Mini Review

Phospholipase Cε as a potential molecular target for anti-inflammatory therapy and cancer prevention

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Phospholipase Cε (PLCε), encoded by *PLCE1*, is the phosphoinositide-specific PLC regulated by the *ras* proto-oncogene product Ras and its relative Rap1. Like other PLC isoforms, PLCε catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate to yield second messengers, diacylglycerol and inositol 1,4,5-trisphosphate. To understand the physiological function of PLCε, we have created and analyzed genetically-modified mice in which PLCε is inactivated or overproduced. PLCε knockout (KO) mice are resistant to two-stage skin chemical carcinogenesis initiated by 7,12-dimethylbenz[a]anthracene and promoted by phorbol 12-myristate 13-acetate (PMA) and to intestinal tumorigenesis caused by the loss-of-function mutation of the *APC* tumor suppressor gene. In PLCε KO mice, inflammation, such as that induced by a single application of PMA, that associated with tumorigenesis, and that in the elicitation phase of allergic contact hypersensitivity, is also attenuated as compared to that in wild-type mice. Conversely, overexpression of PLCε in the epidermis results in the development of skin inflammation that partly shares the features with human psoriasis. In this article, we summarize the data showing the role of PLCε in tumorigenesis and inflammation and discuss the future directions of the study of PLCε for the development of therapies to control inflammation and to prevent cancer progression.

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Introduction

Phosphoinositide-specific phospholipase C (PLC) plays a pivotal role in regulation of intracellular signaling pathways. Upon activation of cell-surface receptors, such as serpentine receptors (also known as G protein-coupled receptors) and tyrosine kinase receptors, PLCs hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG binds to a variety of its target proteins including

Table 1 Phenotypes of PLCε mutant mice

Mutant mouse types	Experimental models	Phenotypes	References
Knockout	Two-stage skin chemical carcinogenesis (DMBA/PMA)	-Reduced tumor number -Inhibition of malignant progression	10)
	Intestinal tumorigeneis (*APC*$^{Min/+}$ mice)	-Reduced tumor number -Inhibition of malignant progression -Decreased expression of proinflammatory molecules and angiogenic factors	15)
	Hapten-induced contact hypersensitivity (DNFB)	-Suppression of inflammation in elicitation phase -Reduced expression of proinflammatory molecule in the skin -No effect on the sensitization phase	12)
	PMA-induced skin inflammation	-Suppression of inflammation -Reduced expression of proinflammatory molecule in the skin	11)
Transgenic (skin-specific)		-Development of IL-23-dependent psoriasis-like dermatitis accompanied by aberrant infiltration of inflammatory cells, particularly those producing IL-22	13)

protein kinase C (PKC) isoforms and Ras guanyl nucleotide-releasing proteins (RasGRPs) while IP_3 stimulates the intracellular Ca^{2+} stores to release Ca^{2+} ion to the cytosol[1-4]. In mammals, at least 13 PLC isoforms have been identified, and they are classified into six classes (β, γ, δ, ε, ζ, and η) based on the similarities in the structures and the mechanisms for the regulation[1-4].

PLCε was first identified as a downstream target of the *ras* proto-oncogene product, Ras small GTPase[5-6]. Subsequently, Rap1, another member of the Ras family of small GTPases, was also shown as an upstream molecule of PLCε. PLCε interacts with the GTP-bound active form of these small GTPases, and this interaction induces the translocation of PLCε to the plasma membrane and the Golgi apparatus where PIP_2 is hydrolyzed[6-8]. In addition to Ras and Rap1, another small GTPase RhoA and heterotrimeric G proteins α_{12} and $\beta_1\gamma_2$ subunits are capable of activating PLCε[9]. In mammals, PLCε is expressed in non-immune cells such as epidermal keratinocytes, dermal fibroblasts, and epithelial cells, but not in immune cells such as lymphocytes, granulocytes, macrophages, and dendritic cells[10-13].

In this mini-review article, we summarize the results obtained from the study of genetically-modified mice in which PLCε loses its lipase activity or is overproduced (Table 1). We discuss the future directions of the study of PLCε for the development of therapies that control inflammation and prevent cancer progression.

Tumorigenesis

To address the physiological role of PLCε, PLCε knockout mice were created by disrupting one of the exons encoding the domain responsible for its lipase catalytic activity[14]. The first evidence for the role of PLCε in tumorigenesis was provided by the experiments employing the two-stage skin chemical carcinogenesis protocol using 7,12-dimethylbenz[a]anthracene (DMBA) and phorbol 12-myristate 13-acetate (PMA) as an initiator and a promoter, respectively[10]. PLCε KO mice developed only a small number of benign tumors compared to wild-type (WT) mice, and their malignant progression into carcinomas appeared to be suppressed.

The second evidence was provided by the study using *APC*$^{Min/+}$

mice[15], which develop multiple intestinal neoplasms (Min) due to the loss-of-function mutation in the *APC* tumor suppressor gene[16]. Intestinal tumors developed in PLCε-deficient $APC^{Min/+}$ mice were markedly decreased in number, and their malignant progression was strongly suppressed compared to those in PLCε-sufficient $APC^{Min/+}$ mice. Notably, infiltration of inflammatory cells and expression of proinflammatory molecules (e.g., cyclooxygenase-2, chemokine (C-X-C motif) ligand (Cxcl)-1, and Cxcl-2) in the intestinal tumors of PLCε-deficient $APC^{Min/+}$ mice were considerably decreased compared to those in the grade-matched tumors of PLCε-sufficient $APC^{Min/+}$ mice. These results suggest that the oncogenic effects of PLCε are mediated by augmentation of inflammation.

Recent genome-wide studies have identified susceptibility loci of gastric cardia adenocarcinoma and esophageal squamous cell carcinoma in *PLCE1*, the gene encoding PLCε[17,18]. Although the impacts of the single nucleotide polymorphisms identified in these human cases are not understood, these findings suggest that PLCε is involved in tumorigenesis also in humans.

PMA-induced skin inflammation and PLCε activation[11]

In $PLC\varepsilon^{-/-}$ mice, infiltration of leukocytes in the skin after a single application of PMA was substantially suppressed compared to that in $PLC\varepsilon^{+/+}$ mice. This alteration highly correlated with the attenuation of PMA-induced expression of interleukin (IL)-1α in the skin. Such *PLC*ε-dependent IL-1α induction was reproduced in primary-cultured dermal fibroblasts. Similarly, Cxcl-2 induction after PMA treatment in cultured dermal fibroblasts was also found to be *PLC*ε-dependent. These results suggested that PLCε plays a role in PMA-triggered upregulation of cytokine expression in dermal fibroblasts.

The above-presented results also suggested that PLCε is activated in dermal fibroblasts after PMA treatment. In order to examine this possibility, we assayed PLC activity in PMA-stimulated cells by measuring the cytosolic free Ca^{2+} concentration whose increase is mediated by the PLC product IP_3. In $PLC\varepsilon^{-/-}$ fibroblasts, the elevation of the cytosolic free Ca^{2+} concentration after PMA application was substantially attenuated as compared to that in $PLC\varepsilon^{+/+}$ fibroblasts, indicating that PMA activates PLCε. As an upstream activator of PLCε, Rap1 appears to play a major role because Rap1 was activated upon PMA treatment and the siRNA-mediated knockdown of Rap1 expression almost totally abolished PMA-induced PLCε activation. The PMA-induced Rap1 activation requires RasGRP3, which is the DAG/PMA-responsive Rap1 GEF, and PKCs (Fig.1).

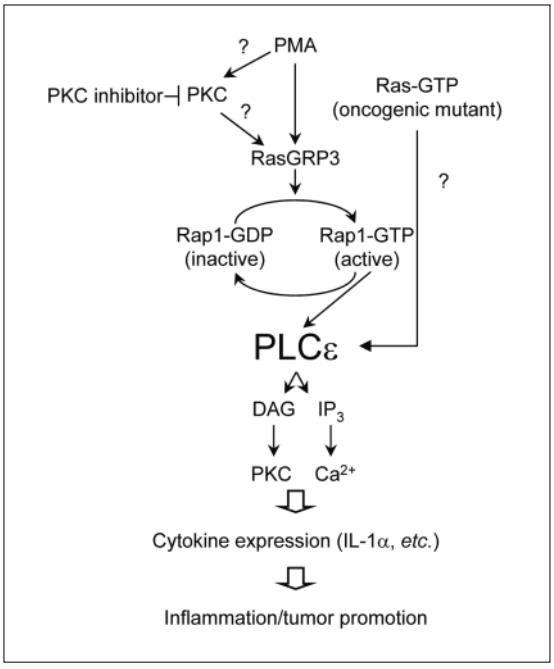

Fig.1 PMA-induced activation of PLCε

PMA activates Rap1 through the DAG/PMA-responsive Rap1 GEF, RasGRP3, and PMA-responsive PKC. The GTP-bound active Rap1 in turn activates PLCε. Oncogenically activated Ras may also be involved in PLCε activation. Activation of PLCε results in the production of proinflammatory molecules such as IL-1α, causing inflammation and promoting tumor formation.

Allergic contact hypersensitivity (CHS)[12]

In the elicitation phase of CHS induced by the hapten 2,4-dinitrofluorobenzene (DNFB), PLCε-deficiency attenuated the expression of proinflammatory cytokines by epidermal keratinocytes as well as dermal fibroblasts *in vivo* without interfering with the infiltration of $CD4^+$ T cells in the hapten-painted sites. Adoptive transfer of $CD4^+$ T cells from DNFB-sensitized $PLC\varepsilon^{-/-}$ mice could induce allergic CHS in naïve mice on the $PLC\varepsilon^{+/+}$ background. On the other hand, naïve mice receiving the hapten-primed $CD4^+$ T cells on the $PLC\varepsilon^{-/-}$ background exhibited reduced CHS response as compared to those on the $PLC\varepsilon^{+/+}$ background (Fig.2). These results indicated that the elicitation phase is PLCε-dependent while the sensitization phase does not require PLCε at all. Experiments using primary-cultured cells demonstrated that both fibroblasts and keratinocytes express proinflammatory cytokine genes after stimulation with T-cell-derived cytokines such as IL-17, IL-4, tumor necrosis factor-α, and interferon-γ in a PLCε-dependent manner. How-

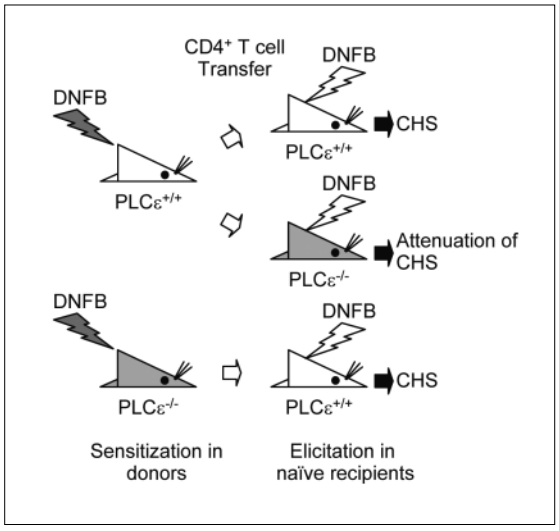

Fig.2 Role of PLCε in the elicitation phase of CHS demonstrated by adoptive transfer of CD4+ T cells

CD4+ T cells were adoptively transferred form the DNFB-sensitized mice on the different *PLCε* background to naïve mice on the different *PLCε* background as illustrated. PLCε-deficiency in the recipient mice attenuated CHS response.

ever, the T-cell-derived cytokines tested failed to function as a ligand that triggers the activation of PLCε, suggesting that certain soluble factors secreted upon cytokine stimulation may mediate PLCε activation leading to proinflammatory gene activation in a *PLCε*-dependent manner.

Inflammation in PLCε-overexpressing transgenic mice[13)]

Role of PLCε in inflammation has been further proven with the transgenic mice overexpressing PLCε in the epidermis using the tissue-specific expression system, in which Cre recombinase induces the expression of the transgene by removing the Cre-recognizable silencing sequences. Epidermis-restricted overexpression was achieved by expressing Cre under the control of the basal layer-specific keratin 5 promoter. The resulting mice spontaneously developed skin inflammation sharing features with human psoriasis, such as formation of adherent silvery scale, excessive growth of keratinocytes, and aberrant infiltration of immune cells to the skin. The development of the skin symptoms in the PLCε transgenic mice was accompanied by increased production of IL-23 from keratinocytes and the accumulation of IL-22-producing CD4+ lymphocytes whose nature has not been fully characterized. IL-23 appeared to be crucial for the skin inflammation caused by PLCε overexpression because intradermal injection of a neutralizing antibody against IL-23 totally reversed these symptoms. These results suggest a possible role of the PLCε-mediated signaling in the pathogenesis of inflammatory diseases involving aberrant IL-23 production, such as psoriasis.

Discussion

As reviewed in this article, our studies using PLCε knockout mice have demonstrated that PLCε plays an important role in regulation of proinflammatory molecule expression in non-immune cells such as keratinocytes and fibroblasts, thereby augmenting inflammation and probably promoting tumorigenesis. The data showing the skin inflammation in the PLCε-overexpressing mice imply that deregulation of the PLCε-mediated signaling may be involved in the pathogenesis of chronic inflammatory diseases. These results suggest that PLCε could be a molecular target for the therapies of inflammatory diseases and for the prevention of carcinogenesis. However, to develop the therapeutic methods targeting PLCε, there are a number of problems to be solved. Which ligands trigger the activation of PLCε in inflammatory processes? How does PLCε operate on inducing proinflammatory cytokine expression? Further studies are underway to address these questions.

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