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RESEARCH LETTER

# Niche formed by bone morphogenetic protein antagonists gremlin 1 and gremlin 2 in human hair follicles

**Shiro Niiyama**[1] | **Yumiko Ishimatsu-Tsuji**[2] | **Jiro Kishimoto**[2]

[1]Department of Dermatology, Toho University Ohashi Medical Center, Tokyo, Japan

[2]Shiseido Global Innovation Center, Yokohama, Japan

**Correspondence**
Shiro Niiyama, Department of Dermatology, Toho, University Ohashi Medical Center, 2-22-36 Ohashi, Meguro-ku, Tokyo, 153-8515 Japan.
Email: sniiyama@aol.com

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It has been proposed that cytokine niches in the hair bulb play an important role in the precise regulation of the hair cycle. Noteworthy analyses of the niche in the telogen-anagen phase formed by *WNT*-related factors have been reported recently.[1,2] In the present study, we analyzed the localization of bone-morphogenic-protein (*BMP*)-related factors that might be involved in niche formation.

The *BMP* family provides some of the most critical signals for the cycling of hair follicles (HFs), which has been reported to suppress cell proliferation and promote differentiation and migration of epithelial cells.[3,4] Previously, we found that expression of a *BMP* antagonist, gremlin 2 (*GREM2*), in the dermal sheath cup (DSC) was higher than that in the dermal papilla (DP) and upper area of the dermal sheath in human HFs by transcriptome analysis.[5] The DSC is the peribulbar component of the DS, which has unique characteristics from the clinical viewpoint that cells derived from the DSC possessed a hair-inductive potential in a rodent model and clinical study in humans.[6,7]

Written informed consent was obtained from the donors participating in this study that was conducted with the approval of the Ethics Committee of Toho University Ohashi Medical Center and Shiseido Global Innovation Center. Skin pieces containing occipital scalp HFs were obtained from a 37-year-old female donor, suffering from female pattern boldness. In situ hybridization was performed on paraffin-embedded tissue sections. *GREM1*-, *GREM2*-, *BMP4*-, lymphoid enhancer-binding factor 1 (*LEF1*)-, SRY box transcription factor 2 (*SOX2*)-specific probes according to the sequence information of Entrez gene ID26585, 64 388, 51 776, 6657, 652 based on highly specific branched DNA signal amplification technology in a View RNATM ISH Tissue 1-Plex Assay kit were supplied from Thermo Fisher Scientific Inc (Waltham, Massachusetts). Signals were detected with Fast Red substrate, followed by fluorescence observation. Nuclear counterstaining was performed with Hoechst 33342 (Thermo Fischer Scientific Inc). Image acquisition was performed using an LSM880 confocal microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). All experiments were performed repeatedly using different scalp tissues to confirm reproducibility. For the validation of the results, the expression of *GREM2* was confirmed in more than 10 HFs derived from different male donors, and expression of *GREM1*, *BMP4*, *LEF1*, and *SOX2* was seen in at least three HFs per two individuals or more (data not shown).

We examined the localization of *GREM2* and other *BMP*-related genes by in situ hybridization on tissue sections. *GREM2* expression was robust in the DSC during the hair cycle in anagen, early catagen, and the miniaturized anagen HF (Figure 1A,D,E), which was restricted to the DSC and absent in the upper area of the DS and the infundibulum in the entire HF.

Next, we analyzed the localization of other *BMP*-related molecules in the hair bulb. Signals of another gremlin subtype, gremlin 1 (*GREM1*), were observed in the DSC and the epithelial matrix of normal and miniaturized anagen HFs, unlike *GREM2* signals. A few signals of *GREM1* were observed in the DP as well as the DSC and epithelial matrix in the catagen phase (Figure 1B,F). *BMP4* was expressed in the epithelial matrix, inner root sheath (IRS), and DP of anagen follicles (Figure 1C). Relatively low expression of *BMP4* was also observed in a restricted area of the DSC adjacent to the DP. This expression pattern of *BMP4* was maintained in the early stage of catagen (Figure 1G).

Shiro Niiyama and Yumiko Ishimatsu-Tsuji contributed equally and should be considered as the first author.

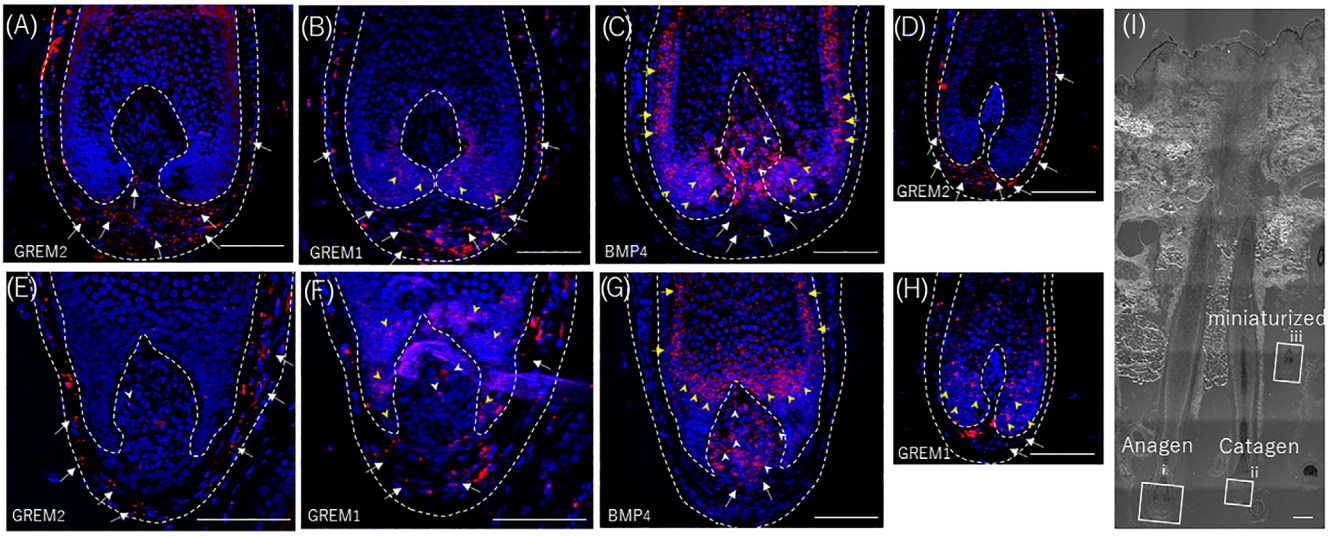


**FIGURE 1** *GREM1*, *GREM2*, and *BMP4* expression in the different hair cycle. *GREM2* (A, D, E), *GREM1* (B, F, H), and *BMP4* (C, G) expression in anagen (A-C), early catagen (E-G) and the miniaturized hair bulbs (D, H) were analyzed. Phase contrast image of whole HF shows the position of the hair bulbs of A-H (I). Boxed areas i-iii: positions of A-C, E-G, D and H, respectively. White arrows: expression in the DSC. White arrowheads: expression in the DP. Yellow arrows: expression in the IRS and inner layers of the ORS. Yellow arrowheads: expression in the epithelial matrix. Scale bars: 100 μm (A-H) and 200 μm (I)

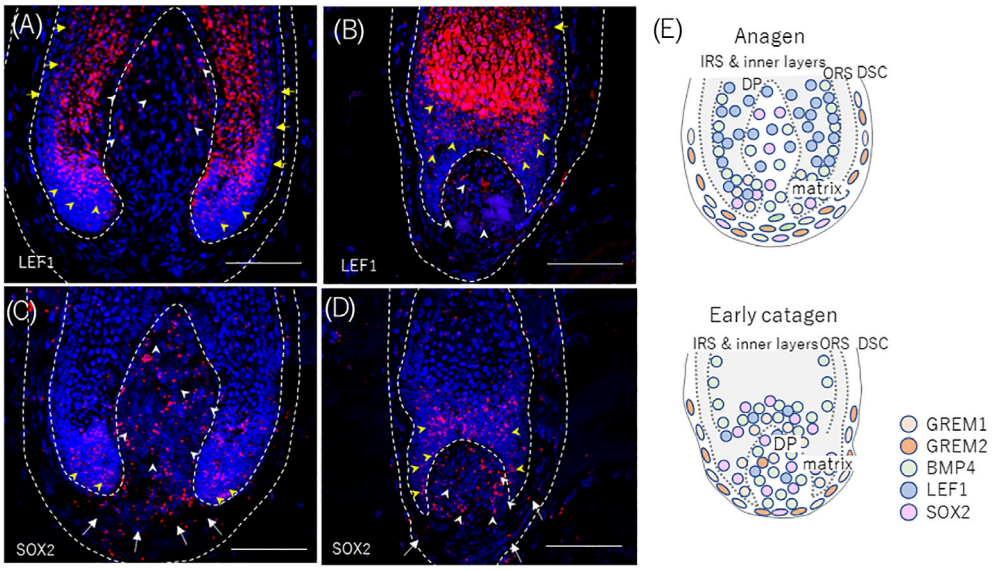


**FIGURE 2** Expression of *LEF1* and *SOX2* in anagen and early catagen hair bulbs. *LEF1* (A, B) and *SOX2* (C, D) expression were analyzed in anagen (A, C) and early catagen (B, D). Putative BMP-related cytokine niche in anagen and early catagen hair bulbs (E). White arrows: expression in the DSC. White arrowheads: expression in the DP. Yellow arrows: expression in the IRS and inner layers of the ORS. Yellow arrowheads: expression in the epithelial matrix. Scale bars: 100 μm

Considering the paracrine function of *BMP4* and extracellular inhibition of *GREM1* and *GREM2*, the growth and differentiation of the adjacent outer root sheath (ORS) and epithelial matrix sandwiched between the areas that expressed *BMP4* and *GREMs* might be regulated by the balance of *BMPs* and their antagonists such as *NOGGIN*, a well-known *BMP* antagonist.[3,8] The specificity and robustness of *GREM2* expression in the DSC suggest its functional contribution to unique regulations in the region, which may tune *BMP* signaling from the DSC, epithelial matrix, and adipose layer.[4] However, *GREM1*, which was expressed in the epithelial matrix region in addition to the DSC, might regulate hair growth more directly, which is similar to its contribution to chicken feather formation.[9]

Moreover, we found distinctive localization of *LEF1* and *SOX2*, which regulate *GREM2*,[10] and another *BMP* antagonist, sclerostin domain containing 1 (*SOSTDC1*),[11] respectively. *LEF1* was detected in the DP and epithelial matrix (Figure 2A,B), which is consistent with previous reports,[1,12] while *SOX2* was expressed in the epithelial matrix in human HFs unlike its expression in mice, which is restricted to the DP and DSC (Figure 2C,D).[13]

Taken together, essential gene expression profiles of the DSC, DP, and epithelial matrix in the hair bulb are coordinately maintained by the niche formed by differentially localized *BMPs* and *BMP* antagonists (Figure 2E), which might be crucial to maintain a normal hair cycling process.

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## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization: Shiro Niiyama, Yumiko Ishimatsu-Tsuji, Jiro Kishimoto.
Formal Analysis: Yumiko Ishimatsu-Tsuji.
Funding Acquisition: Shiro Niiyama.
Writing-Original Draft: Shiro Niiyama, Yumiko Ishimatsu-Tsuji.
Writing-Review and Editing: Shiro Niiyama, Yumiko Ishimatsu-Tsuji, Jiro Kishimoto.

All authors have read and approved the final version of the manuscript.

Shiro Niiyama had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

## ETHICS STATEMENT

Written informed consent was obtained from the patient participating in this study that was conducted with the approval of the Ethics Committee of Toho University Ohashi Medical Center and Shiseido Global Innovation Center.

## ORCID

*Shiro Niiyama* https://orcid.org/0000-0003-1525-4874
*Jiro Kishimoto* https://orcid.org/0000-0002-4479-5701

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