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Hairless controls hair fate decision via Wnt/β–catenin signaling

Kuicheng Zhu [a, b], Cunshuan Xu [a], Mengduan Liu [c], Jintao Zhang [c, *, 1]

[a] College of Life Science and Key Laboratory for Bioengineering, Henan Normal University, Xinxiang 453007, China
[b] Laboratory Animal Center of Zhengzhou University, Zhengzhou 450052, China
[c] Institute of Medical and Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450052, China

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ABSTRACT

The hairless (*Hr*) gene plays a central role in the hair cycle, considering that mutations in the gene result in hair loss with the exception of a few vibrissae after the first hair growth cycle in both mice and humans. This study examinedthe uncommon phenotype and using microarray analyses and functional studies, we found that β-catenin was mediated by *Hr*. Progenitor keratinocytes from the bulge region differentiate into both epidermis and sebaceous glands, and fail to adopt the hair keratinocytes fate in the mutant scalp, due to the decreased Wnt/β-catenin signaling in the absence of the hairless protein. This may be attributed to the dysfunction of normal epithelial-mesenchymal interactions in the hair follicle (HF).

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1. Introduction

Alopecia is a group of common dermatologic diseases that tend to lead to hair loss. This includes diseases such as androgenetic alopecia (AGA) and alopecia areata (AA) both of which involve the alopecia moving outward in a circular pattern on the scalp, which is similar to a beard where hair growth frequently starts above the mouth and on the chin until it extends across the face. A beard typically develops normally in men with AGA or AA; however, the molecular basis of this largely remained unexplored [1,2]. Unidentified gene mutations may play a role in the development of this disorder as it is frequent in people with a family history of alopecia [3,4]. Hairless (*Hr*), is a potentially important regulatory gene for the hair cycle control since an autosomal recessive mutation of *Hr* in mice and humans has been reported to evoke severe, irreversible HF abnormalities except for a few vibrissae [5–7]. In our previous study, a similar phenotype was observed in mice that harbored a mutation of the *Hr* gene named rhinocerotic (symbol: hr^{rhsl}) [8]. To identify the differentially expressed genes that are responsible for this unusual phenotype, we performed a microarray analysis on body sites-specific skins around the corners of the mouth and the scalp at postnatal day 12 (P12) in hr^{rhsl}/hr^{rhsl} mouse. Our findings demonstrate that the Wnt pathway gene (β-catenin) regulated by *Hr* could play a central role in hair regeneration.

2. Materials and methods

2.1. Animals and tissue collection

Hr^{rhsl}/Hr^{rhsl}mice were maintained as previously reported [8]. Skin samples were collected from 12-day-old mutant mice (n = 3). The animal experiments comply with the ARRIVE guidelines and approved by the Animal Ethics Committee of the Zhengzhou University (Zhengzhou, China). Animals were maintained on a 12-h light: 12-h dark circadian schedule in a 20–24 °C housing room, and provided water and mouse chow *ad libitum*.

2.2. Histology and transmission electron microscopy (TEM)

Skin samples from the scalp and chin were collected for both histological and ultrastuctural studies. Skin tissues were cut into pieces, fixed in 10% neutral buffered formalin overnight, embedded in paraffin, sectioned to 5 μm, and stained with hematoxylin and eosin (H&E) for histological studies. The samples were washed in 5% sucrose cacodylate buffer, postfixed with 1% osmium tetroxide, dehydrated, and embedded in epoxy resin after 4 h in Karnovsky's fixative for the transmission electron microscopy (TEM). Ultrathin sections were cut, collected on formvar coated grids, and examined via electron microscopy.

* Corresponding author.
E-mail address: zhangjt_66@126.com (J. Zhang).
[1] The authors contributed equally to this work.

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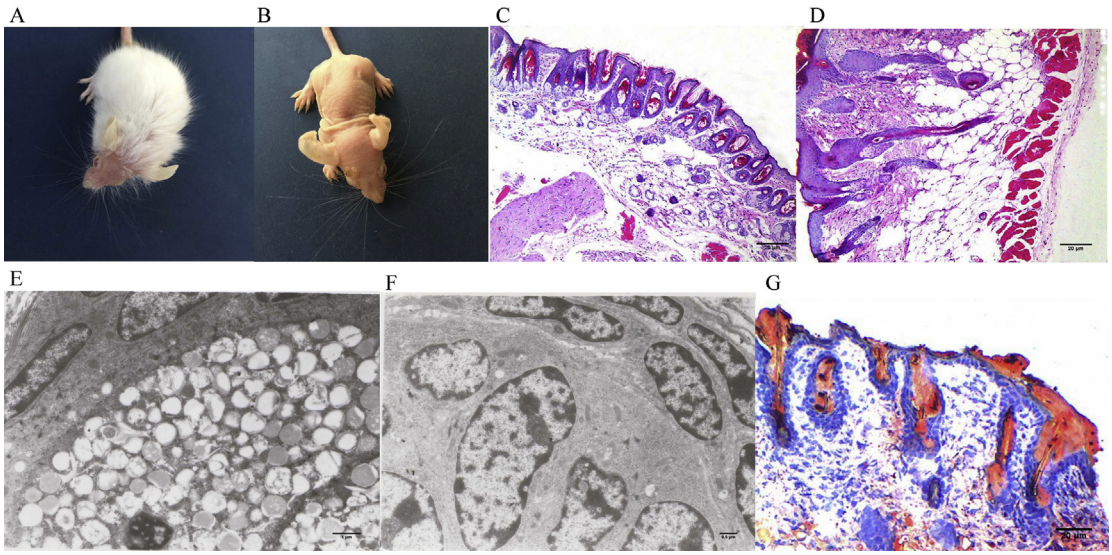

Fig. 1. Phenotypic and structural changes in mutant mice at P12. (A, B) Note the hair loss in the scalp except for vibrissae at P12 and P21, respectively. The dilatation of the pilary and utricle formation in HFs of the scalp was present (C) versus the vibrissae (D) via histology at P12. The central part of the cyst was characterized by an accumulation of lipid droplets in the scalp (E) versus the vibrissae (F) by TEM at P12. Lipids were detected in the cyst contents via Oil Red O staining at P12 (G). Scale bars: 20 μm (C, D and G); 1 μm (E); 0.5 μm (F).

2.3. Oil Red O staining

Samples were embedded in Tissue-TeK® (Sakura, Finetek, Inc., USA) and 6 μm vertical cryosections were stained with Oil Red O solution (Sigma).

2.4. Microarray and real-time PCR

Total RNA was extracted from skin samples extracted from the scalp and chin using the TRIzol® reagent (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Microarray analysis was performed with an Agilent MouseWG-6 v2 Gene Expression BeadChip (Santa Clara, CA, USA) to identify differentially expressed genes. Quantitative PCR (qRT-PCR) was performed with the SYBR Premix Ex Taq (Takara) using aLightCycler480 Software Setup (Roche, USA) to validate the differences. Differences between the scalp and the chin were calculated based on the $2^{-\Delta\Delta Ct}$ method.

2.5. Immunostaining

Skin samples were taken from both the scalp and the chin of three mutant mice for immunohistochemistry. The tissue sections were deparaffinized, and blocked with 10% goat serum. All tissue sections were then incubated overnight at 4 °C with the following primary antibodies: HR (1: 200, Santa Cruz Biotechnology, Inc), β-catenin (1:500, Cell Signaling, Abcam), Caspase 14 (Casp14, 1:100, epidermal differentiation, Abcam), and Keratin 86 (KRT86, 1:200, Keratin, Abcam). The primary antibodies were detected with biotinylated goat anti-rabbit secondary antibodies, and visualized using a DAB Peroxidase Substrate Kit (Sigma). Counterstaining of the sections was performed using hematoxylin.

2.6. Statistical analysis

The data obtained from each mouse were averaged per group, and the standard deviation of the mean values was calculated. An unpaired student's *t*-test was used to determine the significance between two groups: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; ns, not significant.

3. Results

3.1. Histological changes in scalp and chin

The most prominent effect observed in mutant mice is hair loss from P12 (Fig. 1A). Progressive hair loss is initiated around the eyes and progresses caudally, resulting in a completely hairless condition by postnatal day 21 (P21) with the exception of the vibrissae (Fig. 1B). Histology revealed that there was an excessively keratinized layer in the epidermis, with accompanied loss of the hair shaft, prominent dilatation of the pilary canal (infundibulum), and numerous intradermal follicular cysts filled with keratinized material in the scalp (Fig. 1C) versus the chin (Fig. 1D). The ultrastructure of the scalp was also compared with that of the chin. The results showed that in the peripheral part of the cysts, the cytoplasm of the wall epithelial cells that occupied the same position as the initial follicular bulb was filled with lipid droplets at P12 (Fig. 1E). This pattern was not seen in the chin samples (Fig. 1F). The cysts also contain lipids, a component of sebum (Fig. 1G).

3.2. Regional differences in Wnt/β−catenin signaling pathway genes between scalp and chin

To clarify which regional differences from within the signaling mechanisms were unique to the uncommon phenotype of the mutant mouse, we conducted a microarray analysis to investigate the genes with specifically affected expressions by the loss of *Hr* on a body site, specifically on the scalp and the chin. We determined the time point where hair loss (P12) first occurred to clarify the initial events that caused these morphological changes. The results revealed that the WNT signaling pathway gene- β-catenin underwent significant changes (≥ 2, $P < 0.05$) in the scalp versus the chin of the same mouse(Fig. 2A). There was a 0.32-fold decrease in the expression of β-catenin in the scalp versus the chin. In addition, the Casp14, the Keratin (KRT)83, and the KRT86 (that were associated with keratinocyte differentiation and hair-shaft structure) were more highly transcribed in the scalp than in the chin. QRT-PCR confirmed that the above expressed genes in the P12 scalp were higher than in the chin (Fig. 2B).

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