

Letter to the Editor

Progranulin, a secreted tumorigenesis and dementia-related factor, regulates mouse hair growth

To the Editor,

Progranulin (PGRN) is a secreted growth factor implicated in a wide variety of biological processes including tumorigenesis, tissue maintenance, sexual differentiation of the brain, and embryo development [1,2]. It has been recently shown that mutations in PGRN cause a subtype of familial frontotemporal lobar degeneration [3,4]. However, investigation of PGRN functions at the whole organism level is largely limited.

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, CAG-CAT-PGRN mice were crossed with *k5-Cre* mice [5] to generate *k5-PGRN* transgenic mice, in which the PGRN expression is directed to the basal layer of the epidermis and follicular keratinocytes, where PGRN mRNA is normally expressed [6]. 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. 1b). Contrary to the results obtained from cultured cells, keratinocyte-specific overexpression of PGRN did not lead to cancerous transformation or increased susceptibility to chemical carcinogenesis (submitted).

The characteristic feature that distinguished *k5-PGRN* mice from their normal littermates was a hair phenotype, giving the transgenic mice a less fluffy appearance to their coat (Fig. 1c). A similar phenotype was observed in mice from other lines, indicating that the hair phenotype was caused by the increased expression of PGRN, irrelevant to the integration sites of the transgenes. The morphology of the hair follicles of *k5-PGRN* embryos at E15 and E19 was indistinguishable from that of wild-type embryos. The hair phenotype became evident around day 9 when the coat was well formed, and remained obvious between days 10 and 40, during which time the first two hair cycles are highly synchronized in the mouse.

We first asked whether the appearance of *k5-PGRN* mice resulted from abnormalities of hair growth. We compared the length of three

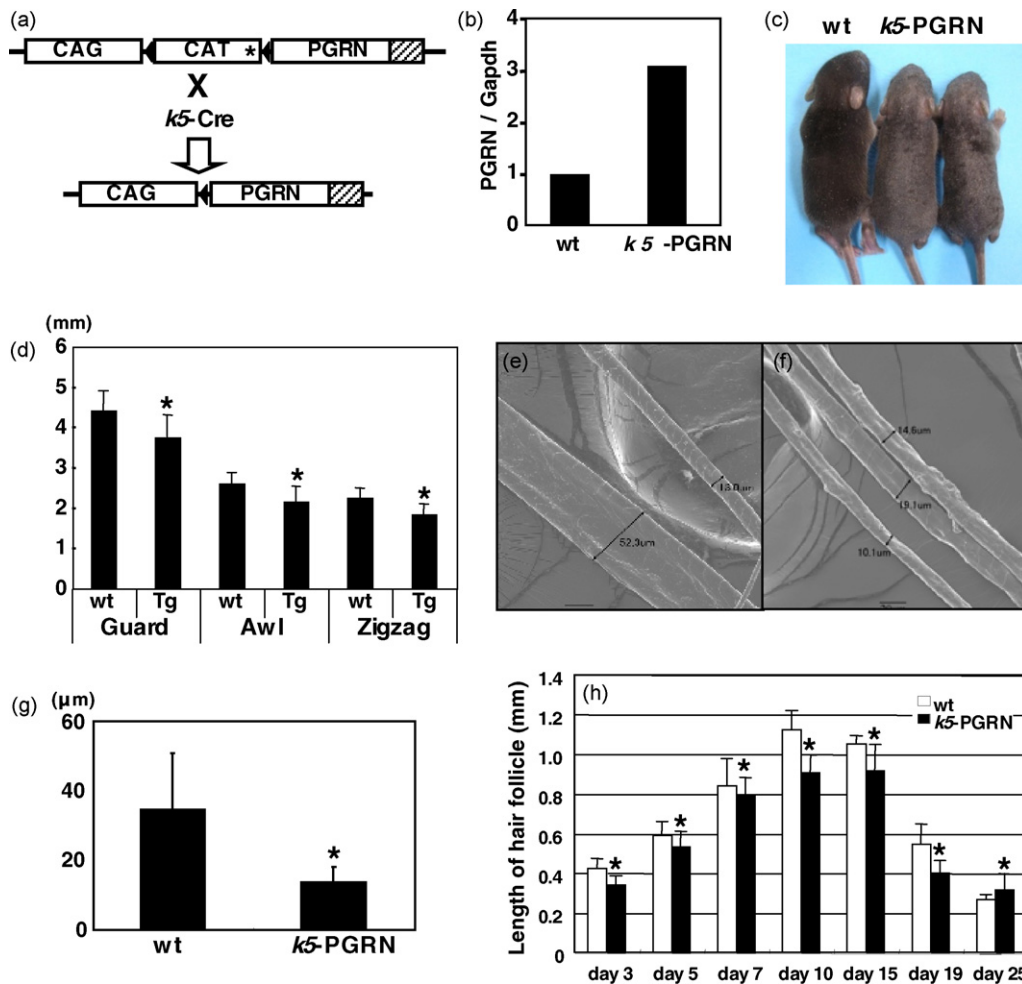


Fig. 1. Hair abnormalities in *k5-PGRN* transgenic mice. (a) Generation of keratinocyte-specific PGRN transgenic mice. The CAG-PGRN transgene is generated by the elimination of the chloramphenicol transferase (CAT) gene through Cre recombination. Asterisk indicates the termination codon. (b) Quantitative real-time RT-PCR for PGRN expression in the epidermis. The PGRN expression level was normalized to *Gapdh* expression. (c) Appearance of *k5-PGRN* mice with their wild-type littermate at day 10. (d) The average length of various types of hair. Error bars, standard deviations. **p* < 0.001. Scanning electron microscopic analysis of hair shafts of wild-type (e) and *k5-PGRN* (f) mice at day 10. (g) The mean diameter of the hair shafts of 10 wild-type and 14 transgenic mouse hairs at a point 500 μm from the hair root on scanning electron micrographs. **p* < 0.01. (h) Comparison of the length of hair follicles between wild-type and *k5-PGRN* mice at multiple time intervals throughout the first hair cycle. The hair follicle length corresponds to the length from the base of the hair bulb to the epidermal surface. Error bars, standard deviations. **p* < 0.05.

types of hair – zigzag, awl, and guard – between wild-type and transgenic animals and found that all hair types of the transgenic mice were shorter than those of wild-type mice (Fig. 1d). In addition, the hairs of the *k5-PGRN* mice were thinner than those of their wild-type counterparts, which was confirmed by scanning electron microscopic analysis (Fig. 1e and f). The average diameter of the transgenic mouse hairs was about one-third that of the wild-type mouse hairs (Fig. 1g). However, the structure of the cuticle of the hairs from *k5-PGRN* mice appeared normal (data not shown).

We next examined skins from the mid-back region of wild-type and *k5-PGRN* mice at multiple time intervals throughout the first hair cycle. As shown in Fig. 1h, in *k5-PGRN* mice, poorer elongation of hair follicles became evident as early as day 3. From days 7 to 15, the transgenic mouse skins showed no significant increase in the length of the hair follicles. After day 19, the thickness of the skin and the length of the hair follicles were indistinguishable from those of wild-type counterparts.

Apoptosis plays a critical role in the regulation of the normal hair cycle and hair development and the presence of apoptotic cells in hair follicles is one of decisive indicators for catagen [7]. Therefore, we investigated whether the suppressed hair growth in transgenic mice was associated with increased apoptosis. Terminal deoxynucleotidyl transferase-mediated dUTP Nick End Labeling (TUNEL) analysis was performed to detect DNA fragmentation, as a marker for apoptotic nuclei, on sections of transgenic and wild-type skins. No TUNEL-positive cells were detected in the wild-type skin at day 3, while TUNEL-positive cells were already present in the bulbs of the transgenic skins at day 3 (Fig. 2a and b). At day 15, the number of TUNEL-positive cells in the transgenic mouse skin was compatible with that of the wild-type skin (Fig. 2c and d). The apoptotic cells were predominantly detected in the inner root sheath cells in the bulge-isthmus portion in both wild-type and transgenic animals. These results suggest that the earlier occurring and sustained catagen is closely associated with suppressed hair growth in *k5-PGRN* mice.

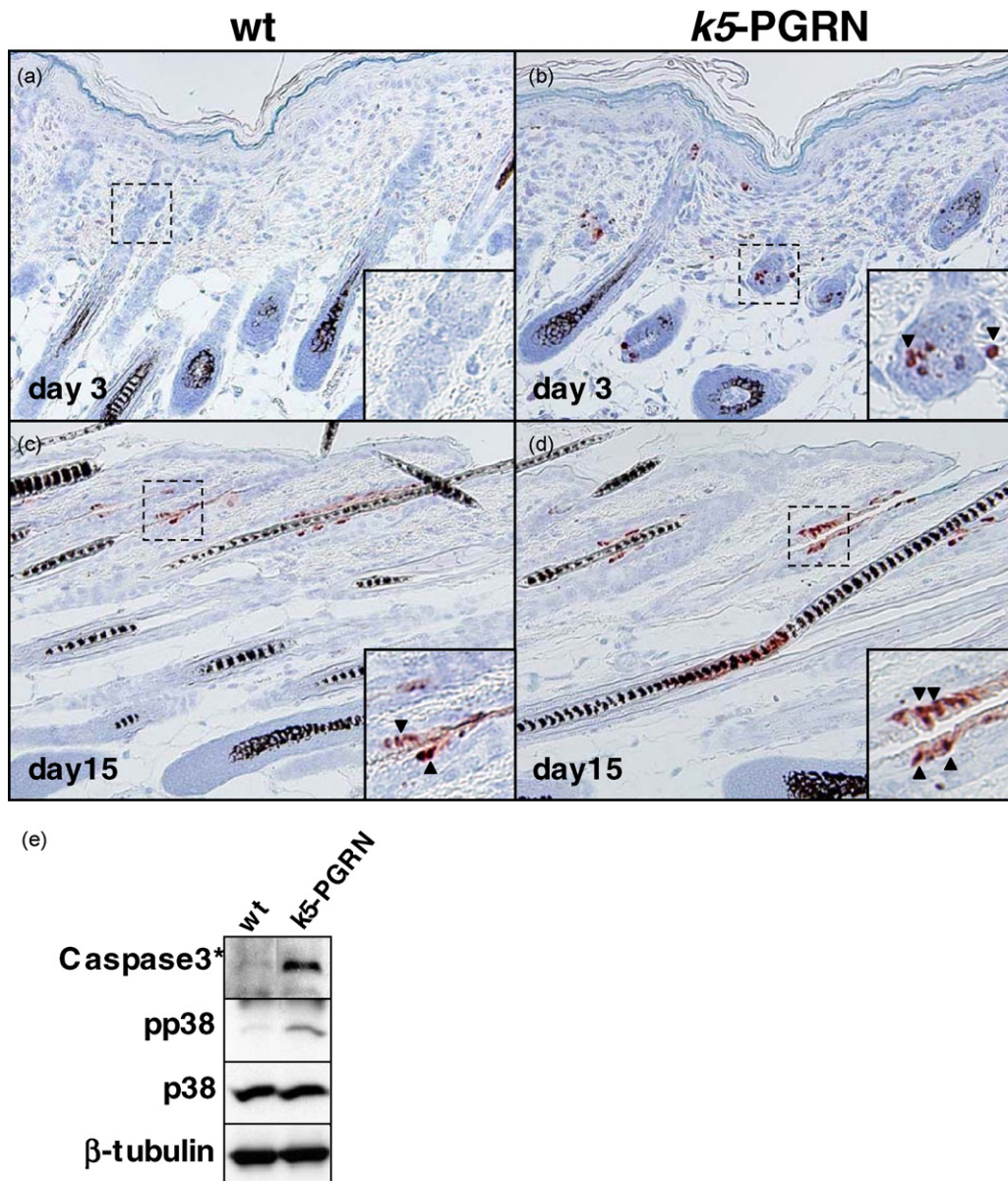


Fig. 2. Accelerated apoptosis in *k5-PGRN* hair follicles. (a–d) Apoptotic cells (red) at days 3 and 15 were detected by TUNEL assay. Arrowheads in insets indicate apoptotic nuclei. (e) Western blot analysis of wild-type and *k5-PGRN* skins for p38, phosphorylated p38 (pp38), and a cleaved form of caspase-3. β -tubulin was used as a loading control.

We further confirmed the increased apoptosis in the transgenic hair follicles by examining the activation status of the p38 signaling pathway, and caspase-3 activity. The p38 pathway is the primary signaling pathway leading to cell death in keratinocytes in response to ultraviolet irradiation and cytotoxic drugs [8,9]. Activation of apoptotic pathways in keratinocytes converges on activation of caspase-3, resulting in cleavage of intracellular substrates and nuclear fragmentation. As shown in Fig. 2e, a phosphorylated (activated) form of p38 and a cleaved (activated) form of caspase-3 were increased in the transgenic mouse skins at day 9, but not in the wild-type mouse skins. Thus, our results imply that PGRN overexpression induces apoptosis via caspase-3 activation through the p38 pathway.

Androgenic alopecia is closely associated with increased sensitivity of hair follicles to androgens. Interestingly, PGRN mRNA is induced by steroid hormones, probably activated through atypical steroid responsive elements [1]. The inner root sheath of the bulge-isthmus portion, where apoptotic cells were intensively detected in *k5-PGRN* mice, is a “hot spot” for apoptosis in human androgenic alopecia [10]. Thus, it would be interesting to examine the relationship between PGRN and steroid hormone expression in the development of hair follicles and the progression of alopecia.

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Letter to the Editor

The effect of DNMTs and MBPs on hypomethylation in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an agnogenic progressive and recurrent autoimmune disease involving poly-organs of human beings. The epigenetic aberration plays an important part in the immune disorder of SLE [1]. It is well known that methylation of CpG dinucleotides in the promoter and first exon of genes is a crucial epigenetic modulation for transcriptional regression [2].

Correspondingly, hypomethylation contributes a lot for the over expression of some genes that might lead to autoimmunity in SLE [3]. Maintenance of the methylation patterns mainly depends on methylating and demethylating processes. DNA methyltransferases (DNMTs: DNMT-1, DNMT-3A, and DNMT-3B) [4] are responsible for formation and maintaining of methylation pattern. Methyl cytosine-binding proteins (MBPs: MBD-1, MBD-2, MBD-3, MBD-4, and MeCP-2) are another potential factors responsible for transcription regression [5]. Interestingly, MBD-2 is the only member that was reported with functions of both transcriptional repressor and DNA demethylase. To determine their influence in the onset of SLE, this paper comparatively analyzed mRNA transcriptional levels of DNMTs, MBPs, lymphocyte function-associated antigen-1 (LFA-1) genes and their relationship in active SLE (SLE-a) and relieved SLE (SLE-r).

21 patients met the 1982 ARA lupus criteria for SLE were recruited from Tongji Hospital and Ruijin Hospital, and assessed using SLE disease activity index (SLE-DAI). SLE-a was defined as a SLE-DAI over 5 and SLE-r between 0 and 5. 21 controls were from