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 (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG binds to a variety of its target proteins including
Table 1 Phenotypes of PLCε mutant mice

<table>
<thead>
<tr>
<th>Mutant mouse types</th>
<th>Experimental models</th>
<th>Phenotypes</th>
<th>References</th>
</tr>
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<tr>
<td>Knockout</td>
<td>Two-stage skin chemical carcinogenesis (DMBA/PMA)</td>
<td>-Reduced tumor number</td>
<td>10)</td>
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<td></td>
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<td>-Inhibition of malignant progression</td>
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<td></td>
<td>Intestinal tumorigenesis (APC&lt;sup&gt;min&lt;/sup&gt;- mice)</td>
<td>-Reduced tumor number</td>
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<tr>
<td></td>
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<td>-Inhibition of malignant progression</td>
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<td></td>
<td></td>
<td>-Decreased expression of proinflammatory molecules and angiogenic factors</td>
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<td></td>
<td>Hapten-induced contact hypersensitivity (DNFB)</td>
<td>-Suppression of inflammation in elicitation phase</td>
<td>12)</td>
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<td></td>
<td></td>
<td>-Reduced expression of proinflammatory molecule in the skin</td>
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<td>-No effect on the sensitization phase</td>
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<td>PMA-induced skin inflammation</td>
<td>-Suppression of inflammation</td>
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<tr>
<td></td>
<td></td>
<td>-Reduced expression of proinflammatory molecule in the skin</td>
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<td>Transgenic</td>
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<td>-Development of IL-23-dependent psoriasis-like dermatitis accompanied by aberrant infiltration of inflammatory cells, particularly those producing IL-22</td>
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protein kinase C (PKC) isoforms and Ras guanyl nucleotide-releasing proteins (RasGRPs) while IP<sub>3</sub> stimulates the intracellular Ca<sup>2+</sup> stores to release Ca<sup>2+</sup> ion to the cytosol<sup>14</sup>. 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<sup>14</sup>.

PLCε was first identified as a downstream target of the ras proto-oncogene product, Ras small GTPase<sup>56</sup>-<sup>60</sup>. 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 PIP2 is hydrolyzed<sup>60</sup>. In addition to Ras and Rap1, another small GTPase RhoA and heterotrimeric G proteins α12 and βγ2 subunits are capable of activating PLCε<sup>6</sup>. 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<sup>10</sup>-<sup>19</sup>.

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<sup>14</sup>. 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<sup>10</sup>. 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<sup>M<sub>Min</sub></sup>
mice\textsuperscript{15}, which develop multiple intestinal neoplasms (Min) due to the loss-of-function mutation in the APC tumor suppressor gene\textsuperscript{16}. Intestinal tumors developed in PLC\(\varepsilon\)-deficient \(\text{APC}^{\text{Min/}\varepsilon}\) mice were markedly decreased in number, and their malignant progression was strongly suppressed compared to those in PLC\(\varepsilon\)-sufficient \(\text{APC}^{\text{Min/}\varepsilon}\) 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 PLC\(\varepsilon\)-deficient \(\text{APC}^{\text{Min/}\varepsilon}\) mice were considerably decreased compared to those in the grade-matched tumors of PLC\(\varepsilon\)-sufficient \(\text{APC}^{\text{Min/}\varepsilon}\) mice. These results suggest that the oncogenic effects of PLC\(\varepsilon\) are mediated by augmentation of inflammation.

Recent genome-wide studies have identified susceptibility loci of gastric cardia adenocarcinoma and esophageal squamous cell carcinoma in PLCE\(1\), the gene encoding PLC\(\varepsilon\)\textsuperscript{17,18}. Although the impacts of the single nucleotide polymorphisms identified in these human cases are not understood, these findings suggest that PLC\(\varepsilon\) is involved in tumorigenesis also in humans.

**PMA-induced skin inflammation and PLC\(\varepsilon\) activation\textsuperscript{11}**

In \(\text{PLC}\varepsilon^{+}\) mice, infiltration of leukocytes in the skin after a single application of PMA was substantially suppressed compared to that in \(\text{PLC}\varepsilon^{-}\) mice. This alteration highly correlated with the attenuation of PMA-induced expression of interleukin (IL)-1\(\alpha\) in the skin. Such PLC\(\varepsilon\)-dependent IL-1\(\alpha\) 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\(\varepsilon\)-dependent. These results suggested that PLC\(\varepsilon\) plays a role in PMA-triggered upregulation of cytokine expression in dermal fibroblasts.

The above-presented results also suggested that PLC\(\varepsilon\) 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 PLC\(\varepsilon\). As an upstream activator of PLC\(\varepsilon\), 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 PLC\(\varepsilon\) activation. The PMA-induced Rap1 activation requires RasGRP3, which is the DAG/PMA-responsive Rap1 GEF, and PKCs (Fig.1).

**Allergic contact hypersensitivity (CHS)\textsuperscript{12}**

In the elicitation phase of CHS induced by the hapten 2,4-dinitrofluorobenzene (DNFB), PLC\(\varepsilon\)-deficiency attenuated the expression of proinflammatory cytokines by epidermal keratinocytes as well as dermal fibroblasts in vivo without interfering with the infiltration of CD\(4^{+}\) T cells in the hapten-painted sites. Adaptive transfer of CD\(4^{+}\) 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 CD\(4^{+}\) 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 PLC\(\varepsilon\)-dependent while the sensitization phase does not require PLC\(\varepsilon\) 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-\(\alpha\), and interferon-\(\gamma\) in a PLC\(\varepsilon\)-dependent manner. How-
Inflammation in PLCε-overexpressing transgenic mice

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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