

Disruption of Protein Kinase C η Results in Impairment of Wound Healing and Enhancement of Tumor Formation in Mouse Skin Carcinogenesis¹

Kazuhiro Chida,² Takeshi Hara, Takaaki Hirai, Chieko Konishi, Kenji Nakamura, Kazuki Nakao, Atsu Aiba, Motoya Katsuki, and Toshio Kuroki

Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan [K. C., T. Ha., T. Hi.]; Department of Cancer Cell Research, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan [C. K.]; Mitsubishi Kagaku Institute of Life Sciences, Tokyo 194-8511, Japan [K. Nakam.]; RIKEN Center for Developmental Biology, Hyogo 650-0047, Japan [K. Nakao]; Department of Molecular and Cellular Biology, Kobe University Graduate School of Medicine, Hyogo 650-0017, Japan [A. A.]; National Institute of Basic Biology, Aichi 444-8585, Japan [M. K.]; and Gifu University, Gifu 501-1193, Japan [T. K.]

ABSTRACT

We have generated a mouse strain lacking protein kinase C (PKC) η to evaluate its significance in epithelial organization and tumor formation. The PKC η -deficient mice exhibited increased susceptibility to tumor formation in two-stage skin carcinogenesis by single application of 7,12-dimethylbenz(a)anthracene (DMBA) for tumor initiation and repeated applications of 12-O-tetradecanoylphorbol-13-acetate (TPA) for tumor promotion. The tumor formation was not enhanced by DMBA or TPA treatment alone, suggesting that PKC η suppresses tumor promotion. Epidermal hyperplasia induced by topical TPA treatment was prolonged in the mutant mice. The enhanced tumor formation may be closely associated with the prolonged hyperplasia induced by topical TPA treatment. In the mutant mice, after inflicting injury by punch biopsy, wound healing on the dorsal skin, particularly reepithelialization, was significantly delayed and impaired in structure. Impairment of epithelial regeneration in wound healing indicates a possibility that PKC η plays a role in maintenance of epithelial architecture. Homeostasis in epithelial tissues mediated by PKC η is important for tumor formation *in vivo*. We propose that PKC η is involved in tumor formation modulated by regulation of proliferation and remodeling of epithelial cells *in vivo*.

INTRODUCTION

PKC³ is a family of phospholipid-dependent serine/threonine kinases that are activated by interactions with polar head groups of membrane lipids produced by various extracellular stimuli (reviewed in Refs. 1–3). The family consists of 10 isoforms, which are classified into three groups based on their structure and activation mechanisms. The conventional PKC α , β I, β II, and γ isoforms are activated by intracellular transducers Ca²⁺ and DG, the latter of which is produced by phospholipase C. The novel PKC δ , ϵ , η , and θ isoforms are activated by DG in the absence of Ca²⁺. The atypical PKC ζ and λ isoforms are not activated by Ca²⁺ or DG. The expression pattern of the isoforms depends on the tissue, cell type, and differentiation state. Potent mouse skin tumor promoters, such as phorbol diester TPA, strongly bind to and activate PKCs by substituting for DG, indicating that PKC isoforms may be causally involved in tumor formation.

Of these isoforms, PKC η was originally cloned from skin cDNA libraries (4, 5) and is expressed predominantly in epithelial tissues (6). In the skin, it is localized in the epidermal granular layer (6, 7). Overexpression of PKC η induces differentiation of human keratinocytes (8). PKC η is activated specifically by CS that is produced in the

granular layer of the epidermis (9). Furthermore, CS induces cell differentiation in mouse and human keratinocytes in culture (10, 11), induces scaling in the mouse epidermis (12, 13), and inhibits tumor promotion in mouse skin carcinogenesis (13). These observations imply that PKC η plays roles in epidermal differentiation and tumor formation. However, multiple isoforms of PKC are expressed simultaneously in epidermal keratinocytes, and a specific function of these isoforms *in vivo* remains unclear.

To clarify functions of a protein *in vivo*, one of the promising strategies is gene targeting in ES cells and subsequent generation of knockout mice (14). Some PKC knockout mice exhibited impairment of a variety of cell functions, especially in the central nervous system and immune systems (15–21). PKC γ -null mice show deficits in spatial and contextual learning (15). Disruption of PKC ϵ induces supersensitivity of γ -aminobutyric acid receptors to ethanol and allosteric modulators (16). PKC β -null mice develop an immunodeficiency characterized by impaired humoral immune responses (17). PKC θ -null mice show deficits in T-cell activation (18). Disruption of PKC δ prevents B-cell tolerance and proliferation (19, 20). PKC ζ -null mice show phenotypic alterations in secondary lymphoid organs (21). PKC α -null mice show enhanced insulin signaling through phosphatidylinositol 3'-kinase in muscles (22). However, no report is available on PKC η or on tumor formation in these knockout mice with PKC isoforms.

In the present study, to obtain further insight into the role of PKC η in tumorigenesis of epithelial cells, we generated mice that lack this enzyme and subjected them to tumor formation in two-stage skin carcinogenesis. Furthermore, we examined the wound healing process after skin injury to analyze maintenance of epithelial tissue architecture.

MATERIALS AND METHODS

Generation of PKC η -deficient Mice. The mouse PKC η gene (*Prkch*) is a unigene, containing 14 exons spanning 197 kb at 62 Mb distal from the centromere of chromosome 12 (Ensembl gene ID, ENSMUG21108), in which exon 9 encodes the ATP-binding site essential for enzymatic activity. Genomic DNA clones including exons 5–9 from nucleotide numbers 614–1279 of PKC η cDNA were isolated previously (23). The targeting vector was constructed using the following fragments (Fig. 1A): a 4.3-kb *Bgl*II-*Sal*I fragment located 5' of exon 8; a 4.6-kb *Xba*I-*Eco*RV fragment 3' of exon 9; a neomycin resistance gene (*neo*^r); and a diphtheria toxin A gene (*DT-A*) for negative selection. The linearized vector was electroporated into 129/SvJ-derived CCE ES cells (24, 25). DNA from G418-resistant ES clones was digested with *Kpn*I or *Ssp*I and hybridized with 5'- or 3'-probe (Fig. 1A). A targeted clone was injected into C57BL/6J blastocysts, and chimerical mice were generated. Males were mated with C57BL/6J females, and DNA from tail biopsies of the agouti offspring was analyzed to confirm the germ-line transmission of the mutant allele by Southern blot analysis and PCR. Heterozygous mutant (*Prkch*^{+/-}) mice with a genetic background of 129/SvJ and C57BL/6J were interbred to generate homozygous (*Prkch*^{-/-}) mice lacking PKC η . Experiments were carried out with strict adherence to guidelines for minimizing distress in experimental animals.

Received 9/16/02; accepted 3/12/03.

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¹ Supported by a Grant-in-Aid for Scientific Research on Priority Areas (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a grant from Japan Health Sciences Foundation.

² To whom requests for reprints should be addressed, at Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan. Phone/Fax: 81-3-5841-8152; E-mail: acchida@mail.ecc.u-tokyo.ac.jp.

³ The abbreviations used are: PKC, protein kinase C; DG, diacylglycerol; TPA, 12-O-tetradecanoylphorbol-13-acetate; CS, cholesterol sulfate; DMBA, 7,12-dimethylbenz(a)anthracene; RT-PCR, reverse transcription-PCR; PCNA, proliferating cell nuclear antigen; ES, embryonic stem.

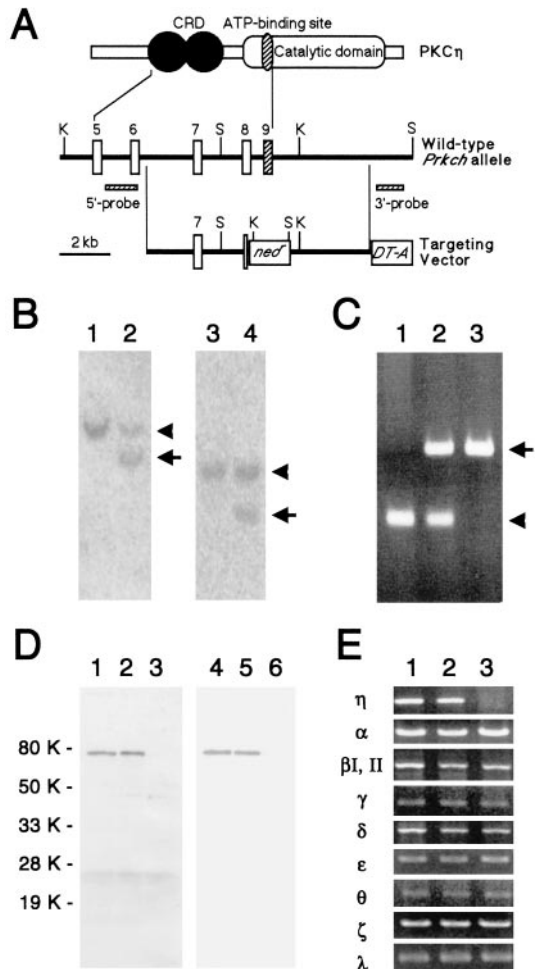


Fig. 1. Targeted disruption of *Prkch*. **A**, targeting strategy. Schematics of PKC η , an allelic region of *Prkch* that contains exons 5–9 (boxes), and a targeting vector are shown. A cysteine-rich domain (CRD, shaded double circle) in the regulatory domain and an ATP-binding site (hatched ellipse) in the catalytic domain of PKC η are indicated. The target exon 9 is shown as a hatched box. The sites of restriction endonucleases are as follows: K, *Kpn*I; and S, *Ssp*I. The targeting vector contains *neo*^r to disrupt exon 8 partly and exon 9 completely, which encodes the NH₂ terminus of the catalytic domain. The *Prkch* 5'- and 3'-probes are shown as hatched rectangles. **B**, Southern blotting of DNA fragments of a targeted ES cell line (Lanes 2 and 4) and the parental cell line (Lanes 1 and 3), using 5'-probe (left panel, Lanes 1 and 2) or 3'-probe (right panel, Lanes 3 and 4). After digestion with *Kpn*I, the 5'-probe hybridizes 10-kb (arrowhead) and 8-kb (arrow) bands as wild-type and targeted alleles, respectively. **C**, PCR for genotyping mice. Genomic DNA was isolated from the tails of mice derived from intercrosses of *Prkch*^{+/-} mice. Lane 1, wild type; Lane 2, heterozygous; Lane 3, homozygous. Amplicons of PKC η exon 9 (arrowhead, 175 bp) and *neo*^r (arrow, 460 bp) are indicated. The absence of an amplicon of exon 9 derived from the wild-type allele of *Prkch* and the presence of an amplicon derived from the *neo*^r gene indicate homozygosity. **D**, immunoblotting of epidermal PKC η with an antibody against the NH₂ terminus (Lanes 1–3) or COOH terminus (Lanes 4–6) region of PKC η . Lanes 1 and 4, wild-type mice; Lanes 2 and 5, heterozygous mice; Lanes 3 and 6, homozygous mice. **E**, RT-PCR of PKC isoforms in the epidermis. Lanes 1–3, wild-type, *Prkch*^{+/-}, and *Prkch*^{-/-} mice, respectively.

PCR for Genotyping. The allele-specific primer sets are as follows: *Prkch* exon 9, 5'-GTGATGCTTGCCAGGATAAAG-3' and 5'-GGAGTCTGA-AAGCAGCAGAAG-3' (GenBank accession number D90402, nucleotide numbers 1164–1336); and *neo*^r, 5'-TCCTGCCGAGAAAGTATCCA-3' and 5'-GTCAAGAAGGCGATAGAAGG-3' (accession number U43612, nucleotide numbers 1064–1523). Amplification was carried out using 35 cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The amplicon corresponding to a band of 175 bp is generated from exon 9, and the targeted amplicon corresponding to a band of 460 bp is generated from *neo*^r.

RT-PCR. RNAs containing poly(A) were isolated from the epidermis of newborn mice. Reverse transcription was performed using an oligo(dT) primer. The resulting cDNA was used as a template for PCR using 20 cycles.

The following primer sets were used, and the sizes of each amplicon are also indicated: (a) PKC α , 5'-GTCCTACCCAAATCCTTG-3' and 5'-TACTGCACCTTTCGCAAGATTGGGTGC-3' (GenBank accession number M25811, nucleotide numbers 1920–2271), 352 bp; (b) PKC β /II, 5'-AAACATCTGGGATGGGGTGACAACCAAG-3' and 5'-CTTGCCTTCGAGTTTCTCCAGTCAATATAC-3' (accession number X53532, nucleotide numbers 1519–1881), 363 bp; (c) PKC γ , 5'-GCAACTGGAATCCGGGCTCTACGTCCG-3' and 5'-CCTCAAACCTTCTGGAGGAGGTCAGTTGTC-3' (accession number L28035, nucleotide numbers 819–1246), 428 bp; (d) PKC δ , 5'-AAGGACATCATGGAGAAGCTATTCGAGAGGG-3' and 5'-AATGTCAGGAATTGCTCAAACCTGGG-3' (accession number X60304, nucleotide numbers 1733–2035), 303 bp; (e) PKC ϵ , 5'-GACCTTGGTGTACTCCAGACAAAATCACC-3' and 5'-CAAGCGTTTGGCCTGGCCTGGCCGGACTTCC-3' (accession number AF028009, nucleotide numbers 922–1219), 298 bp; (f) PKC η , 5'-GAAGTCGTCTACCCCACTGGCTCCATGAAG-3' and 5'-GAGTTGCAATTCCGTGACACATAGGAAAAG-3' (accession number D90402, nucleotide numbers 1767–2108), 342 bp encoded in exons 12–14 of *Prkch*; (g) PKC θ , 5'-GTGAGAGGAGACATCCGCCAGCATCCTTGTTCG-3' and 5'-GGAGCAAATGAGAGTCTCCATCCCTGGG-3' (accession number D11091, nucleotide numbers 1958–2209), 252 bp; (h) PKC ζ , 5'-CATCAGGACAACCCTGACATGAACAC-3' and 5'-CACGGACTCAGCAGACAGCAGAAGTGGG-3' (accession number M94632, nucleotide numbers 1382–1408), 418 bp; and (i) PKC λ , 5'-CACCAGGGAGAGTGGTAAAGCGTCGTCCAG-3' and 5'-GGTCAGGATTGTCAGAGCTCCAACG-3' (accession number D28577, nucleotide numbers 720–1396), 677 bp.

Immunoblotting. The epidermis was prepared from frozen back skin (26). Epidermal extracts were subjected to SDS-PAGE, and the separated proteins were electrically blotted onto a nitrocellulose membrane. PKC η was detected using a rabbit antibody that recognizes the NH₂ terminus (27) or the COOH terminus (Santa Cruz Biotechnology, Santa Cruz, CA) of PKC η .

Carcinogenesis Experiments. Female 6-week-old mice were used. All animals were housed under a 12-h light/12-h dark cycle in a controlled atmosphere. The dorsal skin was shaved 1 week before DMBA treatment, and appropriate chemicals dissolved in 0.2 ml of acetone were applied to the shaved area. Eighteen mice from each group (Table 1) were used for the two-stage carcinogenesis protocol described previously (13). DMBA (100 μ g) was applied topically as an initiator 1 week before promotion. TPA (10 μ g) was repeatedly applied once a week for 20 weeks.

Epidermal Hyperplasia. Female 8-week-old mice were used. The dorsal skin was shaved 1 week before TPA treatment. After topical application of TPA (10 μ g) to the shaved area, the mouse was killed by cervical dislocation, and the dorsal skin was removed for histological examinations. Tissues were fixed in 4% formaldehyde and then embedded in paraffin. Sections (2–3- μ m thick) were subjected to standard H&E staining or immunohistochemistry using an anti-PCNA monoclonal antibody (Dako Japan, Kyoto, Japan).

Wound Healing Analysis. Mice were anesthetized, and their dorsal skins were shaved and cleaned with 70% ethanol. Two full-thickness excisions

Table 1 Tumor formation in two-stage skin carcinogenesis

Eighteen mice (female, 6 weeks old) were used for each group. Tumor formation in all mice except for groups 7–12 was initiated with 100 μ g of DMBA. One week after initiation, the mice were given 10 μ g of TPA once a week for 20 weeks. All of the mice used were alive for the duration of the carcinogenesis experiments.

Group	Initiation	Promotion	<i>Prkch</i>	Tumor formation at 20 wks	
				Incidence (%)	Mean no. of tumors/mouse \pm SD
1	DMBA	TPA	+/+	22	0.39 \pm 0.78
2	DMBA	TPA	+/-	17	0.28 \pm 0.67
3	DMBA	TPA	-/-	78	4.3 \pm 6.7 ^a
4	DMBA		+/+	5.6	0.06 \pm 0.24
5	DMBA		+/-	0	0
6	DMBA		-/-	5.6	0.11 \pm 0.47 ^b
7		TPA	+/+	0	0
8		TPA	+/-	0	0
9		TPA	-/-	0	0
10			+/+	0	0
11			+/-	0	0
12			-/-	0	0

^a Significantly different ($P < 0.02$, t test) from group 1 or 2.

^b Not significantly different ($P > 0.6$) from group 4 or 5.

were obtained from the back of each mouse using a punch biopsy instrument with 2-mm inner diameter by a previously reported method (28), with a slight modification. The wounds were left open and monitored daily. Complete healing was determined based on the following criteria: full closure; colorless reepithelialization; and smooth surface of both healed wounds in the mouse.

RESULTS

PKC η Targeting. We constructed a targeting vector in which exon 9 of *Prkch* was replaced by *neo^r* and obtained ES cell lines in which a single homologous recombination event had occurred at the *Prkch* locus (Fig. 1, A and B). Genotyping revealed generation of *Prkch*^{-/-} mice (Fig. 1C). Thirteen pairs of *Prkch*^{+/-} mice that were hybrids of 129/SvJ and C57BL/6J generated 870 offspring in 10 months, of which 226 were *Prkch*^{+/+}, 437 were *Prkch*^{+/-}, and 207 were *Prkch*^{-/-}. These are consistent with the Mendelian inheritance pattern, indicating that mice homozygous for the disrupted *Prkch* allele are not subject to prenatal lethality.

By immunoblotting, an 80-kDa band corresponding to the native PKC η was clearly detected in epidermal proteins from wild-type and *Prkch*^{+/-} mice, but not from *Prkch*^{-/-} mice (Fig. 1D). In these mutant mice, significant accumulation of flanking molecules of PKC η was not detected. In addition, expression levels of other PKC isoforms in the epidermis of *Prkch*^{-/-} mice were determined by RT-PCR and found not to be significantly different from those of the wild-type mice (Fig. 1E).

Ninety-six percent of *Prkch*^{-/-} mice with a mixed background of 129/SvJ and C57BL/6J were alive, developed, appeared healthy, and of comparable weight with wild-type littermates. However, 3.8% of the *Prkch*^{-/-} mice showed fatal abnormalities, including a lower body weight at one-third that of normal mice and defective eyelid opening at around 2 weeks of age (data not shown). These phenotypes emerged unpredictably in each *Prkch*^{-/-} mouse. Wild-type and *Prkch*^{+/-} mice did not exhibit these phenotypes in breeding. These results may suggest that some genes associated with PKC η functions exist in genetic combinations of 129/SvJ and C57BL/6J.

When interbred, *Prkch*^{-/-} mice produced normal-sized litters that suckled normally and were weaned without difficulty. No difference in apparent behavior in breeding, feeding, and social activity among

the three genotypes was observed. The oldest *Prkch*^{-/-} mice were more than 30 months of age and appeared indistinguishable from their wild-type littermates.

Tumor Formation. To examine the potential effect of PKC η disruption on tumor formation, we performed two-stage skin carcinogenesis. In *Prkch*^{-/-} mice, tumors appeared at 8 weeks of promotion and then increased continuously in number (Fig. 2). The incidence of tumor-bearing mice reached 78% and 4.3 tumors/mouse at 20 weeks of promotion (Table 1). In wild-type and *Prkch*^{+/-} mice, tumors appeared around 10 weeks of promotion, and lower incidences (22% and 17%, respectively) and fewer tumors (0.39 and 0.28 tumor/mouse, respectively) were observed at 20 weeks. All tumors were papillomas. Evidently, *Prkch*^{-/-} mice were significantly more susceptible to skin tumor formation than the wild-type and *Prkch*^{+/-} mice ($P < 0.02$).

DMBA treatment alone without the promotion stage induced tumors at 5.6% incidence and development of 0.06 and 0.11 tumor/mouse in *Prkch*^{-/-} and wild-type mice, respectively; no tumor developed in *Prkch*^{+/-} mice (Table 1). No significant difference ($P > 0.6$) in the number of tumors formed among the three genotypes was observed. Repeated treatments with TPA or acetone as a solvent control did not induce any tumor formation in any of the genotypes. These results suggest that PKC η functions as a negative regulator of the promotion stage.

Moreover, repeated treatments with DMBA (100 μ g) at 4-week intervals induced tumor formation at 20 weeks at an incidence of 39% and 0.61 tumor/mouse in *Prkch*^{-/-} mice, 44% and 0.67 tumor/mouse in *Prkch*^{+/-} mice, and 39% and 1.1 tumor/mouse in wild-type mice. There were no significant differences ($P > 0.3$) among the three genotypes. These data suggest that PKC η is not involved in mutational events in the epidermis.

Epidermal Hyperplasia. We examined epidermal hyperplasia to determine the potential effect of PKC η disruption on keratinocyte proliferation *in vivo*. A single topical application of TPA at 10 μ g induced formation of 40- μ m-thick hyperplastic epidermis on day 2 in all genotypes (Fig. 3). The epidermal thickness returned to the basal level of 15 μ m within 7 days in wild-type mice. However, in *Prkch*^{-/-} mice, hyperplasia lasted up to 10 days. On day 7, basal layer cells highly expressed PCNA. These data suggest that PKC η functions as a negative regulator of keratinocyte proliferation in the epidermis *in vivo*.

Wound Healing of Punched Skin. Effects of PKC η disruption on the wound healing process were examined. In this study, we used congenic mice backcrossed with C57BL/6J for eight generations. In wild-type and *Prkch*^{+/-} mice, contraction of the wound completed around day 5, reepithelialization proceeded, and complete healing with a smooth surface required around 11 and 9 days in the male and female groups, respectively (Table 2). In the *Prkch*^{-/-} mice, healing was delayed for approximately 2 more days, although the contraction of the wound was completed in almost the same period of time as those of the wild-type and *Prkch*^{+/-} mice.

As shown in Fig. 4, a healed wound on day 12 in male wild-type and *Prkch*^{+/-} mice showed full recovery of the tissue structure, which consisted of the dermal connective tissue and epidermis with a two-keratinocyte layer. In *Prkch*^{-/-} mice, although the wound healed macroscopically on day 14, epidermal remodeling was still proceeding, with extensive hyperplasia and papilla structure of epithelial tissues. PCNA was mainly detected in basal cells but not in suprabasal cells, similar to the case of normal tissues. Inflammation was also proceeding. Many leukocytes and PCNA-positive fibroblasts were observed in the repaired dermis. However, no differences in repair of connective tissue were observed among the three genotypes of mice 5 days after injury (data not shown). These results suggest that PKC η

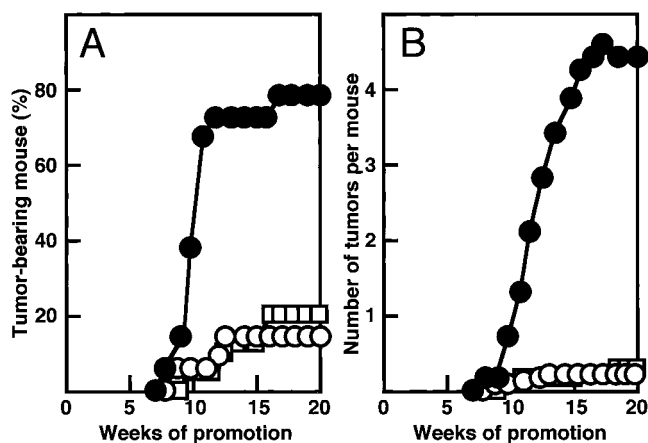


Fig. 2. Time-dependent changes of tumor formation in two-stage skin carcinogenesis. Data for *Prkch*^{-/-} mice (●; group 3 in Table 1), *Prkch*^{+/-} mice (○; group 2 in Table 1), or wild-type mice (□; group 1 in Table 1) are shown. Tumors were initiated with DMBA (100 μ g) and promoted by repeated topical applications of TPA (10 μ g) once a week for 20 weeks. A, incidence of tumors (percentage of tumor bearers). B, average number of tumors/mouse. *Prkch*^{-/-} mice were significantly different from wild-type mice in the number of tumors at weeks 10, 15, and 20 ($P < 0.02$) as determined by Student's *t* test. *Prkch*^{+/-} mice were not significantly different from wild-type mice at weeks 10, 15, and 20 ($P > 0.6$).

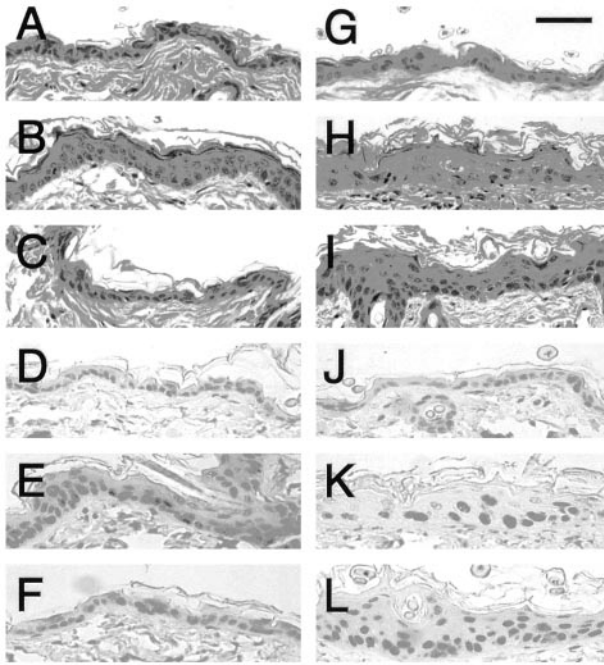


Fig. 3. Epidermal hyperplasia induced by topical treatment with TPA (10 μ g). Wild-type mice, A–F; *Prkch*^{-/-} mice, G–L. H&E staining (A–C and G–I) and immunostaining with anti-PCNA antibody (D–F and J–L) are shown. Representative images on day 0 (A, D, G, and J), day 2 (B, E, H, and K), and day 7 (C, F, I, and L) after treatment are shown. Thickened epidermis was observed in B, H, and I. Cells strongly stained by the anti-PCNA antibody were observed in E, K, and L. Bar, 50 μ m.

Table 2 Wound healing in PKC η -deficient mice

Prkch mutant mice were backcrossed to C57BL/6J for eight generations. Wounds were made at the center dorsal skin of 8-week-old mice using a punch biopsy instrument. Wound healing was monitored daily. All of the mice were alive for the duration of the wound healing experiments.

Group	Sex	<i>Prkch</i>	No.	Healing duration (days)
				Mean \pm SD
A	Male	+/+	16	11.1 \pm 2.1
B		+/-	27	11.0 \pm 1.8
C		-/-	17	13.5 \pm 2.0 ^a
D	Female	+/+	14	9.4 \pm 1.8
E		+/-	25	9.2 \pm 1.6
F		-/-	16	11.1 \pm 1.9 ^b

^a Significantly different ($P < 0.01$, t test) from group A or B.

^b Significantly different ($P < 0.05$) from group D or E.

regulates growth of keratinocytes in remodeling of epithelial tissues during the wound-healing process.

DISCUSSION

The absence of PKC η has little effect on the viability, development, and fertility of a mouse, although a fatal abnormal phenotype is observed very rarely. In the epidermis, although the expression levels of other PKC isoforms are not changed, a possibility of compensation of PKC η functions by other isoforms cannot be excluded. Because gene targeting results in loss of function, the apparent resulting normal phenotype in a knockout mouse may imply that a backup system may function for homeostasis in an animal. Existence of multiple PKC isoforms within a cell could be important in regulation of a lipid-dependent signal transduction (29). This hypothesis may be tested by generating multiple deletions. For example, to study the epidermis, it is now possible to generate mice that are doubly or more multiply deficient in PKC η , α , δ , ϵ , and ζ isoforms, which are expressed in squamous cells.

PKC has been suggested to play essential roles in tumor formation in mouse multiple-stage skin carcinogenesis, which is one of the most studied *in vivo* approaches in experimental cancer research (reviewed in Refs. 30 and 31). The initiation is attributed to defined molecular events such as mutations of specific genes, e.g., c-H-*ras* (32, 33), whereas the promotion stage is attributed to long-term modulation of intracellular signal processing through activation of PKC by tumor promoters and progression to malignant conversion of tumors (31, 34). In transgenic mice with PKC β II and ϵ isoforms, sensitivities to colon carcinogenesis and epidermal carcinoma formation, respectively, increase (35, 36). However, transgenic mice with PKC δ are resistant to tumor promotion (37). These findings, together with those for the PKC η knockout mice in this study, indicate that functions of PKC isoforms vary in tumor formation.

Previous reports suggest that PKC η is involved in differentiation of epithelial cells and that its activation is specifically mediated by CS *in vitro* and *in vivo*. Although repeated applications of CS to the skin stimulate terminal differentiation of the epidermis *in vivo* (12, 13), we found that the PKC η -deficient mice respond to CS in the form of scaling induction (data not shown), indicating that not only PKC η but also other isoforms respond to the differentiation-associated lipid. In fact, CS activates PKC ϵ as well as PKC η in a cell-free system (10). In the PKC η knockout mice, signal transduction on terminal differentiation may be mediated by PKC ϵ .

The prolonged epidermal hyperplasia in the PKC η -deficient mice induced by TPA treatment may be closely associated with increased tumor formation. Overexpression of PKC η inhibits cell growth in culture due to blockage of cell cycle progression from G₁ to S phase (38, 39). Furthermore, PKC η phosphorylates p21, an inhibitor of cyclin-dependent protein kinase, and inhibits cyclin-dependent protein kinase 2, resulting in inhibition of cell cycle progression of keratinocytes in culture (40). However, in TPA-treated epidermis, lack of PKC η did not affect inductions of ornithine decarboxylase and c-Jun (data not shown), which are thought to be important in tumor promotion. In addition, there was no significant difference in phosphorylations of mitogen-activated protein kinases, extracellular signal-regulated kinase 1/2, and p38 (data not shown). PKC η might function as an inhibitor of cell cycle machinery *in vivo*, rather than as a signaling molecule in gene expression through the mitogen-activated protein kinase pathway.

Disruption of PKC η impaired epithelial regeneration during cutaneous wound repair. Wound healing is a complex process requiring the collaborative functions of many different tissues and cells, including those involved in inflammation and regeneration of defective cells, and finally resulting in remodeling (reviewed in Ref. 41). One of the mechanisms underlying all of these process is the actions of

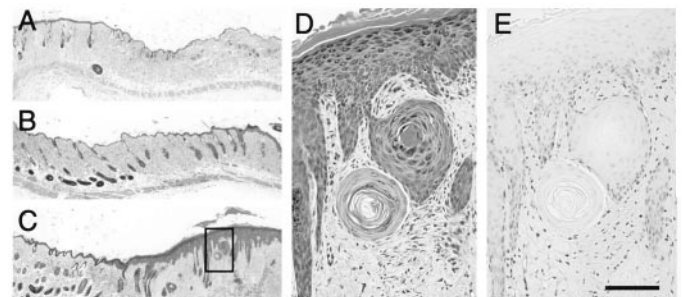


Fig. 4. Wound healing in male mice of wild-type (A), *Prkch*^{+/-} (B), and *Prkch*^{-/-} (C) with C57BL/6J background. Skin wound healing on day 12 (A and B) or day 14 (C–E) after infliction of a 2-mm-diameter wound with a punch biopsy instrument. H&E staining (A–D) and anti-PCNA antibody staining (E) are shown. Boxed area in C is enlarged in D and E. Bar, 100 μ m.

various growth factors. Epidermal growth factor is known to stimulate migration and proliferation of keratinocytes, whereas transforming growth factor β inhibits their growth. However, no significant differences in mRNA levels of epidermal growth factor and transforming growth factor $\beta 1$ in the skin were observed between wild-type and PKC η mutant mice (data not shown). Some recent works using knockout mice clearly showed importance of inflammation in wound healing (42–44). Additional studies are required to clarify whether PKC η is involved in the production of and/or response to inflammatory cytokines in epithelial regeneration.

Most human cancers originate from epithelial tissues. Studies using epithelial tissues of animal models are important in understanding human carcinogenesis. PKC η knockout mice can be useful tools for this purpose. Additional studies are necessary to elucidate molecular and cellular mechanisms by which PKC η , as well as other PKC isoforms, proliferation, differentiation, and carcinogenesis of epithelial cells are regulated.

ACKNOWLEDGMENTS

We thank Dr. M. Seiki (Institute of Medical Science, The University of Tokyo) for helpful discussions, H. Otsuka and T. Nakada for technical assistance, and I. Sato for secretarial assistance.

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