Biochemical and Biophysical Research Communications 414 (2011) 106–111

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

PLCε cooperates with the NF-κB pathway to augment TNFα-stimulated CCL2/MCP1 expression in human keratinocyte

Yuko Harada, Hironori Edamatsu, Tohru Kataoka *

Division of Molecular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe, Japan

ARTICLE INFO

Article history:
Received 2 September 2011
Available online 17 September 2011

Keywords:
PLCε
NF-κB
Keratinocyte
Inflammation
CCL2

ABSTRACT

Phospholipase Cε (PLCε) is a unique class of PLC regulated by both Ras family small GTPases and heterotrimeric G proteins. We previously showed by using mice bearing its null or transgenic allele that PLCε plays a crucial role in various forms of skin inflammation through upregulation of proinflammatory cytokine production from keratinocytes. However, molecular mechanisms how PLCε augments cytokine production were largely unknown. We show here using cultured human keratinocyte PHK16-0b cells that induction of the expression of chemokine (C–C motif) ligand 2 (CCL2) following stimulation with tumor necrosis factor (TNF)α, which primarily depends on the activation of the NF-κB pathway, is abrogated by small interfering RNA-mediated knockdown of PLCε. Enforced expression of PLCε causes substantial CCL2 expression and cooperates with low level TNFα stimulation to induce marked overexpression of CCL2, both of which are only partially blocked by pharmacological inhibition of the NF-κB signaling. However, PLCε knockdown exhibits no effect on both the NF-κB-*cis*-element-mediated transcription *per se* and the post-translational modifications of NF-κB implicated in transcriptional regulation, suggesting that PLCε constitutes a yet unknown signaling pathway distinct from the NF-κB pathway. This pathway can cooperate with the NF-κB pathway to achieve a synergistic TNFα-stimulated CCL2 induction in keratinocytes.

1. Introduction

Phosphoinositide-specific phospholipase C (PLC) plays a pivotal role in intracellular signaling by catalyzing the hydrolysis of phosphatidylinositol 4,5-bisphosphate into two vital second messengers, diacylglycerol and inositol 1,4,5-trisphosphate. Among 13 isoforms of PLC, which are organized into six classes: β, γ, δ, ε, ζ, and η [1], PLCε is unique in that its lipase activity is activated by direct association with Ras family small GTPases: Ras, Rap1, and Rap2 [1,2]. Subsequent studies demonstrated that PLCε is also activated by another small GTPase RhoA and heterotrimeric G proteins α12 and β1γ2 subunits [2]. By using *PLCε*$^{-/-}$ mice, homozygous for the allele devoid of the lipase activity, we showed that PLCε plays a crucial role in carcinogenesis and inflammation of the skin [3–7]. In the two-stage skin chemical carcinogenesis using the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as a promoter, *PLCε*$^{-/-}$mice exhibited marked resistance to tumor formation [4]. Subsequent studies showed that TPA-induced skin inflammation, which is intimately involved in tumor promotion, was attenuated in *PLCε*$^{-/-}$ mice [5] and that PLCε is required for efficient production of proinflammatory cytokines from intrinsic skin cells in the elicitation stage of the allergic dermatitis [6]. Moreover, transgenic mice overexpressing PLCε in epidermal keratinocytes spontaneously developed skin inflammation, which correlated well with increased production of cytokines implicated in human inflammatory skin diseases from keratinocytes [7]. These results implied that PLCε in keratinocyte plays a crucial role in skin inflammation through upregulation of proinflammatory cytokine expression. However, molecular mechanisms underlying the PLCε's action on cytokine expression remain to be clarified.

Tumor necrosis factor (TNF)α is a proinflammatory cytokine having pleiotropic functions, such as stimulation of the transcription of the genes encoding proinflammatory cytokines, adhesion molecules, and growth factors as well as the activation of caspases causing apoptosis [8]. Upon binding of TNFα to TNFα receptor-1, the ligand–receptor complex aggregates and recruits TNFR-associated death domain (TRADD), TNFR-associated factor 2 (TRAF2), and receptor-interacting protein (RIP), leading to the activation of the inhibitor of NF-κB (IκB) kinase (IKK) complex, which consists of IKKα, IKKβ, and the regulatory subunit IKKγ [also known as NF-κB essential modulator (NEMO)]. The IKK complex phosphorylates IκBα at Ser32 and Ser36 in its N-terminal regulatory domain, and the phosphorylated IκBα is targeted for ubiquitination and subsequent proteasomal degradation, thereby causing nuclear translocation of NF-κB and transcriptional activation of its target

* Corresponding author. Address: Division of Molecular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Fax: +81 78 382 5399.
E-mail address: kataoka@people.kobe-u.ac.jp (T. Kataoka).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.09.032

genes [8–10]. On the other hand, p65/RelA, the primary NF-κB family member responsible for transcriptional activation of the target genes, is subject to post-translational modifications, such as phosphorylation and acetylation, which are involved in regulation of the transcriptional activity, DNA-binding affinity, and stability. For instance, phosphorylations at Ser276, Ser311, and Ser536 by mitogen- and stress-activated kinase-1/2 (MSK1/2), protein kinase C-ζ (PKCζ), and IKK, respectively, are implicated in regulation of the p65/RelA-mediated transcription [9–11]. Besides NF-κB, TNFα also activates mitogen-activated protein (MAP) kinases, such as Jun N-terminal kinase (JNK) and p38 MAP kinase.

In the present study, we examine the role of PLCε in upregulation of proinflammatory cytokine expression by using a culture of immortalized human keratinocytes. We show that TNFα-stimulated expression of chemokine (C–C motif) ligand 2 [CCL2; also known as monocyte chemotactic protein-1 (MCP-1)], which primarily depends on the activation of the NF-κB pathway, is under the great influence of the PLCε activity. However, PLCε exhibits no effect on both the NF-κB-*cis*-element-mediated transcription *per se* and the post-translational modifications of NF-κB implicated in transcriptional regulation. These results suggest that PLCε constitutes a signaling pathway distinct from the NF-κB pathway and that this pathway and the NF-κB pathway exert a synergistic effect on the TNFα-stimulated CCL2 expression in keratinocytes.

2. Materials and methods

2.1. Inhibitors and antibodies

Cell permeable C3 transferase (CT04; Cytoskeleton), JNK inhibitor II (420119; Calbiochem), SB203580 (559389; Calbiochem), MG-132 (474790; Calbiochem), and BAY11-7085 (196872; Calbiochem) were commercially obtained. Recombinant human TNFα (300-01A) was purchased from PeproTech. Antibodies used are as follows: anti-HA (3F10) (1867423; Roche), anti-IκBα (4812; Cell Signaling Technology), anti-phospho-Ser32-IκBα (2859; Cell Signaling Technology), anti-NF-κB (sc-109; Santa Cruz Biotechnology), anti-phospho-Ser276-NF-κB (3037; Cell Signaling Technology), anti-phospho-Ser311-NF-κB (sc-101748; Santa Cruz Biotechnology), and anti-phospho-Ser536-NF-κB (3033; Cell Signaling Technology).

2.2. Cell culture

PHK16-0b cells, human keratinocytes immortalized with human papilloma virus, were obtained from Japanese Collection of Research Bioresources (JCRB0141) and maintained in Defined Keratinocyte Serum Free Medium supplemented with growth factors (Invitrogen).

2.3. Gene silencing with small interfering RNA (siRNA)

PHK16-0b cells (6×10^6 cells) were transfected with Stealth siRNA (Invitrogen) targeting PLCε (HSS121828) or Gα12 (HSS178466) by electroporation using GenePulser (Bio-Rad) as described before [5].

2.4. Transfection of expression vectors

PHK16-0b cells were transfected by electroporation with the following expression vectors: pFLAG-CMV2-PLCε [12], pEF-BOS-HA-H-Ras(G12V) [12], pRK-myc-RhoA(Q63L) [13], pEF-BOS-HAx3-RhoA [14], pCMV5-Gα12(Q229L) [15], pCMV-Flag-IKKα [16], pGL4.32-[luc2p/NF-κB-RE/Hygro] (Promega), pEF-RL [17], and pEGFP-C1 (invitrogen). pFLAG-CMV2-Rap1A(G12V) was constructed by inserting the *rap1A*(*G12V*) cDNA into pFLAG-CMV2 (Sigma). pEF-BOS-Rap2B(G12V) was constructed by inserting the *rap2B(G12V)* cDNA into pEF-BOS.

2.5. Luciferase reporter assay

pGL4.32[luc2p/NF-κB-RE/Hygro], containing five copies of an NF-κB response element that drive the transcription of the firefly luciferase reporter, and pEF-RL, expressing renilla luciferase as an internal control, were co-transfected into cells and, 3 days later, the luciferase activity was determined using Dual luciferase assay kit (Promega).

2.6. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Total cellular RNA isolation, cDNA synthesis, RT-PCR, and qRT-PCR were performed as described previously [6,7]. Relative mRNA levels of each transcript were determined by the ΔΔCt method with the reference genes, β-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers used are listed in Supplementary Table S1.

2.7. Immunoprecipitaion and immunoblotting

Cells were solubilized in RIPA buffer [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% (w/v) Triton X-100, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, and protease inhibitor cocktail (Roche)] and subjected to immunoprecipitation and/or immunoblotting [18].

2.8. Assay for RhoA activity

RhoA activation was monitored in cells transiently transfected with pEF-BOS-HAx3-RhoA by the pull-down assay using a glutathione *S*-transferase (GST) fusion of the Rho-binding domain of Rhotekin.

2.9. Immunofluorescence

Immunofluorescence analyses were performed essentially as described [18].

2.10. Statistical analyses

Data are expressed as means ± SEMs of triplicate determinations unless otherwise mentioned. Unpaired student's *t*-test was performed for determination of *p* values.

3. Results

3.1. Role of PLCε in TNFα-stimulated CCL2 induction in human keratinocytes

By using immortalized human keratinocyte PHK16-0b cells, we first searched for cytokines whose expression were induced by TNFα stimulation. We examined interleukin (IL)-1α, IL-1β, IL-6, chemokine (C-X-C motif) ligand 8 [CXCL8 (also known as IL-8)], CXCL1 [also known as growth-regulated oncogene-α (Groα)], IL-22, IL-23, chemokine (C–C motif) ligand 20 [CCL20; also known as macrophage inflammatory protein-3α (MIP-3α)], CCL2, granulocyte–macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF), most of which had been found elevated in the inflamed skin of the transgenic mice

overexpressing PLCε in keratinocytes [7]. TNFα treatment caused robust elevation of CXCL8, CXCL1, CCL20, and CCL2, but not IL-1α, IL-1β, and IL-6 (Fig. 1A). The other cytokines were not expressed in PHK16-0b cells (data not shown). Among the TNFα-induced cytokines, only the expression of CCL2 was abrogated by the siRNA-mediated knockdown of PLCε (Fig. 1A and B). The elevation of the CCL2 mRNA started in less than 1 h after TNFα administration and its level reached a peak in around 3 h (Fig. 1C and D). Enforced expression of PLCε substantially elevated the basal expression level of CCL2 (Fig. 1E) and, to our surprise, caused vast (>1000-fold) overexpression of CCL2 in combination with low concentration TNFα stimulation (Fig. 1F). The elevation of CCL2 expression was observed in the inflamed skin of the keratinocyte-specific PLCε transgenic mice [7], suggesting that a similar phenomenon occurs *in vivo* (Fig. 1G). These results support a pivotal role of PLCε in the TNFα-stimulated CCL2 expression in keratinocytes.

3.2. Role of NF-κB in TNFα-stimulated CCL2 induction

We next investigated the nature of the intracellular signaling pathways involved in the TNFα-stimulated expression of CCL2 in PHK16-0b cells. Pretreatment with JNK inhibitor II or SB203580, a p38 MAP kinase inhibitor, had no effect on the TNFα-stimulated CCL2 expression (Fig. 2A). On the other hand, the IKK inhibitor BAY11-7085, which abrogates the ligand-dependent activation of NF-κB [19], entirely blocked the TNFα-stimulated CCL2 expression (Fig. 2B). Moreover, the proteasome inhibitor MG-132, which abrogates NF-κB activation by inhibiting the proteasomal degradation of IκB, also showed a similar effect (Fig. 2C) and enforced expression of IKKα caused substantial elevation of CCL2 (Fig. 2D), confirming the essential role of the NF-κB pathway. Like the TNFα-stimulated CCL2 expression, the IKKα-induced CCL2 expression was abrogated by the PLCε knockdown. On the other hand, the PLCε overexpression-dependent CCL2 elevation was only partially inhibited by BAY11-7085 (Fig. 2E, *left panel*). Also, the synergistic CCL2 elevation by the combination of PLCε overexpression and low concentration TNFα stimulation was partially inhibited by BAY11-7085 (Fig. 2E, *right panel*).

3.3. Role of PLCε on NF-κB cis element-dependent transcriptional activation

To examine whether PLCε plays a role in the TNFα-induced NF-κB activation *per se*, we used a luciferase reporter assay driven by the NF-κB *cis* element. The siRNA-mediated knockdown of PLCε failed to show any effects on both the basal and the TNFα-stimulated transcription (Fig. 3A). Also, TNFα-induced nuclear translocation of NF-κB, which is instrumental in its transcriptional activity [8,10], was unaffected by the PLCε knockdown (Fig. 3B). Furthermore, both the basal and the TNFα-induced phosphorylations at Ser276, Ser311, and Ser526, which are implicated in regulation of NF-κB [9–11], were essentially unaffected by the PLCε knockdown (Fig. 3C). These results indicated that PLCε is not involved in the TNFα-induced NF-κB activation *per se*, suggesting that it constitutes a distinct signaling pathway which modulates the NF-κB pathway in a synergistic manner.

3.4. Role of small GTPases and heterotrimeric G proteins in PLCε-dependent CCL2 expression

Finally, we investigated the upstream regulatory molecules of PLCε that could be involved in the TNFα-stimulated CCL2 expression. Overexpression of the constitutively active mutants of Ras [H-Ras(G12V)], Rap1 [Rap1A(G12V)], and Rap2 [Rap2B(G12V)] all failed to elevate the CCL2 expression (Fig. 4A). In contrast, overexpression of the constitutively active RhoA [RhoA(Q63L)] caused robust elevation of the basal CCL2 expression (Fig. 4A). Although TNFα increased the formation of the GTP-bound active RhoA (Fig. 4B), the siRNA-mediated knockdown of PLCε failed to blunt the RhoA(Q63L)-dependent CCL2 expression (Fig. 4C), indicating that RhoA did not act through PLCε activation. Moreover,

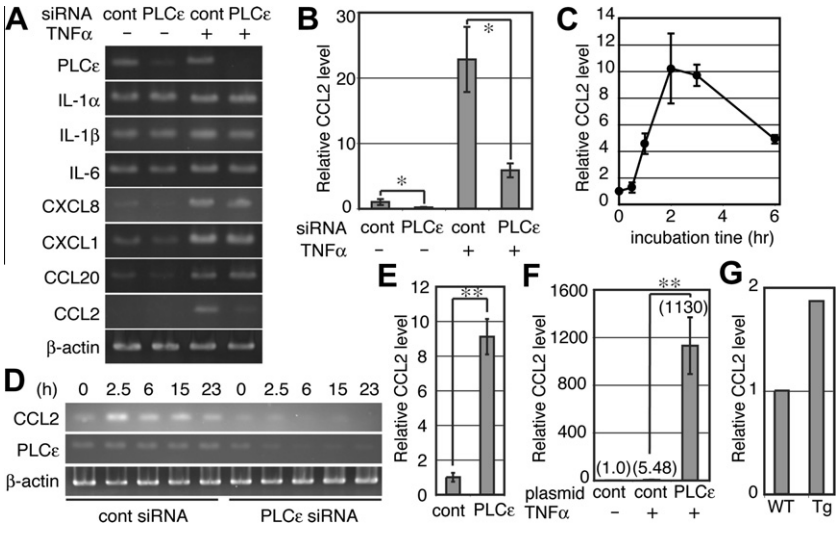

Fig. 1. Analysis of cytokines induced by TNFα stimulation. (A) PHK16-0b cells were transfected with either control or PLCε-specific siRNA, and, 72 h later, stimulated with 100 ng/mL TNFα for 2.5 h. Cytokine mRNA levels were analyzed by RT-PCR. (B) PHK16-0b cells, transfected as in (A), were stimulated with 20 ng/mL TNFα for 2.5 h. Fold induction of the *CCL2* mRNA over unstimulated control cells was determined by qRT-PCR. $^{*}p < 0.05$. (C) PHK16-0b cells were stimulated with 20 ng/mL TNFα and the kinetics of the *CCL2* mRNA induction was analyzed by qRT-PCR. The mRNA levels are shown relative to that of unstimulated cells. (D) PHK16-0b cells, transfected as in (A), were stimulated with 4 ng/mL TNFα for the indicated time periods and subjected to analysis of the CCL2 expression by RT-PCR. (E) PHK16-0b cells were transfected with either pFLAG-CMV2-PLCε (*PLCε*) or the empty vector (*control*) and collected 48 h later for qRT-PCR analysis for the CCL2 expression. The mRNA levels are shown relative to that of control cells. $^{**}p < 0.01$. (F) PHK16-0b cells, transfected as in (E), were stimulated with 4 ng/mL TNFα for 2 h. The CCL2 mRNA levels determined by qRT-PCR are shown as in (B) and the mean values are indicated in parentheses. $^{*}p < 0.05$, $^{**}p < 0.01$. (G) The dorsal skins of wild-type (*WT*) and the keratinocyte-specific PLCε transgenic mice (*Tg*) were collected at postnatal day 26 for qRT-PCR analysis of CCL2 expression. The mRNA levels are shown relative to that of wild-type.

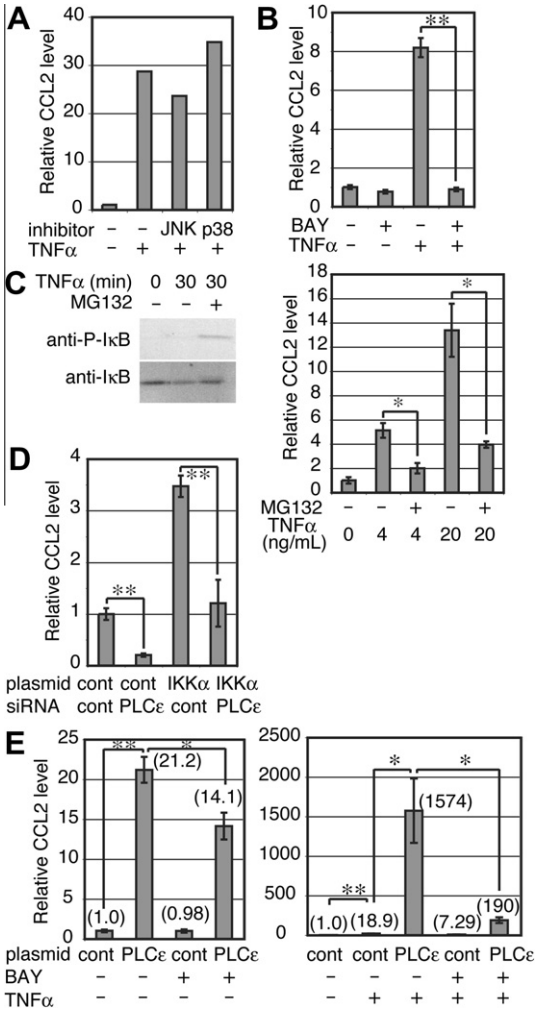

Fig. 2. Involvement of NF-κB in TNFα-stimulated CCL2 expression. (A) PHK16-0b cells were treated with 30 μM JNK inhibitor II or 10 μM SB203580 for 1 h and subsequently stimulated with 20 ng/mL TNFα for additional 2.5 h. Fold induction of the *CCL2* mRNA over unstimulated control cells was determined by qRT-PCR. (B) PHK16-0b cells, pretreated with 20 μM BAY11-7085 for 1 h, were stimulated with 20 ng/mL TNFα for 2 h and subjected to determination of the CCL2 expression by qRT-PCR. Data are shown as in (A). $^{**}p < 0.01$. (C, *left*) PHK16-0b cells, pretreated with 5 μM MG-132 for 1 h, were stimulated with 20 ng/mL TNFα for 30 min and subjected to immunoblotting with anti-phospho-IκB antibody (*upper panel*) and anti-IκB antibody (*lower panel*). (C, *right*) PHK16-0b cells, pretreated with MG-132, were stimulated with the indicated concentrations of TNFα for 2.5 h. The *CCL2* mRNA levels determined by qRT-PCR are shown as in (A). $^{*}p < 0.05$. (D) PHK16-0b cells were co-transfected with a combination of either pCMV-Flag-IKKα (*IKKα*) or the empty vector (*control*) and the indicated siRNAs, and incubated for 72 h. The *CCL2* mRNA levels were determined by qRT-PCR, which are shown relative to that of control cells. $^{**}p < 0.01$. (E) PHK 16-0b cells were transfected with pFLAG-CMV2-PLCε (*PLCε*) or the empty vector (control). Forty-eight hours later, cells were pretreated with 20 μM BAY11-7085 for 1 h and subsequently stimulated with 4 ng/mL TNFα for 2 h. Fold induction of the *CCL2* mRNA over untreated control cells was determined by qRT-PCR. Mean values are shown in parentheses. $^{*}p < 0.05$, $^{**}p < 0.01$.

TNFα-stimulated CCL2 expression was not inhibited by treatment with the RhoA inhibitor C3 transferase (Fig. 4D), ruling out the involvement of RhoA. On the other hand, overexpression of the constitutively active Gα12 [Gα12(Q229L)] caused robust elevation of the basal CCL2 expression (Fig. 4E), which was significantly attenuated by the PLCε knockdown (Fig. 4E). Moreover, siRNA-mediated knockdown of Gα12 caused substantial inhibition of the TNFα-stimulated CCL2 expression (Fig. 4F). These results suggested that Gα12 is at least partially mediates the signal from the TNFα receptor to PLCε, leading to the CCL2 induction.

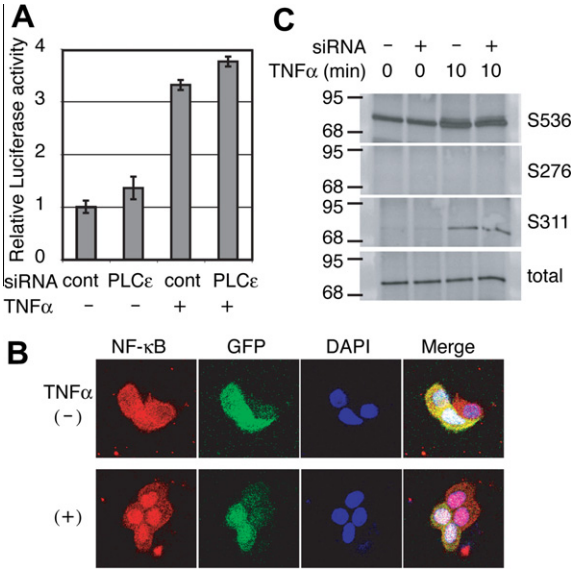

Fig. 3. Effects of PLCε knockdown on NF-κB activities. (A) PHK16-0b cells were co-transfected with a combination of control or PLCε-specific siRNA and both pGL4.32[luc2p/NF-κB-RE/Hygro] and pEF-RL. Seventy-two hours later, cells were stimulated with 20 ng/mL TNFα for 2.5 h and subjected to the luciferase assay. The luciferase activities are shown relative to that of unstimulated control cells. (B) PHK16-0b cells, co-transfected with PLCε-specific siRNA and the green fluorescent protein expression vector pEGFP-C1, were incubated for 72 h. After stimulation with 20 ng/mL TNFα for 10 min, NF-κB and the nuclei were visualized with anti-NF-κB antibody (*red*) and 4′,6-diamidino-2-phenylindole (DAPI) (*blue*), respectively. (C) PHK16-0b cells, transfected by siRNAs as in Fig. 1A, were stimulated with 20 ng/mL TNFα for 10 min. NF-κB protein was immunoprecipitated from the cell extracts by using anti-NF-κB antibody and subjected to immunoblotting with various phospho-specific antibodies recognizing the indicated phosphorylated residues.

4. Discussion

In this study, we have shown that PLCε plays a pivotal role in the TNFα-stimulated CCL2 expression in cultured human keratinocytes, PHK16-0b cells, which are known to maintain intrinsic properties of normal keratinocytes such as the ability to differentiate into keratin 1- and involucrin-positive keratinocytes when cultured in the high calcium-containing media (data not shown). Further, our results suggest that PLCε constitutes a yet unknown signaling pathway which is distinct from but effectively cooperate with the NF-κB pathway to achieve a synergistic induction of the CCL2 expression upon TNFα stimulation. Among the NF-κB target cytokines tested in this study, only CCL2 exhibits a PLCε-dependency of TNFα-induced expression (Fig. 1). This suggests that other *cis*-acting elements in the *CCL2* gene promoter may be targeted by the hypothetical PLCε pathway to induce synergistic activation of *CCL2* transcription with the NF-κB *cis* element. Our result that the PLCε overexpression-dependent CCL2 elevation is only partially inhibited by pharmacological blockade of the NF-κB signaling may also support this notion. The *CCL2* gene promoter is reported to contain *cis*-acting elements for AP-1, Sp-1, C/EBP, and NF-AT [20–22]. The involvement of AP-1 homodimeric or heterodimeric complexes consisting of the c-Jun, c-Fos, and ATF family members [23] is likely to be ruled out considering that the JNK and p38 MAP kinase inhibitors fail to block the TNFα-stimulated CCL2 expression. However, it must be noted that we cannot rule out the possibility that PLCε might be involved in regulation of yet unknown aspects of the NF-κB pathway which are not addressed in this study. As for upstream regulators, Gα12 seems to play a certain role in mediating the signal from the TNFα receptor to PLCε, leading to the CCL2 induction. This function of Gα12 must be independent of its activity to interact with RhoGEFs and thereby activate

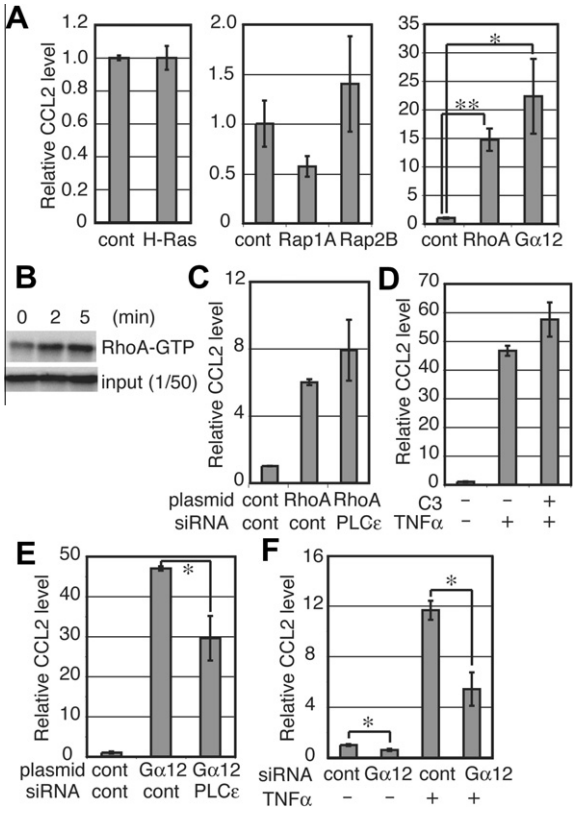

Fig. 4. Effects of various small GTPases and Gα12 on the TNFα-stimulated CCL2 expression. (A) PHK16-0b cells were transfected with either pEF-BOS-HA-H-Ras(G12V), pEF-BOS-Rap2B(G12V), pRK-myc-RhoA(Q63L), pCMV5-Gα12(Q229L), or pEF-BOS (*control*) and cultured for 48 h. The *CCL2* mRNA levels determined by qRT-PCR are shown relative to that of control cells. $^{*}p < 0.05$, $^{**}p < 0.01$. (B) PHK16-0b cells, transfected with pEF-BOS-HAx3-RhoA, were stimulated with 20 ng/mL TNFα and harvested at the indicated time points. The GTP-bound RhoA was quantitated by the pull-down assay. (C) PHK16-0b cells were co-transfected with a combination of either pRK-myc-RhoA(Q63L) (*RhoA*) or pFLAG-CMV2 (*control*) and the indicated siRNAs, and cultured for 72 h. The *CCL2* mRNA levels determined as in (A). (D) PHK16-0b cells, pretreated with or without 1.0 μg/mL C3 transferase for 1.5 h, were stimulated with 20 ng/mL TNFα for 2.5 h and subjected to determination of the CCL2 expression by qRT-PCR. The mRNA levels are shown relative to that of unstimulated control cells. (E) PHK16-0b cells were co-transfected with pCMV5-Gα12(Q229L) or pEF-BOS (*control*) and the indicated siRNAs, and cultured for 72 h. The *CCL2* mRNA levels determined by qRT-PCR are shown as (A). $^{*}p < 0.05$. (F) PHK16-0b cells were transfected with the indicated siRNAs and, 72 h later, stimulated with 20 ng/mL TNFα for 2.5 h and subjected to determination of the CCL2 expression by qRT-PCR. Data are shown as in (D). $^{*}p < 0.05$.

RhoA [2] because the involvement of RhoA in the TNFα-stimulated CCL2 expression is ruled out. It is presently unknown whether TNFα stimulation leads to Gα12 activation.

The chemokine CCL2 regulates the migration and infiltration of monocytes/macrophages, memory T lymphocytes, and natural killer cells [24]. CCL2-deficient mice show impairment of both the monocyte recruitment and the cytokine expression [25]. Moreover, CCL2 is reported to be involved in various inflammatory diseases, such as asthma and rheumatoid arthritis [24]. Considering the extraordinary production of CCL2 caused by the synergistic action of PLCε and weak TNFα stimulation, activation of PLCε may exert a profound effect on the development of inflammation by predisposing keratinocytes highly sensitive to certain proinflammatory stimuli, which is likely to be the case with the keratinocyte-specific PLCε transgenic mice [7]. TNFα is known to play an important role in the development of chronic inflammatory diseases and targeting TNFα by neutralizing antibodies has been found quite effective in curing chronic inflammatory diseases like rheumatoid arthritis [8]. Further studies including those clarifying the nature of the hypothetical PLCε pathway may provide a new insight into the regulatory mechanism of inflammation.

Acknowledgments

We are grateful to Drs. Jun Ninomiya-Tsuji, Xi He, Takaya Satoh and Hiroshi Itoh for providing the mammalian expression vectors. This work was supported by Grants-in-Aid to TK (20390080, 23390071, and Global COE Program A08) and to HE (22790290) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.09.032.

References

[1] C. Song, C.D. Hu, M. Masago, et al., Regulation of a novel human phospholipase C, PLCε, through membrane targeting by Ras, J. Biol. Chem. 276 (2001) 2752–2757.
[2] P.G. Suh, J.I. Park, L. Manzoli, et al., Multiple roles of phosphoinositide-specific phospholipase C isozymes, BMB Rep. 41 (2008) 415–434.
[3] H. Edamatsu, N. Takenaka, L. Hu, et al., Phospholipase Cε as a potential molecular target for anti-inflammatory therapy and cancer prevention, Inflamm. Regen., in press.
[4] Y. Bai, H. Edamatsu, S. Maeda, et al., Crucial role of phospholipase Cε in chemical carcinogen-induced skin tumor development, Cancer Res. 64 (2004) 8808–8810.
[5] S. Ikuta, H. Edamatsu, M. Li, et al., Crucial role of phospholipase Cε in skin inflammation induced by tumor-promoting phorbol ester, Cancer Res. 68 (2008) 64–72.
[6] L. Hu, H. Edamatsu, N. Takenaka, et al., Crucial role of phospholipase Cε in induction of local skin inflammatory reactions in the elicitation stage of allergic contact hypersensitivity, J. Immunol. 84 (2010) 993–1002.
[7] N. Takenaka, H. Edamatsu, N. Suzuki, et al., Overexpression of phospholipase Cε in keratinocytes upregulates cytokine expression and causes dermatitis with acanthosis and T-cell infiltration, Eur. J. Immunol. 41 (2011) 202–213.
[8] B.B. Aggarwal, Signalling pathways of the TNF superfamily: a double-edged sword, Nat. Rev. Immunol. 3 (2003) 745–756.
[9] S.T. Smale, Hierarchies of NF-κB target-gene regulation, Nat. Immunol. 12 (2011) 689–694.
[10] A. Oeckinghaus, M.S. Hayden, S. Ghosh, Crosstalk in NF-κB signaling pathways, Nat. Immunol. 12 (2011) 695–708.
[11] J. Ruland, Return to homeostasis: downregulation of NF-κB responses, Nat. Immunol. 12 (2011) 709–714.
[12] T.G. Jin, T. Satoh, Y. Liao, et al., Role of the CDC25 homology domain of phospholipase Cε in amplification of Rap1-dependent signaling, J. Biol. Chem. 276 (2001) 30301–30307.
[13] R. Habas, Y. Kato, X. He, Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1, Cell 107 (2001) 843–854.
[14] T. Aoki, S. Ueda, T. Kataoka, et al., Regulation of mitotic spindle formation by the RhoA guanine nucleotide exchange factor ARHGEF10, BMC Cell Biol. 10 (2009) 56.
[15] M. Nagao, Y. Kaziro, H. Itoh, The Src family tyrosine kinase is involved in Rho-dependent activation of c-Jun N-terminal kinase by Gα12, Oncogene 18 (1999) 4425–4434.
[16] J. Ninomiya-Tsuji, K. Kishimoto, A. Hiyama, et al., The kinase TAK1 can activate the NIK-IκB as well as the MAP kinase cascade in the IL-1 signalling pathway, Nature 398 (1999) 252–256.
[17] Y. Sun, J. Yamauchi, Y. Kaziro, et al., Activation of c-*fos* promoter by Gβγ-mediated signaling: involvement of Rho and c-Jun N-terminal kinase, J. Biochem. 125 (1999) 515–521.
[18] D. Wu, M. Tadano, H. Edamatsu, et al., Neuronal lineage-specific induction of phospholipase Cε expression in the developing mouse brain, Eur. J. Neurosci. 17 (2003) 1571–1580.
[19] J.W. Pierce, R. Schoenleber, G. Jesmok, et al., Novel inhibitors of cytokine-induced IκBα phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects *in vivo*, J. Biol. Chem. 272 (1997) 21096–21103.
[20] Y. Wang, G.K. Rangan, B. Goodwin, et al., Lipopolysaccharide-induced MCP-1 gene expression in rat tubular epithelial cells is nuclear factor-κB dependent, Kidney Int. 57 (2000) 2011–2022.
[21] O. Sekine, Y. Nishio, K. Egawa, et al., Insulin activates CCAAT/enhancer binding proteins and proinflammatory gene expression through the phosphatidylinositol 3-kinase pathway in vascular smooth muscle cells, J. Biol. Chem. 2007 (2002) 36631–36639.

[22] R. Kojima, H. Taniguchi, A. Tsuzuki, et al., Hypertonicity-induced expression of monocyte chemoattractant protein-1 through a novel cis-acting element and MAPK signaling pathways, J. Immunol. 184 (2010) 5253–5262.
[23] J. Hess, P. Angel, M. Schorpp-Kistner, AP-1 subunits: quarrel and harmony among siblings, J. Cell Sci. 117 (2004) 5965–5973.
[24] S.L. Deshmane, S. Kremlev, S. Amini, et al., Monocyte chemoattractant protein-1 (MCP-1): an overview, J. Interferon Cytokine Res. 29 (2009) 313–326.
[25] B. Lu et al., Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice, J. Exp. Med. 187 (1998) 601–608.