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Subcellular Localization of Diacylglycerol-responsive Protein Kinase C Isoforms in HeLa Cells

Julhash U. Kazi, Cho-Rong Kim, and Jae-Won Soh*

Biomedical Research Center for Signal Transduction Networks, Department of Chemistry, Inha University, Incheon 402-751, Korea. *E-mail: soh@inha.ac.kr

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Subcellular localization of protein kinase often plays an important role in determining its activity and specificity. Protein kinase C (PKC), a family of multi-gene protein kinases has long been known to be translocated to the particular cellular compartments in response to DAG or its analog phorbol esters. We used C-terminal green fluorescent protein (GFP) fusion proteins of PKC isoforms to visualize the subcellular distribution of individual PKC isoforms. Intracellular localization of PKC-GFP proteins was monitored by fluorescence microscopy after transient transfection of PKC-GFP expression vectors in the HeLa cells. In unstimulated HeLa cells, all PKC isoforms were found to be distributed throughout the cytoplasm with a few exceptions. PKCα was mostly localized to the Golgi, and PKCγ, PKCδ and PKCζ showed cytoplasmic distribution with Golgi localization. DAG analog TPA induced translocation of PKC-GFP to the plasma membrane. PKCα, PKCζ and PKCθ were also localized to the Golgi in response to TPA. Only PKCδ was found to be associated with the nuclear membrane after transient TPA treatment. These results suggest that specific PKC isoforms are translocated to different intracellular sites and exhibit distinct biological effects.

Key Words: PKC, TPA, HeLa, Translocation, Localization

Introduction

Protein kinase C (PKC) is a multi-gene family of at least 10 protein serine/threonine kinases.1-2 PKC isoforms regulate diverse cellular signaling pathways by phosphorylating their downstream kinases and substrate proteins. Activation of PKC isoforms results in cellular transformation, proliferation, differentiation and tumorigenesis. Certain PKC isoforms are also reported to be involved in growth inhibition and apoptosis.3 These effects might be regulated by their subcellular localization and stimulus-induced translocation.4,5 PKC isoforms consist of an amino-terminal regulatory domain and a carboxy-terminal catalytic domain. Based on the structural similarity and cofactor requirement, PKC isoforms can be subdivided into three groups. Classical PKC isoforms (α, β1, β2, γ) contain two C1 domains and a C2 domain which are regulated by diacylglycerol (DAG) and Ca2+. Novel PKC isoforms (δ, ε, η, θ) are regulated by DAG, but not by Ca2+. These isoforms contain a novel C2 domain that lacks conserved Ca2+ binding residues. Atypical PKC isoforms (ζ, ι) are not responsive to either DAG or Ca2+. These two isoforms lack C1b and C2 domains.1

Binding of DAG to the C1 domains plays an important role in the subcellular localization and activation of classical and novel PKC isoforms. PKC interacting proteins are also thought to be involved in these processes.6 DAG analog phorbol esters, such as 12-O-tetradecanoylphorbol-13-acetate (TPA), can bind to the C1 domains activating classical and novel PKC isoforms. We and several investigators studied the subcellular localization and TPA-induced translocation of PKC isoforms in different cell lines.6,7 The localization patterns of different PKC isoforms might be isofrom and cell line specific. For example, PKCα was found to be localized to the cytoplasm and TPA induced translocation to the plasma membrane in NIH3T3 cells, whereas PKCδ was reported to be translocated from the cytoplasm to the plasma membrane and the nucleus in rat liver WB cells.6,8 PKCδ was shown to be localized to the cytoplasm and the nucleus in unstimulated CHO-K1 cells and TPA induced translocation to the plasma membrane and the nuclear membrane.9 However, we did not observe any nuclear accumulation of PKCδ in NIH3T3 cells.9 Other C1 domain targeting stimuli such as bryostatin 1 also induced translocation of PKC isoforms from the cytoplasm to the plasma membrane.10 While PKC isoforms have distinct roles in various cancers, the localization patterns of PKC isoforms in cancer cells have not been studied well. Recently the subcellular localization and TPA-induced translocation of PKCζ were studied in breast cancer cells.11 Ceramide-mediated translocation of several PKC isoforms was also investigated in human myelogenous leukemia HL-60 cells.12 In this study, we studied the subcellular localization of DAG-dependent PKC isoforms in HeLa cervical cancer cells. We used green fluorescent protein (GFP)-fused PKC isoforms to visualize the localization patterns in living cells.

Experimental Section

Plasmids. pGFP3-PKCα-WT, pGFP3-PKCβ1-WT, pGFP3-PKCβ2-WT, pGFP3-PKCγ-WT, pGFP3-PKCD-WT, pGFP3-PKCD-WT and pGFP3-PKCζ-WT plasmids were described previously.6 pGFP3-PKCO-WT plasmid was constructed by subcloning the BamHI fragment of full length open reading frame of PKCζ isoform into the pGFP3 6 expression vector.

Cell Transfection. HeLa cells were grown in DMEM containing 10% calf serum. Triplicate of 1 × 105 cells in 35 mm plates were transfected by lipofectin (Gibco BRL) with 5 µg of expression plasmid. pGFP3 was used as empty control vector.

Western Blotting. Six hours after transfection, cells were
**Results**

To explore the expression patterns of PKC-GFP and the stability of intact proteins upon TPA treatment, we transfected HeLa cells with pGFP3-PKC-WT constructs or empty control vector. After transient expression, the expression levels of the overexpressed PKC-GFP proteins were examined by Western blotting using anti-GFP antibody, without TPA stimulation and after 10 minutes of TPA stimulation. No significant difference of PKC-GFP protein levels was observed between the stimulated and unstimulated cells (Fig. 1), which suggests that PKC-GFP proteins were expressed as intact protein in unstimulated cells and transient TPA treatment did not alter that protein levels.

pGFP3-PKC-WT constructs were transfected into the HeLa cells to investigate the subcellular localization of PKC isoforms in cervical cancer cells. The subcellular localization of PKC-GFP was observed with fluorescence microscopy after transient expression. Classical PKC isoforms showed homogeneous distribution within the cytoplasm (Fig. 2A, 2B, 2C, and 2D), suggesting that classical PKC isoforms were present in abundant amounts in the cytoplasm of the unstimulated cells and that these isoforms were inactive in absence of agonist. PKCγ was found to be localized to the Golgi with a cytoplasmic distribution (Fig. 2D) while PKCβ1 showed granular distribution (Fig. 2B).

Novel PKC isoforms showed differential localization patterns within this subfamily. For example, PKCδ was distributed th-
PKC isoforms play central roles in several signal transduction pathways which regulate cell proliferation, differentiation and apoptosis. Several PKC isoforms are expressed abundantly in various cancer cells including cervical cancer cells. A number of factors, such as cell type, stimuli and specific isoforms have the distinct functions in PKC-mediated signal transduction pathways. Subcellular localization of PKC isoforms and their substrate specificity may contribute to this diversity. Localization of PKC isoforms might be mediated by the isoform specific cofactors and the interacting proteins. Several investigators have described the subcellular localization of PKC isoforms using various methods. In this study, we investigated the differential localization of PKC isoforms in the form of GFP fusion protein in HeLa cells. Results presented here suggest that inactive PKC isoforms are mostly localized to the cytoplasm and DAG-responsive PKC isoforms are generally translocated to the plasma membrane upon TPA stimulation.

PKCα, PKCβ1 and PKCβ2 were well distributed throughout the cytoplasm in unstimulated cells, indicating that these isoforms are inactive in unstimulated HeLa cells. A portion of PKCγ, PKCδ, PKCε and PKCθ isoforms might be activated in unstimulated cells as they were found to be localized to the cytoplasmic cell organelles such as Golgi and endoplasmic reticulum. Golgi localization of PKCγ and PKCδ was also observed in NIH3T3 cells. However, PKCε was found to be distributed throughout the cytoplasm in NIH3T3 cells, it was localized to the Golgi in HeLa cells. This finding may suggest that PKCε plays a role in unstimulated HeLa cells. PKCε is known to have oncogenic potential. The nuclear localization of PKCδ was reported in CHO-K1 cells. However, we did not observe any nuclear accumulation of this novel PKC isoform either in HeLa cells or in NIH3T3 cells. Recently it was reported that PKCδ contains a carboxy-terminal bipartite nuclear localization signal and was localized to the nucleus in response to the apoptotic signals inducing apoptosis. How, it is still unclear that how PKCδ is localized to the nucleus in certain unstimulated cells.

PKC inhibitors such as UCN-01 induce growth inhibition in cervical cancer cells by inhibiting endogenous PKC isoforms. PKC isoforms are translocated to the plasma membrane and activate several substrate proteins, resulting in cell proliferation. PKC binding proteins such as RACK might be involved in this process. Localization of activated PKCα to cytoplasmic membranous organelles, nuclear membrane and nucleus was observed in different cell lines. In HeLa cells, we observed that PKCα was translocated to the plasma membrane and to the Golgi. PKCε and PKCθ also showed same translocation patterns. Only PKCδ was translocated to the nuclear membrane with plasma membrane and Golgi co-localization. These observations suggest that subcellular localization PKC isoforms is specific to the PKC isoforms as well as to the cell types.

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References