells resulted in elongation of doubling time, diminished anchorage-independent growth in soft agar, and increased melanin production (56). PKC α also takes part in the chondrogenesis of mesenchymal cells (57). The details of molecular mechanisms of PKC α -mediated differentiation remain to be elucidated. Translocation to the nucleus during differentiation suggests the possibility that PKC α may be implicated in cell cycle control and/or expression of genes necessary for the differentiation phenotype.

The possible involvement of PKC α in oocyte maturation and morphogenesis has also been demonstrated. In porcine oocytes (36), injection of an antibody specific for PKC α blocked cortical granule exocytosis by PMA or fertilization. F9 embryonal carcinoma cells differentiate into parietal endoderm-like cells in response to retinoic acid (RA) (54). Undifferentiated cells express PKC β but not PKC α , whereas differentiated endoderm cells express PKC α but not PKC β . Overexpression of PKC α in F9 cells enhanced RA-induced differentiation, indicating an important role of PKC α in induction and maintenance of endoderm phenotype.

3.4. Cell migration and adhesion. As PKC α translocates and accumulates in focal contacts, it is shown to be involved in the regulation of cell motility and the adhesion of focal contacts. In MDCK cells (18), PKC α is the only PKC isoform that translocates and participates in the formation of Ca²⁺-dependent desmosomes in response to scratch wounding of a confluent cell sheet. An antisense oligonucleotide to PKC α abolishes the development of desmosomes at the wound edge. The role of PKC α in wounding

closure has also been demonstrated in MCF-10A and 2C4 fibrosarcoma cells (13). At the wound edge, PKC α interacts with β 1-integrin and ERZ (ezrin, radixin, and moesin) proteins, F-actin binding proteins which are involved in the maintenance of cell shape and extension of lamellipodia. Stable expression of PKC α increased phosphorylation of ERZ proteins at C-terminal threonine. Overexpression of ezrin mutant at the C-terminal phosphorylation site inhibited PKC α -mediated cell migration, indicating that migration is controlled through the phosphorylation of ERZ proteins by PKC α . Implication of small G protein Rac1 in PKC α -mediated cell migration has been demonstrated in MCF-10 cells (25). Overexpression of PKC α in MCF-10 cells enhanced cell motility. This increased motility is blocked by PKC inhibitors and dominant-negative Rac1, but not by dominant-negative RhoA or dominant-negative Cdc42. As mentioned below, PKC α participates in migration of vascular endothelial and smooth muscle cells.

3.5. Tumorigenesis. The malignant phenotype of carcinoma cells is associated with uncontrolled growth, morphological change and invasion. Therefore, PKC α is implicated in malignant phenotypes of several tumors, such as gliomas and breast cancers. In fact, elevated PKC activity was observed in human breast tumors. Moreover, overexpression of PKC α caused human breast cancer cells to show a more aggressive and metastatic phenotype, anchorage-independent growth in soft-agar and tumorigenicity in nude mice (25, 37). PKC α may also be involved in the drug resistance of cancer cells through phosphorylation and activation of a membrane-bound efflux pump, termed P-glycoprotein, the product of *mdr1* gene. An MCF-7 cell colony isolated on the basis of its resistance to doxorubicin expressed elevated levels of PKC α (37). On the other hand, in doxorubicin-resistant MCF-7 cells transfected with PKC α , the resistance to anti-cancer drugs was increased with enhanced phosphorylation of P-glycoprotein and decreased drug accumulation (58).

A mutant form of PKC α (four point mutations in the regulatory domain) was isolated from a murine fibrosarcoma cell line by ultraviolet irradiation and was reported to have the ability to transform Balb/c fibroblasts (59). However, the tumorigenic activity of this mutant PKC α could not be confirmed by the other investigators (38). A point mutation in the V3 region (D294G) of PKC α was identified in human pituitary and thyroid tumors (60). Ectopic expression of D294G PKC α in rat embryonic fibroblasts displayed a decreased requirement of serum for proliferation, and these cells formed colonies in soft agar (61). However, this mutant failed to transform human pituitary cell line GH3B6 (60). These observations indicate that the function of PKC α is strictly regulated by intracellular conditions.

If unregulated growth and invasiveness are, in part, regulated by PKC α , it could be a target for anti-cancer therapy. In fact, antisense blocking of PKC α reversed the transformed phenotype of human lung carcinoma cells (62) and glioma cells (63). All-*trans*-retinoic acid (ATRA), a vitamin A derivative, has the ability to reverse the malignant to the normal phenotype and to inhibit cancer invasiveness and unregulated growth, and is used to treat several types of cancer. PKC α appears to be a possible target of ATRA (64). ATRA is shown to bind to PKC α through competition with PS and inhibit its activity.

3.6. Cardiac hypertrophy and angiogenesis. PKC α

regulates the hypertrophic growth of neonatal cardiomyocytes, characterized by enhanced sarcomeric organization, increased cell surface area, increased expression of atrial natriuretic factor, and increased [3 H]leucine incorporation. PKC α antisense oligonucleotides inhibited hypertrophic growth of rat neonatal cardiomyocytes induced by myotrophin (65). In contrast, overexpression of wild-type PKC α induced hypertrophic growth of neonatal cardiomyocytes (66). Dominant negative PKC α antagonized phenylephrine-induced hypertrophic growth and PMA-induced activation of ERK1/2. On the other hand, dominant negative MEK1, an upstream regulator of ERK1/2, inhibited hypertrophy induced by wild-type PKC α . Therefore, PKC α mediates hypertrophic growth of neonatal cardiomyocytes, in part through activation of the ERK1/2-dependent signaling pathway.

Overexpression of PKC α in rat capillary endothelial cells enhanced their migration in response to hepatocyte growth factor (HGF), known as "scatter factor" (67). Antisense study indicates that PKC α is required for migration of human umbilical endothelial cells in response to scratch-wounding (68). PKC α is also implicated in migration of vascular smooth muscle cells, since its down-regulation by antisense oligonucleotides resulted in the inhibition of cell spreading toward fibronectin (16). These findings suggest that PKC α plays an important part in vascular formation.

3.7. Inflammation. The possible implication of PKC α in inflammatory responses has also been suggested. Dominant negative PKC α inhibited bacterial lipopolysaccharide-induced cytokine production by macrophages (61). Production of nitric oxide (NO), an inflammatory mediator induced by lipopolysaccharides in vascular smooth muscle cells, is enhanced by overexpression of PKC α (70). Overexpression of PKC α in the epidermis of transgenic mice by keratin 5 promoter resulted in striking alterations of PMA-induced inflammatory responses, edema and infiltration of neutrophils, and expression of genes implicated in inflammation such as cyclooxygenase-2 and tumor necrosis factor- α (71). Overexpression of PKC α in human keratinocytes had no effect on proliferation or differentiation (72). Consistent with this observation, the sensitivity to PMA-induced tumor promotion in the skin of transgenic mice was identical to that of wild-type mice. Inconsistent with these observations, PKC α appeared to inhibit secretory responses and release of arachidonic acid (AA) metabolites in the mast cell line RBL-2H3 cells (73, 74). The possible involvement of PKC α in the inhibition of inflammatory responses is also suggested in HepG2 human hepatocellular cell line stimulated by interleukin-1 (IL-1). Down-regulation of PKC α by antisense oligonucleotides blocked IL-1-induced expression of I κ B (inhibitor of NF κ B) (75). Therefore, PKC α negatively regulates NF κ B-induced expression of genes involved in a variety of inflammatory responses through induction of I κ B.

4. Conclusion

PKC α was first thought to be activated in a Ca $^{2+}$ -mobilizing signal transduction system. Extensive studies in the past two decades have revealed that it functions in a variety of cell responses including one which is initiated by the tyrosine kinase, and that its regulatory mechanisms are more complicated than originally imagined. It is now clear that localization of PKC α at particular cell compartments

is essential for it to exhibit specific functions. Further exploration of the specific substrates and anchoring proteins will lead to the better understanding of the networks of PKC α signal transduction pathways.

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