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Minoxidil activates β-catenin pathway in human dermal papilla cells: A possible explanation for its anagen prolongation effect

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ABSTRACT

Background: It is believed that the length of the actively growing phase of the anagen hair cycle mainly contributes to hair length. Recent studies showed that maintenance of β-catenin activity in the dermal papilla cells (DPCs) enables hair follicles to keep actively growing. Topical minoxidil treatment promotes hair growth in men with androgenetic alopecia, suggesting that minoxidil may prolong the actively growing phase of the anagen hair cycle.
Objective: To investigate whether minoxidil prolongs the anagen hair cycle in mice and, if so, to investigate whether minoxidil activates β-catenin pathway in human DPCs.
Methods: Dorsal skins of C57BL/6 mice were depilated to synchronize the hair cycle. After 10 days, 3% minoxidil were topically applied daily for 10 days. Sections of back skins were stained with hematoxylin and eosin. Hair follicles were graded and hair cycle score (HCS) was calculated. Cultured human DPCs were transiently transfected with the β-catenin responsive TCF reporter plasmid (pTopflash) and corresponding negative control reporter (pFopflash) to assess the activity of β-catenin signaling by minoxidil. Immunofluorescence staining and immunoblot were performed to examine the expression and localization of β-catenin in the presence or absence of minoxidil. Phosphorylation of GSK3β, PKA and PKB were also examined by immunoblot after minoxidil treatment. RT-PCR analysis and immunoblot were employed to investigate the expression of β-catenin pathway targets in DPCs, such as Axin2, Lef-1, and EP2.
Results: Modest extension of anagen phase thereby delay of catagen progression was observed by application of minoxidil in mice. Minoxidil stimulated the transcriptional activity of pTopflash but not pFopflash. Nuclear accumulation of β-catenin was also observed after minoxidil treatment. Immunoblot further showed that minoxidil treatment increases the phosphorylation of GSK3β, PKA and PKB. Moreover, minoxidil induced Axin2, Lef-1, and EP2 expression.
Conclusion: Our results strongly suggest that minoxidil extends the anagen phase by activating β-catenin activity in the DPCs.

1. Introduction

The post-natal hair follicle undergoes a cycle of growth (anagen), regression (catagen) and rest (telogen) [1–3]. The length of the actively growing phase of the anagen hair cycle is believed to mainly contribute to hair length. Androgenetic alopecia (AGA) is the most common type of hair loss in men. One of the key features of AGA is shortening of the anagen period [4]. Therefore, maintenance of anagen phase of hair follicle is a key to develop remedies for treatment and prevention of AGA.

The mammalian hair follicle contains epithelial cells in the outer root sheath, inner root sheath, matrix, and hair shaft that are derived from the epithelium. It also contains dermal papilla (DP) and dermal sheath cells derived from the mesenchyme. The reciprocal interactions between the epithelium and mesenchyme are essential for post-natal hair growth as well as embryonic formation of hair follicle [1–3]. The DP, a cluster of specialized fibroblasts, is known to play a key role in the regulation of hair cycling and growth and is encapsulated by the overlying follicular matrix keratinocytes during anagen. Factors from the DP are believed to stimulate proliferation and differentiation of follicular keratinocytes into the hair shaft [1–3].

Recent studies showed that β-catenin activity is present in the DP of actively growing phase of the anagen [5] and ablation of β-catenin in the DP resulted in premature induction of catagen in mice [6]. It is also shown that maintenance of β-catenin activity

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keeps cultured DPCs in anagen-phase characteristics [7,8]. These results show correlation between β-catenin activity of DP and anagen duration and strongly suggest that actively growing phase of the anagen can be prolonged by maintaining/activating β-catenin activity of DP.

Topical minoxidil treatment promotes hair growth in men with AGA [9,10], suggesting that minoxidil may prolong the anagen hair cycle. Here, we observed that minoxidil prolongs the anagen hair cycle using C57BL/6 mice. Since β-catenin activity of DP is implicated in anagen duration, we next investigated whether minoxidil activates β-catenin pathway in human DPCs.

2. Materials and methods

2.1. Primary culture of dermal papilla cells

Biopsy specimens were obtained from the occipital scalps of male patients with androgenic alopecia during hair transplantation with patients' consent. Dermal papillae were isolated from the bulbs of dissected hair follicles, transferred onto plastic dishes coated with bovine type 1 collagen, and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Gaithersburg, MD, USA) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 20% heat-inactivated fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2. The explants were left for several days, and the medium was changed every three days. After cell outgrowth had become sub-confluent, cells were harvested with 0.25% trypsin/10 mM EDTA in Hank's balanced salt solution (HBSS) and sub-cultured at a split ratio of 1:3. Afterwards, DPCs were maintained in DMEM supplemented with 10% FBS.

2.2. In vivo study of minoxidil and hair cycle staging

7-week-old female C57BL/6 mice in the teloegen stages of the hair cycle [11] were purchased from Orient BioInc. (Seongnam, Korea). Dorsal areas of each mouse were synchronized to the anagen stage by depilation. After 10 days, all hair follicles in the depilated skin area entered the anagen VI. From this time point, vehicle or 3% minoxidil were treated for 10 days. Mice ($n = 5$) were killed and the treated region of dorsal skin were stained with hematoxylin and eosin. To calculate the hair cycle score (HCS), anagen VI hair follicles were arbitrarily attributed a score of 100, hair follicles in early catagen (catagen I–catagen III) a score of 200, in mid-catagen (catagen IV–catagen V) of 300, and in late catagen (catagen VI–VIII) of 400. Fifty hair follicles identified on sections were graded for each mouse and 5 mice were used for each group.

2.3. RT-PCR analysis

Total RNA was isolated using TRIzol reagent, and cDNA was synthesized from 3 μg total RNA using an cDNA synthesis kit containing the ImProm-II™ reverse transcriptase and oligo-dT primers according to the manufacturer's instructions (Promega, Madison, WI, USA). One microliter of cDNA was amplified with each of the forward and reverse primers. For the detection of Axin2, 30 cycles (45 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C) of amplification was performed with forward primer 5′-CAGTGGATGCTGGAGAGTGA-3′ and reverse primer 5′-TGCCAGTTTCTTTGGCTCTT-3′. For the detection of Lef-1, 30 cycles (45 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C) of amplification was performed with forward primer 5′-CCAGCTATTGTAACACCTCA-3′ and reverse primer 5′-TTCAGATGTAGGCAGC TGTC-3′. For the detection of β-actin, 23 cycles (45 s at 94 °C, 45 s at 58 °C, and 45 s at 72 °C) of amplification was performed with 5′-GGGAAATCGTGCGTGACATT-3′ and 5′-GGAGTTGAAGGTAGTTTCGT-3′. PCR products were separated by electrophoresis on 1% agarose gel and visualized under UV light.

2.4. Immunobloting

NE-PER Nuclear and Cytoplasmic extraction reagents (Pierce, Rockford, IL, USA) were used according to the manufacturer's protocol to extract nuclear and cytoplasmic proteins. Proteins (5 μg/lane) were separated by 10% SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were blocked with 5% milk in PBS for 1 h. They were probed with rabbit polyclonal antibody against β-catenin (1:1000 dilution; Cell Signaling, Beverly, MA, USA) or were probed with rabbit polyclonal antibody against p-GSK3β (Ser 90, 1:1000 dilution; Cell Signaling). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit Ig (Jackson Immuno Research, Baltimore, PA, USA) were used as the secondary antibodies at a 1:7000 dilution. The bands were visualized using ECL Plus (Amersham, Buckinghamshire, UK). The membranes were also probed with mouse monoclonal antibody against Lamin B1 (1:500 dilution; Zymed Laboratories, San Franciso, CA, USA) and mouse monoclonal antibody against actin (1:5000 dilution; Chemicon, Temecula, CA, USA), relatively. For the detection of EP2, p-PKA, and p-PKB, total cell lysates (5 μg/lane) were probed with rabbit polyclonal antibody against EP2 (1:500 dilution; Cayman, Ann Arbor, MI), p-PKA R2 (Ser 96, 1:2500 dilution; abCam, Cambridge, UK) and p-PKB (Ser 473, 1:1000 dilution; Cell Signaling).

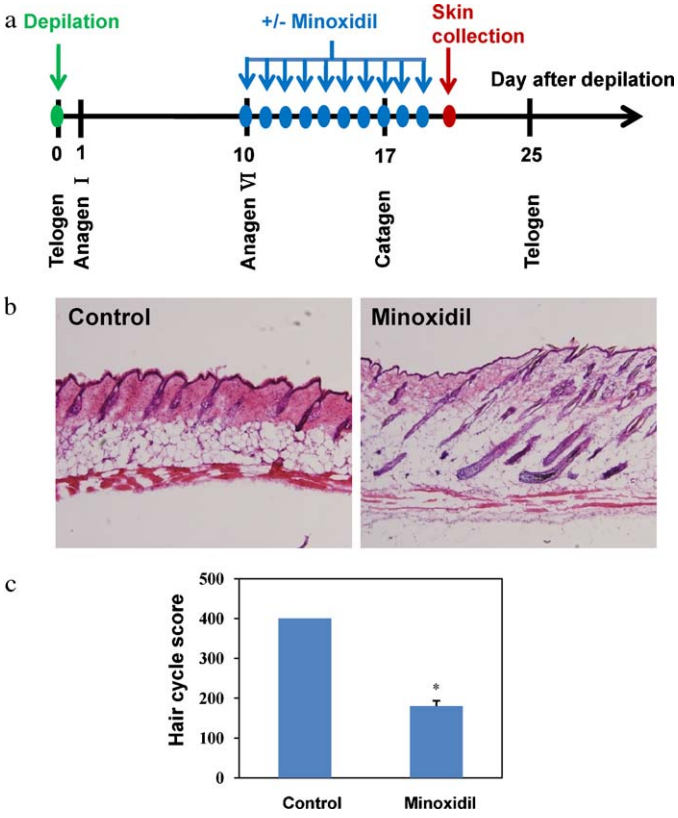

Fig. 1. Prolongation of anagen stage by minoxidil (a) scheme of the experiment. The back skin of C57BL/6 mice ($n = 5$) was treated with the vehicle (left) or 3% topical minoxidil (right) at anagen stage for 10 days. (b) A representative histology from five animals at day 20 is shown. (c) Calculation of the hair cycle scores. Fifty hair follicles (HFs) identified on sections were graded for each mouse and 5 mice were used for each group. HFs of each group were staged and each stage of the hair cycle has been scored as follows: anagen VI = 100, early catagen = 200, mid catagen = 300, late catagen = 400. The score indicates the mean hair cycle stages of all HFs per group. $^{*}p < 0.05$.