OVEREXPRESSION OF PROGRANULIN INCREASES CELLS IN GROWTH PHASE, BUT IS INSUFFICIENT TO INDUCE SKIN TUMOR FORMATION

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Abstract
Progranulin (PGRN), a multifunctional secreted growth factor, is highly expressed in various types of human cancers. However, it is still unknown whether upregulation of PGRN is a cause or a result of cancerous transformation. To assess the consequences of enhanced PGRN expression *in vivo*, we generated transgenic mice in which PGRN is specifically expressed in keratinocytes using the Cre/lox system. PGRN overexpression in the epidermis promoted the entry of basal cells to the proliferation phases, as revealed by Ki-67 immunostaining, but was insufficient to facilitate it to the mitotic phase, as revealed by phosphohistone H3 immunoreactivity. Accordingly, we observed no significant difference in the incidence of spontaneously occurring and chemically induced skin tumors between the transgenic and wild-type mice. These results suggest that overexpression of PGRN alone is insufficient to cause cancerous transformation of keratinocytes.

Key words : progranulin, growth factor, signal transduction, tumorigenesis, apoptosis

Introduction

Progranulin (PGRN) is a pluripotent secreted growth factor implicated in tumorigenesis[1, 2)], tissue maintenance[3, 4)], early embryonic development[5)], sexual differentiation of the brain[6)], modulation of human immunodeficiency virus transcription via Tat protein[7, 8)], chondrocyte differentiation[9)], and neuronal differentiation[10)]. In mammals, PGRN is composed of a signal sequence followed by 7 1/2 tandem repeats of a 12 cysteinyl granulin (GRN) or epithelin (EPI) motif[1)]. The isolation of approximately 6 kDa GRN peptides preceded the isolation of PGRN. GRN peptides were isolated from several biological sources, such as inflammatory cells[11)], bone marrow[12)], and urine[13)]. Although GRN peptides are structurally very similar to one another, their biological activities are not the same ; GRNA/EPI1 peptide is a mitogen for keratinocytes, whereas GRNB/EPI2 peptide antagonizes GRNA/EPI1 activity[14)]. The intact precursor of these peptides is mitogenic to a variety of cultured cells, and is called granulin/epithelin precursor (GEP)[15)], proepithelin[16)], PC cell-derived growth factor[17)], and acrogranin[18)]. Epithelial transforming growth factor (TGFe) appears to be a partial fragment of PGRN[19)].

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Because PGRN expression is elevated in various types of cancer cell lines and human cancers, PGRN has been intensively studied in relation to carcinogenesis. PGRN is highly expressed in invasive cancer cell lines and clinical specimens including breast, ovarian, renal cancers as well as in gliomas[20]. Attenuating the expression level of PGRN mRNA levels of highly tumorigenic progranulin-expressing cell lines using an antisense approach greatly suppresses tumor progression. Consistently, PGRN activates the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3´-kinase (PI3K), pathways[15,21], both of which are central to the regulation of cell proliferation. However, because the PGRN receptor has not yet been identified, it is still unclear how PGRN activates these signaling pathways.

PGRN and GRNs are also implicated in tissues repair, balancing host defenses and cell proliferation by exerting opposing activities[4]. Upon injury, PGRN is induced in dermal fibroblasts and endothelia to promoter fibroblast proliferation and neovasularization[3]. PGRN is proteolysed to generate GRN peptides by elastase released from neutrophils that accumulate in sites of inflammation. GRNs inhibit cell proliferation, but promote further neutrophil accumulation via interleukin 8 secretion[4]. Some fractions of PGRN are protected from proteolysis by forming a complex with secretory leukocyte protease inhibitor (SLPI) and facilitate cell proliferation[4]. Application of purified PGRN to wounded areas promotes healing processes in SLPI-deficient mice, in which wound healing processes are severely impaired[3, 4].

No mutations in the *PGRN* gene have been so far identified in human cancers. However, it has been recently reported that mutations in the *PGRN* gene cause a subtype of familial frontotemporal lobar degeneration (FTLD) with tau-negative and ubiquitin-positive inclusions[22, 23]. FTLD is a common dementia under age 65, second only to Alzheimer's disease, and is characterized by regional degeneration and/or atrophy of the frontal and lateral lobes with progressive changes in social, behavioral and/or language functions. It remains to be elucidated how loss of function mutations of PGRN cause FTLD.

Despite a wealth of studies pointing to a close relationship between high expression of PGRN and cancerous transformation, it is still unclear whether upregulation of PGRN is a cause or a result of cancerous transformation. To directly address this question, we generated transgenic mice in which PGRN is specifically expressed in the basal cells of the epidermis, but we could not find a direct correlation of tumor incidence and PGRN expression in keratinocytes.

Materials and Methods

Generation of transgenic mice

Full-length human PGRN cDNA was introduced into the *Hin*d III site of the CAG-CAT vector, which contains the chicken β-actin gene promoter with the cytomegalovirus enhancer (CAG) followed by a loxP-flanked stop cassette encoding chloramphenicol acetyltransferase (CAT) and a rabbit β-globin polyadenylation signal. The resultant CAG-CAT-PGRN transgene was microinjected into the pronuclei of C57BL/6 zygotes as described. The zygotes were transferred into the oviducts of pseudopregnant female mice to obtain 85 founder mice. The founders were screened for the presence of the CAG-CAT-PGRN transgene by PCR and Southern blotting. For PCR, a 630-base pair fragment spanning between the CAT and PGRN genes was amplified with a forward primer, 5´-CAGCCATACCACATTTGTAGAGG-3´, and a reverse primer, 5´-ACCCACGGAGTTGTTACCTGATC-3´. For Southern blotting, genomic DNA was digested with *Eco*RI, probed with a PGRN cDNA fragment, and visualized with the Gene Images Random-Prime Labeling and Detection System (GE Healthcare UK Ltd., Buckinghamshire, UK). As a result, we found that 20 out of 85 founders carried the transgenes. We established three transgenic lines — lines 11, 61 and 70 — from the transgene-carrying founders and deposited these lines in the RIKEN mouse bank under accession numbers CDB0419T-1, CDB0419T-2, and CDB0419T-3, respectively.

K5-PGRN mice were obtained by crossing the CAG-CAT-PGRN mice with *k5-Cre* mice, in which Cre recombinase is expressed under the control of the keratin 5 promoter. Elimination of the CAT gene and correct alignment of the CAG and PGRN genes were confirmed by PCR with a primer pair of 5´-GCTGGTTATTGTGCT-

GTCTCATC-3´ (forward) and 5´-ACCCACGGAGTTGTTACCTGATC-3´ (reverse) under the following conditions : 50 cycles at 94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec, giving rise to a 412-base pair fragment. The Institutional Review Board of the Akita University School of Medicine approved all animal experiments, and all subsequent animal experiments adhered to the "Guidelines for Animal Experimentation" of the University.

Quantitative real-time RT-PCR

Newborn mouse skins were dissected and treated with 100 ng/ml dispase (Roche Diagnostics, Mannheim, Germany) for 2 hours at 37°C to separate the epidermis and dermis. Total RNA was prepared from the isolated epidermis using the High Pure RNA Tissue Kit (Roche Diagnostics) according to the manufacturer's protocol. The isolated RNA was reverse-transcribed using SuperScript III (Invitrogen, Tokyo, Japan) with random nanomers (Takara Bio Inc., Shiga, Japan). Real-time PCR amplification was carried out on a Light Cycler (Roche Diagnostics) using SYBR Premix Ex Taq (Takara Bio Inc.). Primers for PGRN were : forward, 5´-ACACTGTGTGTGACCTGATCCAGAG-3´ ; reverse, 5´-CGACTGTAGACGGCAGCAGGTA-3´. Primers for mouse glyceraldehyde 3-phosphate dehydrogenase (Gapdh) were : forward, 5´-AAATGGTGAAGGTCGGTGTG-3´ ; reverse, 5´-TGAAGGGGTCGTTGATGG-3´. The expression of PGRN was normalized to that of Gapdh.

Generation of PGRN-speãfic antibody

Two rabbits were immunized with a synthetic peptide, CLRREAPRWDAPLRDPALRQ, corresponding to the C-terminal 20 amino acids of human PGRN. After collecting sera, their specificity was determined by Western blotting and immunoprecipitation, and it was found that the sera specifically recognized PGRN, but not GRN peptides (data not shown).

Tissue histology and immunohistochemistry

For histological analysis, tumors and dorsal skin samples at various postnatal days were fixed in 4% paraformaldehyde for 24 hours at 4°C, embedded in paraffin, and sectioned at 5 μm. Hematoxylin and eosin staining was performed using standard procedures. Immunohistochemical staining was performed using an avidin-biotin immunoperoxidase technique. In brief, paraffin embedded sections were deparaffinized in four changes of xylene and progressively rehydrated through gradients of ethanol. Endogenous peroxidase activity was quenched with 0.3% H_2O_2 in anhydrous methanol for 20 minutes. For antigen retrieval, slides were microwaved for a total of 9 minutes (3 minutes × 3 times) in citrate buffer (pH 6.0) and blocked with 10% normal goat serum (NITIREI Biosciences, Tokyo, Japan). The slides were then incubated with primary antibodies to keratin 5 (COVANCE, Berkeley, CA, USA), keratin 10 (COVANCE ; 1 : 500), and phosphohistone H3 (SIGMA, Tokyo, Japan ; 1 : 500) at room temperature for 1 hour. Biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) was used as a secondary antibody in 1 × PBS containing 2% bovine serum albumin at room temperature for 30 minutes. After a 10 minute incubation with Streptavidin-HRP (Dako, Glostrup, Denmark), the slides were incubated for 20 minutes at room temperature with a 3-amino-9-ethyl carbazole solution (Dako). Sections were counter-stained with hematoxylin and mounted with fluorescence mounting medium (Dako).

The Ki67 index is the ratio of the number of Ki67-positive cells over the total cell number. To calculate the Ki67 index, the number of Ki67-positive cells and the total cell number in the basal layer of the epidermis from eight independent eye fields (860 × 1,155 μm^2 / field) per mouse were counted under a light microscope. Both cell numbers were calculated from three pairs of wild-type and transgenic mice (24 eye fields in total).

Two-stage mouse skin carãnogenesis

The chemical carcinogenesis experiment started with 10 6-week-old wild-type mice and 11 age and sex-matched *k5-PGRN* transgenic mice. The dorsal skin was shaved with an electric clipper. The shaved area was treated with 100 μg of 7, 12-dimethylbenzanthracene (DMBA ; SIGMA) dissolved in 100 μl acetone as an tumor initiator, followed by topical treatment with 10 μg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA ; SIGMA) in 100 μl ethanol once a week. Because tumor incidence was very low even after 20 TPA treatments, a second

shot of DMBA (100 μg) was applied followed by TPA (10 μg) treatment to the 30th week. Mice treated with DMBA or TPA only were used as controls. For quantitative analysis, tumors with a diameter larger than 1 mm were counted.

Western blot analysis

Mouse back skins were homogenized with a pestle in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P40, and 0.25% sodium deoxycholate) containing protease and phosphatase inhibitor cocktails (Roche) and centrifuged to obtain cell lysates. The protein concentrations of the lysates were measured using the BCA Protein Assay Kit (PIERCE, Rockford, IL, USA). Equal amounts of the cell lysates were electrophoresed on an 8%-10% SDS-polyacrylamide gel and then electrically transferred to Hybond-P polyvinylidene difluoride membranes (GE Healthcare). After blocking in 1 × Tris-buffered saline containing 0.1% Tween 20 (TBST) and 5% dry fat milk, the membranes were incubated with primary antibodies against ERK, phospho-ERK, Akt, phospho-Akt, p38, phospho-p38, cleaved caspase 3 (Cell Signaling Technology, Beverly, MA, USA), β-tubulin (SIGMA), or PGRN. After washing in 1 × TBST, the membranes were incubated with appropriate secondary antibodies conjugated with horseradish peroxidase. Bands were detected using the ECL Plus Western Blotting Detection System (GE Healthcare UK Ltd.).

Results

Generation of keratinocyte-speãfic PGRN transgenic mice

To investigate the consequences of PGRN overexpression *in vivo*, we generated keratinocyte-specific PGRN transgenic mice using the Cre/lox system. As shown in Fig. 1A, in the transgenic CAG-CAT-PGRN strain, the transgene-derived PGRN is not expressed until a loxP-flanked stop cassette encoding chloramphenicol acetyltransferase (CAT) is excised by Cre-mediated recombination. After mating with *Keratin 5*-Cre transgenic mice (*k5*-Cre), in which Cre recombinase is under the control of the *keratin 5* promoter[24], the PGRN gene expression is directed to the basal layer of the epidermis and the outer root sheath of hair follicles. We selected three CAG-CAT-PGRN mouse lines, nos. 11, 61, and 70, which have high copies of the transgene as revealed by Southern blot (Fig. 1B) and polymerase chain reaction (PCR) analysis (Fig. 1C). These CAG-CAT-PGRN mice were crossed with *k5-Cre* mice to generate *k5-PGRN* transgenic mice. Elimination of the stop cassette of the CAT gene was confirmed by PCR using a primer pair spanning between the CAG and PGRN genes (Fig. 1D). Quantitative real-time PCR analysis showed that the expression level of *PGRN* in the epidermis of *k5-PGRN* transgenic mice was about three times higher than that of wild-type littermates (Fig. 1E). The enhanced expression of PGRN in *k5-PGRN* mice was also confirmed by Western blot analysis (Fig. 1F) and immunohistochemistry on sections from back skin of transgenic mice using an anti-PGRN antibody (Fig. 1G) that can discriminate PGRN from GRN peptides. Consistent with data in cultured cells[15,21], activated forms of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and Akt kinase were increased in *k5-PGRN* mice compared with wild-type littermates (Fig. 1F), indicating that the exogenously introduced PGRN was functional in the transgenic mice. The data described below were obtained with the transgenic mice derived from line no. 61 and their wild-type littermates. We obtained similar results from mice derived from line nos. 11 and 71.

Overexpression of PGRN is insuffiãent to induce mitosis in keratinocytes *in vivo*

Based on several lines of evidence that the expression of PGRN is upregulated in many tumor cell lines and human tumors[2], we predicted that *k5*-PGRN transgenic mice would show an increased incidence of spontaneous tumor formation. We monitored 10 wild-type and 10 *k5*-PGRN mice for spontaneous tumorigenesis. Contrary to our prediction, no skin tumors were detected on both the wild-type and *k5*-PGRN mice during a 16-month observation period.

We next compared susceptibility to chemical carcinogens between wild-type and transgenic mice. A two-stage chemical carcinogenesis protocol using DMBA as an initiator and TPA as a promoter was applied to 10 wild-type and 11 sex-matched *k5*-PGRN mice. No tu-

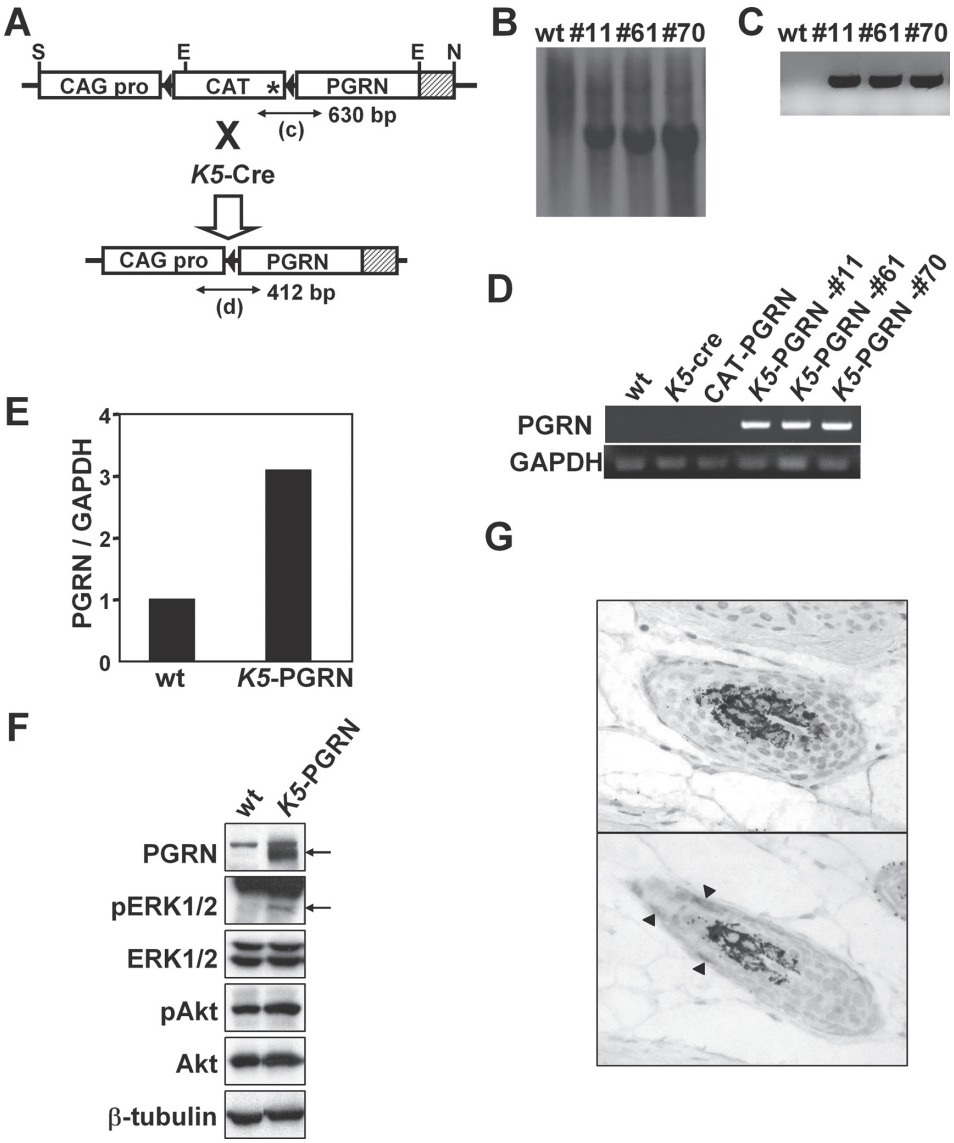

Fig. 1. Generation of keratinocyte-specific PGRN transgenic mice. A : Schematic representation of the CAG-CAT-PGRN and CAG-PGRN transgenes. The CAG-PGRN transgene is generated by the elimination of the CAT gene through Cre recombination. The positions and lengths of the primer pairs for genotyping are indicated by bidirectional arrows. E, *Eco*RI ; N, *Not*I ; S, *Sal*I. Asterisk, a stop codon ; filled triangles, loxP sites ; hatched box, rabbit β-globin polyadenylation signal. B : Genotyping of CAG-CAT-PGRN transgenic mice (nos. 11, 61, and 70) by Southern blot analysis. *Eco*RI digested-tail DNAs were hybridized with a full-length PGRN cDNA. wt, wild-type. C : Genotyping of CAG-CAT-PGRN transgenic mice by PCR with the primer pair indicated in A. D : Genotyping of CAG-PGRN transgenic mice with the primer pair indicated in A. E : Quantitative real-time RT-PCR for *PGRN* expression in the epidermis. The *PGRN* expression level was normalized to *Gapdh* expression. F : Western blot analysis of the skin specimens from wild-type and *k5-PGRN* mice for PGRN, ERK1/2, phospho-ERK1/2 (p-ERK1/2), Akt, and phospho-Akt (p-Akt). β-tubulin was used as a loading control. Arrowheads indicate specific bands. G : Immunostaining of the transgenic skins with an anti-PGRN antibody (lower panel). Pre-immune serum was used as a control antibody (upper panel). Arrowheads indicate outer root sheath cells.

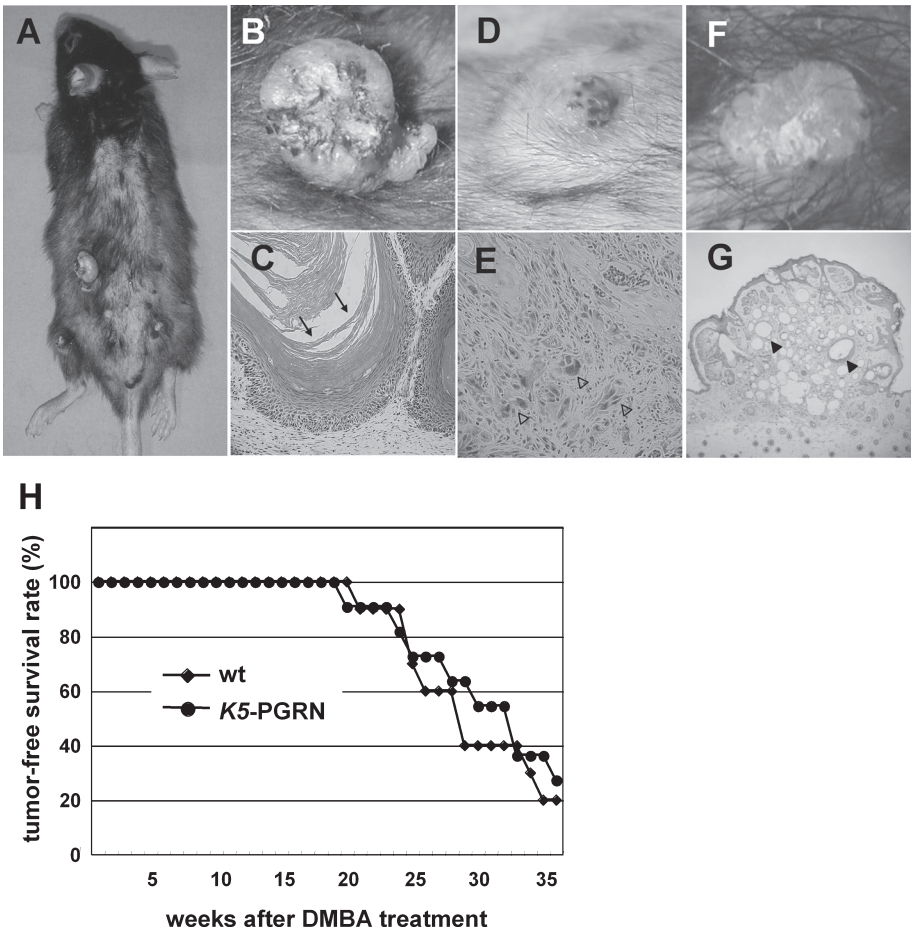

Fig. 2. PGRN overexpression did not result in increased skin tumor incidence. A: A representative of *k5-PGRN* transgenic mice harboring chemically-induced tumors on the back. B, D, F: Higher magnification views of the tumors developed on *k5-PGRN* transgenic mice. C, E, G: Hematoxylin and eosin staining of a section of the tumors in B, D, and F, respectively. Arrow, hyperkeratosis; open arrowheads, infiltrating carcinoma cells in the dermis; filled arrowheads, sebaceous gland lumens. H: Chemically-induced tumor incidence after DMBA/TPA treatment. The percentage of visible-tumor-free animals in each group was plotted.

mor formation was detected in the mice treated with DMBA alone and TPA alone over the 35-week observation period (data not shown). Tumor formation was observed after about 20 topical applications of TPA following DMBA treatment in both wild-type and *k5-PGRN* mice (Fig. 2, A-G). Histologically, the tumors developed included papillomas (Fig. 2, B and C), squamous cell carcinomas (Fig. 2, D and E), sebaceous gland tumors (Fig. 2, F and G), keratoacanthomas, sebaceous hyperplasia and nevi (data not shown). However, there was no significant difference in the tumor incidence and the number of tumors per mouse between wild-type and transgenic mice (Fig. 2H).

To explore the effects of overexpression of PGRN on cellular proliferation in the epidermis and hair follicles, we performed immunohistochemistry using a Ki67 antibody, a marker for proliferating cells in all active phases of the cell cycle (G1, S, G2 and M phases), and an antibody against phosphohistone H3, a marker for cells in the mitotic phase. As shown in Fig. 3A and B, the number of

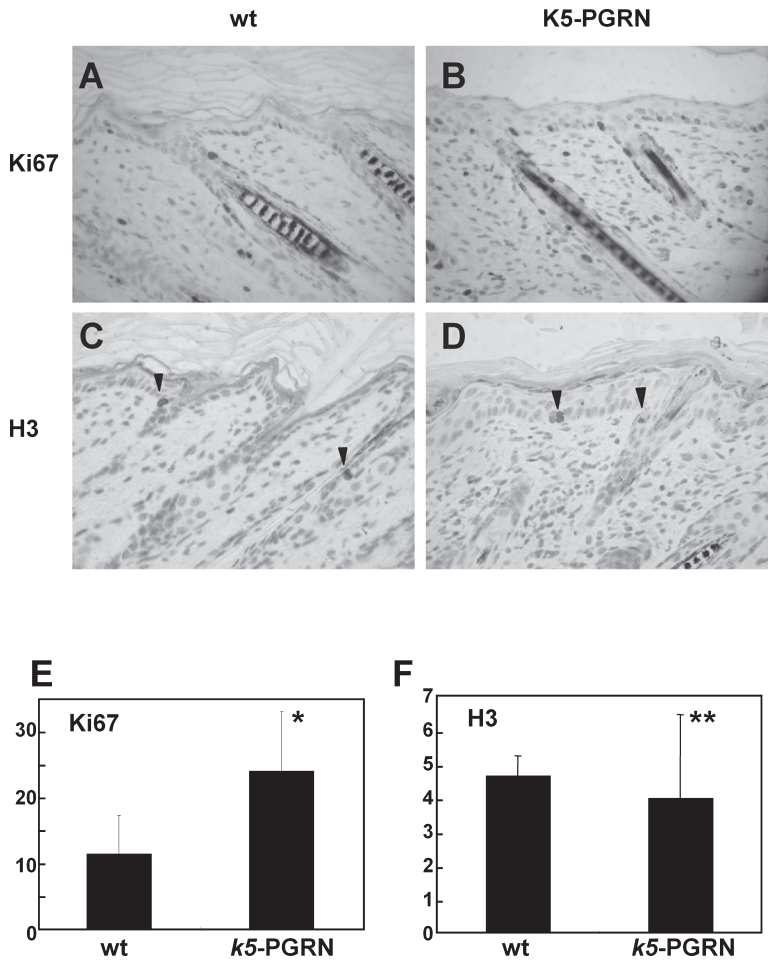

Fig. 3. A–D : Immunohistochemistry of the back skins of wild-type and *k5-PGRN* mice at day 10 with an antibodies to Ki67 and phosphohistone H3. Arrowheads indicate phosphohistone H3-positive cells (red). E : A comparison of the Ki67 indices between wild-type and *k5-PGRN* mice. $*p<0.05$. $**p=0.668$. F : A comparison of the number of phosphohistone H3-positive cells in the basal layer of the epidermis between wild-type ($n=3$) and *k5*-PGRN ($n=5$) mice at day 10.

Ki67-positive nuclei in the basal layer of the epidermis and the outer root sheath cells, where PGRN was overexpressed, was markedly increased in *k5*-PGRN mice. Quantitative analysis showed that the Ki67 index in *k5*-PGRN mice was about 2.5 times higher than that in wild-type mice (Fig. 3E). In contrast, no significant difference in the number of phosphohistone H3-positive nuclei in the basal layer of the epidermis and hair follicle cells was observed between wild-type and *k5*-PGRN mice (Fig. 3C, D, and F).

Discussion

Although PGRN is highly expressed in several human epithelial cancers[3], it has been unclear whether increased expression of PGRN is a cause or result of tumorigenesis *in vivo*. PGRN is normally expressed in the rodent embryonic and adult epidermis and human cultured keratinocytes[25,26]. By using the Cre/lox system, we forced overexpression of PGRN in the basal cells, which are the most actively proliferating cells in the epidermis.

Our study suggests that overexpression of PGRN does not cause tumorigenic transformation *in vivo*, at least in keratinocytes, or facilitate chemically induced tumor formation. These results suggest that overexpression of PGRN alone is insufficient to cause cancerous transformation of keratinocytes.

It may be claimed that the expression level of PGRN in the *k5-PGRN* mice used here was not high enough to cause keratinocyte transformation. In the Cre/lox system we used here, most transgenes tandemly inserted in a certain position in the genome can be eliminated by Cre recombination. The expression level of *PGRN* transcripts in *k5-PGRN* mice was about three times higher than that of wild-type at the mRNA level (Fig. 1E), whereas the PGRN protein in transgenic animals was much higher than that in wild-type (Fig. 1F), suggesting that the exogenously introduced PGRN protein is remarkably stable. It should be stressed that the PGRN protein level established in the *k5-PGRN* mice was enough to upregulate the MAPK/ERK and Akt pathways (Fig. 1F). Because there is no general rule for a certain gene to determine the minimum expression level required for causing transformation, it is prudent to conclude that augmented expression and stability of PGRN does not lead to cancerous transformation, at least in keratinocytes.

It may be too early to generalize our results from keratinocytes alone. Because our transgenic system can be applied to a wide variety of cells by using different tissue-specific Cre mice, it is important to verify the effects of PGRN overexpression in other cells. It would be also interesting to see whether overexpression of GRN peptide A in keratinocytes causes tumor formation using this system. It has been reported that epithelin 1, equivalent to GRN peptide A, stimulates keratinocyte proliferation, whereas epithelin 2, equivalent to GRN peptide B, inhibits their proliferation.

In general, the immunoreactivities of Ki67 and phosphohistone H3 are used as cell proliferation markers. We observed a remarkable increase in Ki67-positive cells in the basal layer of the *k5*-PGRN mouse epidermises (Fig. 3, A, B, E), but no significant difference in the number of phosphohistone H3-positive cells between wild-type and *k5*-PGRN mice (Fig. 3, C, D, F). Because phosphohistone H3 immunoreactivity is specific to cells in mitosis, these results suggest that PGRN overexpression in the epidermis promotes epidermal basal cells to enter the proliferation phases, but is insufficient to lead them to mitosis. No significant increase in tumor incidence in k5-PGRN mice (Fig. 2H) may be attributable to this discrepancy between Ki67 and phosphohistone H3 immunoreactivities. Thus, PGRN may be involved in enhancing the competence of cells to receive further proliferation signals.

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References

1) Bateman, A. and Bennett, H.P. (1998) Granulins : the structure and function of an emerging family of growth factors. *J. Endocrinol.,* **158**, 145-151.
2) He, Z. and Bateman, A. (2003) Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. *J. Mol. Med*., **81**, 600-612.
3) He, Z., Ong, C.H., Halper, J. and Bateman, A. (2003) Progranulin is a mediator of the wound response. *Nat. Med*., **9**, 225-229.
4) Zhu, J., Nathan, C., Jin, W. *et al.* (2002) Conversion of proepithelin to epithelins : roles of SLPI and elastase in host defense and wound repair. *Cell,* **111**, 867-878.
5) Diaz-Cueto, L., Stein, P., Jacobs, A., Schultz, R.M. and Gerton, G.L. (2000) Modulation of mouse preimplantation embryo development by acrogranin (epithelin/granulin precursor). *Dev. Biol.,* **217**, 406-418.
6) Suzuki, M. and Nishiahara, M. (2002) Granulin precursor gene : a sex steroid-inducible gene involved in sexual differentiation of the rat brain. *Mol. Genet. Metab.*, **75**, 31-37.
7) Hoque, M., Tian, B., Mathews, M.B. and Pe'ery, T. (2005) Granulin and aranulin repeats interact with the Tat.P-TEFb complex and inhibit Tat transactiva-

tion. *J. Biol. Chem.*, **280**, 1364–1367.

8) Hoque, M., Young, T.M., Lee, C.G., Serrero, G., Mathews, M.B. and Pe'ery, T. (2003) The growth factor granulin interacts with cyclin T1 and modulates P-TEFb-dependent transcription. *Mol. Cell Biol.*, **23**, 1688–1702.

9) Xu, S.Q., Tang, D., Chamberlain, S., Pronk, G., Masiarz, F.R., Kaur, S., Prisco, M., Zanocco-Marani, T. and Baserga, R. (1998) The granulin/epithelin precursor abrogates the requirement for the insulin-like growth factor 1 receptor for growth in vitro. *J. Biol. Chem.*, **273**, 20078–20083.

10) Van Damme, P., Van Hoecke, A., Lambrechts, D., Vanacker, P., Bogaert, E., van Swieten, J., Carmeliet, P., Van Den Bosch, L. and Robberecht, W. (2008) Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. *J. Cell Biol.*, **181**, 37–41.

11) Bateman, A., Belcourt, D., Bennett, H., Lazure, C. and Solomon, S. (1990) Granulins, a novel class of peptide from leukocytes. *Biochem. Biophys. Res. Commun.*, **173**, 1161–1168.

12) Bhandari, V., Palfree, R.G. and Bateman, A. (1992) Isolation and sequence of the granulin precursor cDNA from human bone marrow reveals tandem cysteine-rich granulin domains. *Proc. Natl. Acad. Sci. U S A.*, **89**, 1715–1719.

13) Sparro, G., Galdenzi, G., Eleuteri, A.M., Angeletti, M., Schroeder, W. and Fioretti, E. (1997) Isolation and N-terminal sequence of multiple forms of granulins in human urine. *Protein Expr. Purif.*, **10**, 169–174.

14) Shoyab, M., McDonald, V.L., Byles, C., Todaro, G.J. and Plowman, G.D. (1990) Epithelins 1 and 2 : isolation and characterization of two cysteine-rich growth-modulating proteins. *Proc. Natl. Acad. Sci. USA.*, **87**, 7912–7916.

15) Zanocco-Marani, T., Bateman, A., Romano, G., Valentinis, B., He, Z.H. and Baserga, R. (1999) Biological activities and signaling pathways of the granulin/epithelin precursor. *Cancer Res.*, **59**, 5331–5340.

16) Plowman, G.D., Green, J.M., Neubauer, M.G., Buckley, S.D., McDonald, V.L., Todaro, G.J. and Shoyab, M. (1992) The epithelin precursor encodes two proteins with opposing activities on epithelial cell growth. *J. Biol. Chem.*, **267**, 13073–13078.

17) Zhou, J., Gao, G., Crabb, J.W. and Serrero, G. (1993) Purification of an autocrine growth factor homologous with mouse epithelin precursor from a highly tumorigenic cell line. *J. Biol. Chem.*, **268**, 10863–10869.

18) Baba, T., Hoff, H.B., 3rd, Nemoto, H., Lee, H., Orth, J., Arai, Y. and Gerton, G.L. (1993) Acrogranin, an acrosomal cysteine-rich glycoprotein, is the precursor of the growth-modulating peptides, granulins, and epithelins, and is expressed in somatic as well as male germ cells. *Mol. Reprod. Dev.*, **34**, 233–243.

19) Parnell, P.G., Wunderlich, J., Carter, B. and Halper, J. (1992) Transforming growth factor e : amino acid analysis and partial amino acid sequence. *Growth Factors*, **7**, 65–72.

20) Liau, L.M., Lallone, R.L., Seitz, R.S., Buznikov, A., Gregg, J.P., Kornblum, H.I., Nelson, S.F. and Bronstein, J.M. (2000) Identification of a human glioma-associated growth factor gene, granulin, using differential immuno-absorption. *Cancer Res.*, **60**, 1353–1360.

21) Lu, R. and Serrero, G. (2001) Mediation of estrogen mitogenic effect in human breast cancer MCF-7 cells by PC-cell-derived growth factor (PCDGF/granulin precursor). *Proc. Natl. Acad. Sci. USA.*, **98**, 142–147.

22) Baker, M., Mackenzie, I.R., Pickering-Brown, S.M., *et al.* (2006) Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature*, **442**, 916–919.

23) Cruts, M., Gijselinck, I., van der Zee, J. *et al.* (2006) Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*, **442**, 920–924.

24) Sano, S., Itami, S., Takeda, K. *et al.* (1999) Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J.*, **18**, 4657–4668.

25) Daniel, R., Daniels, E., He, Z. and Bateman, A. (2003) Progranulin (acrogranin/PC cell-derived growth factor/granulin-epithelin precursor) is expressed in the placenta, epidermis, microvasculature, and brain during murine development. *Dev. Dyn.*, **227**, 593–599.

26) Daniel, R., He, Z., Carmichael, K.P., Halper, J. and Bateman, A. (2000) Cellular localization of gene expression for progranulin. *J. Histochem. Cytochem.*, **48**, 999–1009.