Dihydrotestosterone-Inducible IL-6 Inhibits Elongation of Human Hair Shafts by Suppressing Matrix Cell Proliferation and Promotes Regression of Hair Follicles in Mice

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Autocrine and paracrine factors are produced by balding dermal papilla (DP) cells following dihydrotestosterone (DHT)-driven alterations and are believed to be key factors involved in male pattern baldness. Herein we report that the IL-6 is upregulated in balding DP cells compared with non-balding DP cells. *IL-6* was upregulated 3 hours after 10–100 nM DHT treatment, and ELISA showed that IL-6 was secreted from balding DP cells in response to DHT. IL-6 receptor (IL-6R) and glycoprotein 130 (gp130) were expressed in follicular keratinocytes, including matrix cells. Recombinant human IL-6 (rhIL-6) inhibited hair shaft elongation and suppressed proliferation of matrix cells in cultured human hair follicles. Moreover, rhIL-6 injection into the hypodermis of mice during anagen caused premature onset of catagen. Taken together, our data strongly suggest that DHT-inducible IL-6 inhibits hair growth as a paracrine mediator from the DP.

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INTRODUCTION

The hair follicle contains epithelial cell populations of outer root sheath, matrix, and its derivatives—inner root sheath and hair shaft. The hair follicle also contains dermal papilla (DP) and dermal sheath (DS) cells derived from the mesenchyme. The reciprocal interactions between the epithelium and mesenchyme are essential for postnatal hair growth and cycling of hair follicles (reviewed by Hardy, 1992; Millar, 2002; Botchkarev and Kishimoto, 2003). The postnatal hair follicle undergoes a cycle of growth (anagen), regression (catagen), and rest (telogen; reviewed by Stenn and Paus, 2001; Botchkarev and Kishimoto, 2003).

Male pattern baldness is the most common type of hair loss in men (reviewed by Hoffmann, 2002; Rathnayake and Sinclair, 2010). One of the key features of male pattern baldness is follicular miniaturization, in which thick terminal hairs are transformed into thin vellus-like hairs (Rushton *et al.*, 1991). In addition, in developing male pattern baldness, hair follicles undergo premature transition from anagen to catagen (reviewed by Jahoda, 1998; Kaufman, 2002); thus, hair follicles spend more time in telogen with a short anagen phase, resulting in shorter hairs.

Dihydrotestosterone (DHT) dependence of male pattern baldness has been well demonstrated (Imperato-McGinley *et al.*, 1974; Kuttenn *et al.*, 1979; Drake *et al.*, 1999). Circulating androgens, including DHT, enter the follicle via the DP capillaries. In addition, a significant portion of DHT is produced by DP cells from testosterone. Androgens bind to the androgen receptor within the DP cells and then activate or repress target genes (Randall *et al.*, 1994; reviewed by Randall, 2008; Inui and Itami, 2011). Therefore, it is believed that DP cells secrete diffusible factors that affect follicular keratinocytes and DP cells in response to androgens.

In this study, we report that the IL-6 is upregulated in balding DP cells compared with non-balding DP cells. We showed that IL-6 is produced by balding DP cells in response to DHT. In addition, we examined expression of IL-6 receptors in human hair follicles, investigated the effects of IL-6 on cultured human hair follicles, and assessed the role of recombinant human IL-6 (rhIL-6) in postnatal hair cycling in mice.

RESULTS

Upregulation of IL-6 mRNA and protein in balding DP cells

In our preliminary experiments using an ELISA strip for profiling angiogenesis proteins, we found higher levels of IL-6 in conditioned medium of balding DP cells compared with non-balding DP cells (data not shown). To verify the upregulation of IL-6 in balding DP cells, we therefore examined the expression of *IL-6* transcript by reverse

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Abbreviations: DHT, dihydrotestosterone; DP, dermal papilla; DS, dermal sheath; gp130, glycoprotein 130; IL-6R, IL-6 receptor; rhIL-6, recombinant human IL-6

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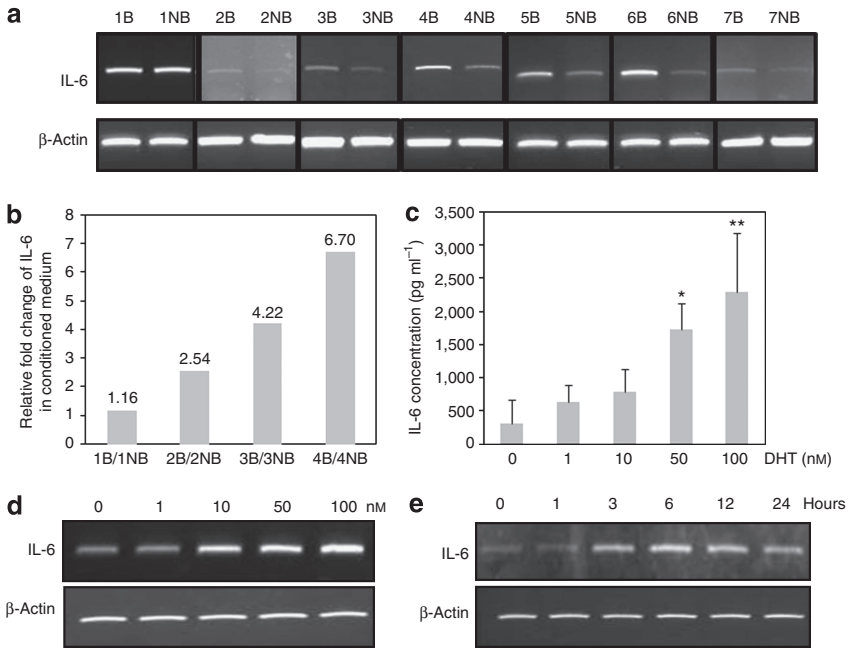

Figure 1. Upregulation of IL-6 in cultured balding DP cells compared with the non-balding DP cells and IL-6 induction in response to DHT in balding DP cells. Seven pairs of matched balding and non-balding dermal papilla (DP) cells were analyzed by reverse transcription (RT)-PCR (**a**). IL-6 levels in conditioned medium of balding (B) and non-balding (NB) DP cells from four matched pairs in duplicate measurements were made by ELISA and the relative fold change of IL-6 in each cases (B/NB) are shown (**b**). Balding DP cells were treated with varying concentrations of dihydrotestosterone (DHT), and concentrations of IL-6 were measured by ELISA (**c**); values represent the mean ± SD of five experiments using five different DP cell strains and duplicate measurements were made for each experiment (*$P<0.005$, **$P<0.001$). Cells were also treated with varying concentrations of DHT for 6 hours (**d**) or 100 nM DHT for varying times (**e**) and analyzed by RT-PCR.

transcription-PCR analysis using seven pairs of balding DP cells and matched non-balding DP cells. Higher levels of *IL-6* expression were observed in balding DP cells in six of seven cases (Figure 1a). Next, we measured the concentration of IL-6 in the conditioned medium produced by balding DP cells and non-balding DP cells using ELISA. We found that all of balding DP cells secrete higher levels of IL-6 compared with matched non-balding DP cells (Figure 1b).

IL-6 induction in balding DP cells by DHT

We next examined whether or not IL-6 expression is affected by DHT. As androgen receptor expression is downregulated significantly in cultured balding DP cells (Kwack *et al.*, 2008), early-passage (p2–p3) DP cells were treated with varying physiological concentrations of DHT or vehicle control (ethanol). ELISA showed that IL-6 is secreted from balding DP cells in response to DHT and maximum secretion was observed in 100 nM DHT. The mean concentration of IL-6 was 2,286 pg ml^{-1} in the presence of 100 nM DHT and 361 pg ml^{-1} in the absence of DHT (Figure 1c). Reverse transcription-PCR analysis showed that *IL-6* is upregulated after 10–100 nM DHT treatment and maximum induction was observed at 100 nM DHT (Figure 1d). We also observed that *IL-6* is upregulated 3 hours after DHT treatment (Figure 1e).

IL-6R and gp130 is expressed in human follicular keratinocytes

To identify the target cells of IL-6 in human hair follicles, we examined the expression of IL-6 receptor (IL-6R) and glycoprotein 130 (gp130), a critical IL-6 family member protein, in human hair follicles. We found that IL-6R and gp130 are expressed in matrix cells, as well as the outer and inner root sheaths of human scalp hair follicles (Figure 2a–f). Immunoblot analysis also showed that IL-6R and gp130 are expressed in cultured matrix cells and outer root sheath keratinocytes (Figure 2g). Occasionally, we observed weak expression of IL-6R and gp130 in the DP and DS of hair follicles and in cultured DP and DS cells. When expression of the IL-6R (Figure 3a–d) and gp130 (Figure 3e–h) in balding versus non-balding hair follicles was examined, no significant difference was observed.

IL-6 inhibits hair shaft elongation and proliferation of matrix cells in cultured human hair follicles

We next investigated the effects of IL-6 on hair shaft elongation using a hair follicle organ culture system (Philpott *et al.*, 1994). Isolated scalp hair follicles were cultured for 6 days in the presence or absence of rhIL-6. In this study, we used 5–50 ng ml^{-1} of IL-6. Although we were not sure as to whether these doses were physiologic, 25–100 ng ml^{-1} of IL-6 is commonly used in *in vitro* studies (Hernández-Quintero *et al.*, 2006; Wang *et al.*, 2007). We observed that hair shaft elongation is inhibited by IL-6 treatment in a dose-dependent manner. The average elongation of hair shafts after 6 days was 1.96 mm in the absence of IL-6, whereas 1.62 and 1.33 mm in the presence of 5 and 50 ng ml^{-1} of rhIL-6, respectively (Figure 4a). We also observed that the number of

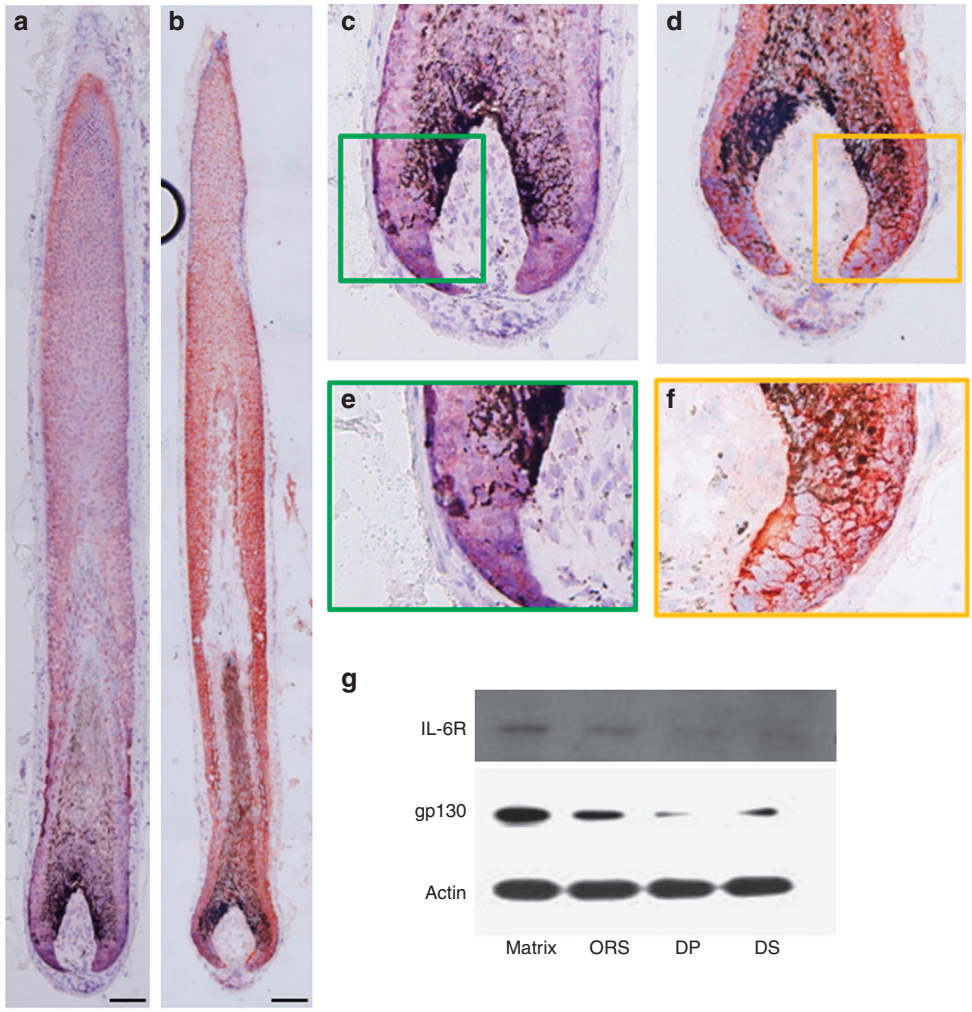

Figure 2. Expression of IL-6R and gp130 in hair follicles and cultured cells. Human scalp hair follicles were immunostained with antibodies against IL-6 receptor (IL-6R, **a**) and glycoprotein 130 (gp130, **b**). High-powered images of IL-6R and gp130 expression in hair bulb regions are also shown in **c** and **d**, respectively. Close-up images of boxed regions of **c** and **d** with emphasis on the follicular matrix are also shown in **e** and **f**. Bar = 0.1 mm. Immunoblot analysis (**g**) of IL-6R and gp130 protein expression in cultured hair cells. Actin expression was measured to check the quantity and integrity of the protein samples. DP, dermal papilla; DS, dermal sheath; ORS, outer root sheath.

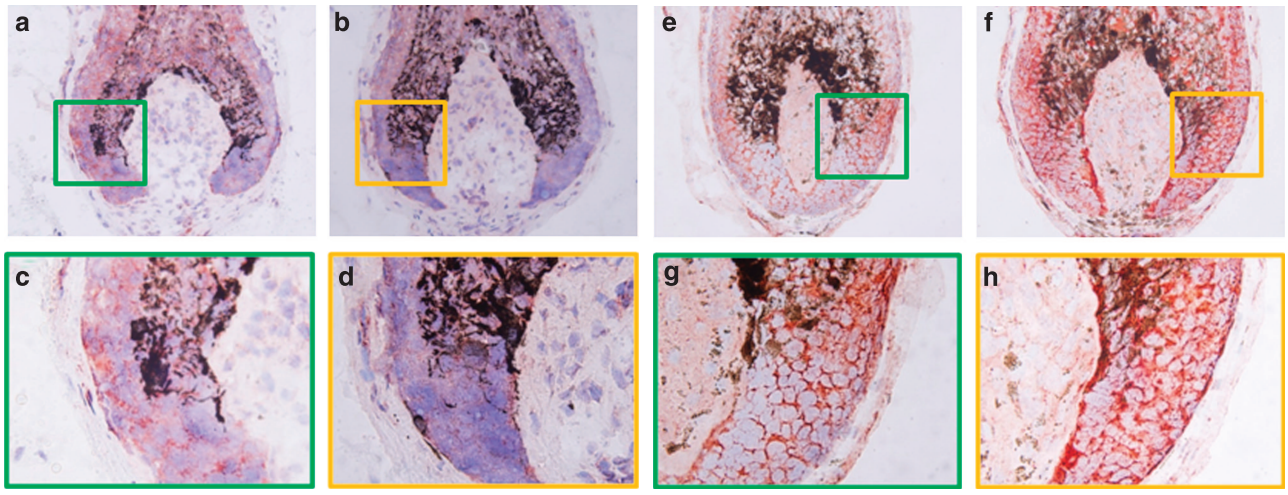

Figure 3. Immunohistochemical staining for IL-6R and gp130 in balding versus non-balding hair follicles. Matched balding (**a** and **e**) and non-balding (**b** and **f**) scalp hair follicles were immunostained with antibodies against IL-6R (**a** and **b**) and gp130 (**e** and **f**). Close-up images of boxed regions of **a**, **b**, **e**, and **f** with emphasis on the follicular matrix are also shown in **c**, **d**, **g**, and **h**. gp130, glycoprotein 130; IL-6R, IL-6 receptor.

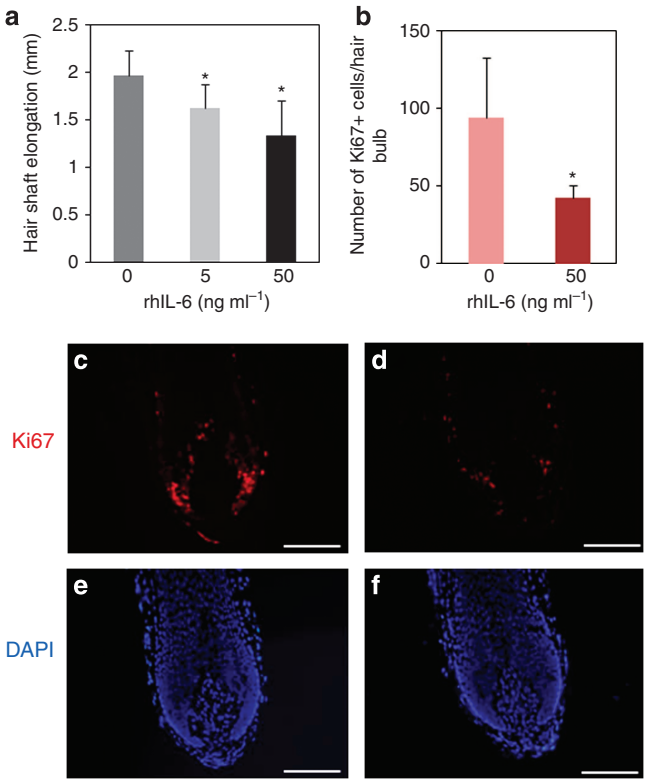

Figure 4. Effect of rhIL-6 on cultured human hair follicles. Isolated human hair follicles were cultured for 6 days in the absence or presence of 5 or 50 ng ml^{-1} recombinant human IL-6 (rhIL-6), and hair shaft elongation was measured (**a**). Values are average of four mean ± SD of six determinations per experiment from four experiments (*$P<0.05$). Human hair follicles were treated in the absence (**c** and **e**) or presence of 50 ng ml^{-1} rhIL-6 (**d** and **f**) for 2 days and stained with Ki-67 immunofluorescence staining. Corresponding 4,6-diamidino-2-phenylindole (DAPI) nuclear staining is also shown (**e** and **f**). Ki-67-positive proliferative cells (red) in the hair bulb were counted and data are mean ± SD from five hair follicles (*$P<0.05$; **b**). Bar = 0.1 mm.

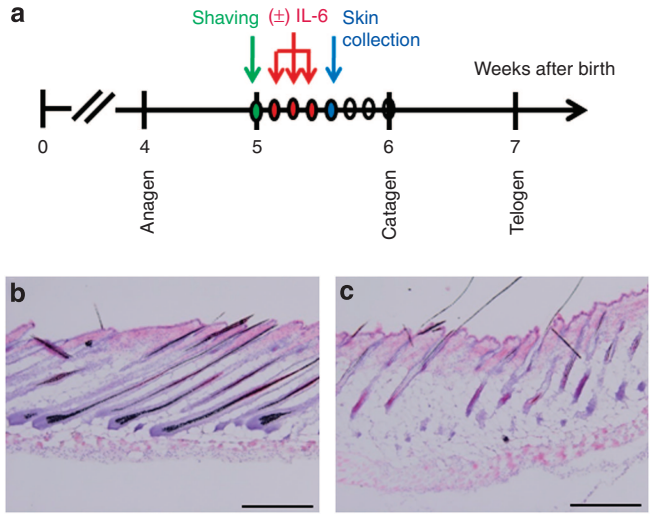

Figure 5. Effect of rhIL-6 on catagen onset. Scheme of the experiment (**a**). The back skins of C57BL/6 mice ($n=6$) were shaved at 5 weeks of age and the vehicle (**b**) or 100 µl of 500 ng ml^{-1} of recombinant human IL-6 (rhIL-6; **c**) was injected once a day for 3 days. The skin samples were stained with hematoxylin and eosin and a representative histology is shown. The same experiment was repeated twice using different mice. Bar = 0.1 mm.

Ki-67-positive matrix cells around DP was markedly decreased in the presence of 50 ng ml^{-1} of IL-6 (Figure 4b–d).

IL-6 treatment accelerates catagen onset from anagen in mice

To determine whether or not rhIL-6 induces premature transition from anagen to catagen *in vivo*, the dorsal surfaces of 5-week-old female C57BL/6 mice in the anagen stage of the hair cycle (Müller-Röver *et al.*, 2001) were shaved and 100 µl of 500 ng ml^{-1} of rhIL-6 was injected once a day for 3 days. Histological examination of sections of the back skins showed that hair follicles from the rhIL-6-injected skins entered the catagen phase, whereas hair follicles from the vehicle-treated skin remained in the anagen phase (Figure 5).

DISCUSSION

Although the underlying molecular, pathogenic mechanism of male pattern baldness remains to be elucidated, recent published data strongly suggest that inhibitory paracrine and autocrine factors produced by the balding DP cells by DHT-driven alterations may be key to male pattern baldness (reviewed by Randall, 2008; Inui and Itami, 2011). Specifically, transforming growth factors-β1/β2, which inhibit epithelial cell growth, are increased from DP cells from the balding scalp in response to androgens (Inui *et al.*, 2002; Hibino and Nishiyama, 2004), and DHT-inducible dickkopf 1 from balding DP cells causes apoptosis in follicular keratinocytes (Kwack *et al.*, 2008). In addition, balding DP cells secrete inhibitory autocrine factors that inhibit the growth of DP cells (Hamada and Randall, 2006). With the hope of identifying such paracrine and/or autocrine factors, in a preliminary study we performed ELISA array screening and found that higher levels of IL-6 are secreted from balding DP cells compared with non-balding DP cells.

In this study, we observed that IL-6 mRNA and protein are upregulated in six of seven (85%) and three of four (75%) balding DP cells in male pattern baldness patients, respectively. In addition, we observed that the level of expression of IL-6 mRNA and protein was upregulated in response to DHT in balding DP cells. This result is in agreement with the finding that DHT increases IL-6 expression in SKOV-3 ovarian cancer cell lines (Wang *et al.*, 2007). Further investigation is needed to elucidate the mechanism of DHT-induced IL-6 expression.

Previous studies have reported that IL-6 is expressed in the DP and DS of human hair follicles and in cultured DP cells and DS cells, whereas IL-6R and gp130 are expressed in the inner and outer root sheaths of human hair follicles (Ahmed *et al.*, 1996; Chiu *et al.*, 1996; Yu *et al.*, 2008). In this study, we also showed that IL-6R and gp130 are expressed in the inner and outer root sheaths of human hair follicles. However, in contrast to the reports of Yu *et al.* (2008) and Ahmed *et al.* (1996), we observed that IL-6R and gp130 are also expressed on matrix cells of hair follicles and in cultured matrix cells. In agreement with this observation, we found a

significant reduction of Ki67-positive matrix cells and inhibition of hair shaft elongation by IL-6 treatment in cultured human hair follicles. These results strongly suggest that IL-6 is released from DP cells and inhibits the activity of follicular keratinocytes that express IL-6R *in situ*. However, our finding is inconsistent with the report from Limat *et al.* (1993), who found no correlation between outer root sheath cell proliferation and IL-6 production in the coculture system, and the report from Hernández-Quintero *et al.* (2006), who found promotion of human epidermal keratinocyte proliferation by IL-6. We suggest that the discrepancy is due to the culture conditions or cell types used.

In developing male pattern baldness, hair follicles undergo premature transition from anagen to catagen induced by androgens (Jahoda, 1998), resulting in a short anagen phase. Because we observed that IL-6 inhibits matrix cell proliferation, which expresses IL-6R resulting in hair shaft elongation in cultured human hair follicles, we reasoned that IL-6 promotes anagen-to-catagen transition *in vivo*. Indeed, we observed that IL-6 injection into the hypodermis during anagen causes premature onset of catagen in mice. These results, together with DHT-induced IL-6 expression in balding DP cells, suggest that IL-6 is one of the paracrine factors from DP, thereby shortening anagen hair growth (Figure 6).

Our finding of a hair growth-inhibitory action for IL-6 is in agreement with the retarded hair growth in transgenic mice that overexpress IL-6 under the control of keratin-14 promoter (Turksen *et al.*, 1992), and with the hair growth retardation in mice receiving oncostatin M, an IL-6 cytokine family member (Yu *et al.*, 2008). Our data are also in agreement with the recent report by Hamada and Randall (2006), who found that conditioned medium of human balding DP cells delay the onset of anagen in mice. However, our finding differs with the recent report from Tanabe *et al.* (2006) who found a hair growth-promoting effect of IL-6. Although further investigation is needed, we suggest that the discrepancy might be due to the doses of IL-6 used.

In summary, we have provided evidence, which to our knowledge is previously unreported, that balding DP cells secrete higher levels of IL-6 compared with the non-balding DP cells. We also have shown that IL-6 is inducible by DHT in balding DP cells. In addition, IL-6 inhibits hair shaft elongation and inhibits proliferation of matrix cells that express IL-6R in cultured hair follicles. These data, together with the early catagen induction effect of IL-6, strongly suggest that DHT-inducible IL-6 inhibits hair growth as a paracrine mediator from the DP.

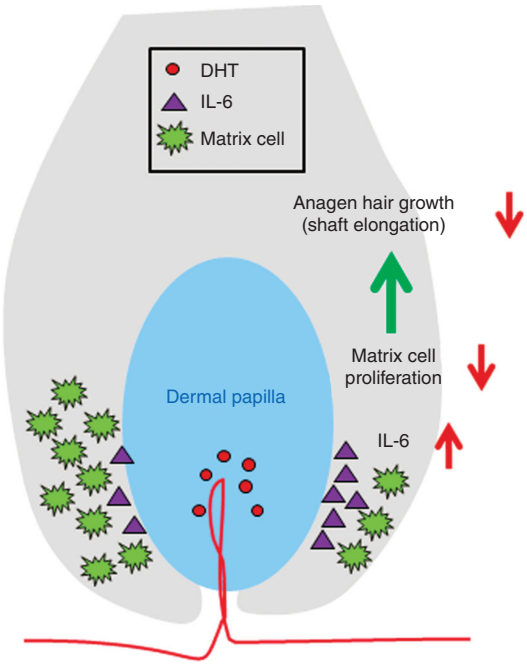

Figure 6. Proposed model based on this study. Dihydrotestosterone (DHT)-inducible IL-6 from dermal papilla functions as an inhibitory paracrine mediator that inhibits hair shaft growth by suppressing matrix cell proliferation.

MATERIALS AND METHODS

Isolation and culture of human hair follicles

Non-balding scalp specimens were obtained from patients undergoing hair transplantation surgery, and matching balding scalp specimens were obtained by punch biopsy (3 mm) from the same individuals. The Medical Ethical Committee of the Kyungpook National University Hospital (Daegu, Korea) approved all of the described studies. The study was conducted according to the Declaration of Helsinki Principles. Informed written consent was obtained from the patients. Hair follicles from non-balding scalps were used for organ culture studies. The hair follicles were isolated and cultured as described before (Kwack *et al.*, 2008).

Cell culture

DP and DS were isolated from the bulbs of the dissected hair follicles, transferred onto plastic dishes coated with bovine type 1 collagen, and cultured in DMEM (Gibco BRL, Gaithersburg, MD), supplemented with penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}), and 20% heat-inactivated fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2. The explants were left for several days and the medium was changed every 3 days. After the cell outgrowth became subconfluent, cells were harvested with 0.25% trypsin/10 mM EDTA in Hanks' balanced salt solution and subcultured with a split ratio of 1:3. Subsequently, DP and DS cells were maintained in DMEM supplemented with 10% fetal bovine serum.

Matrix cells were isolated and cultured as described before (Roh *et al.*, 2004). Briefly, hair follicles at anagen phase were selected, and matrix cell-containing tissue fragments were dissected out. Hair fragments were digested with 0.05% trypsin–EDTA for 10 minutes, and Versene (0.53 mM, Gibco BRL) was added for an additional 10 minutes. Cells were centrifuged and plated onto tissue culture plastic dishes. The isolated cells were then cultured in keratinocyte growth medium on a feeder layer of NIH3T3 fibroblasts that had been pretreated with mitomycin C for 2 hours. Outer root sheath cells were cultured as described before (Kwack *et al.*, 2008). Briefly, the hair shaft and hair bulb regions of the hair follicle were cut off to prevent contamination with other cells. Trimmed hair follicles were immersed in DMEM supplemented with 20% fetal bovine serum. On the third day of culture, the medium was changed to keratinocyte growth medium containing penicillin, streptomycin, and fungizone.

Reverse transcription-PCR analysis

Total RNA was isolated using TRIzol reagent and complementary DNA was synthesized from 3 μg of total RNA using the complementary DNA synthesis kit containing the ImProm-IITM reverse transcriptase and oligo-dT primer, according to the instructions of the manufacturer (Promega, Madison, WI). Complementary DNA (1 μl) was amplified with each of the forward and reverse primers. For the detection of *IL-6*, 30 cycles (45 seconds at 94 °C, 45 seconds at 56 °C, and 45 seconds at 72 °C) of amplification were performed with forward primer 5′-TACCCCCAGGAGAAGATTCC-3′ and reverse primer 5′-GAGGTGCCCATGCTACATTT-3′. For the detection of β-actin, 23 cycles (45 seconds at 94 °C, 45 seconds at 58 °C, and 45 seconds at 72 °C) of amplification was performed with 5′-GGGAA ATCGTGCGTGACATT-3′ and 5′-GGAGTTGAAGGTAGTTTCGT-3′. PCR products were separated by electrophoresis on a 1% agarose gel and visualized under UV light.

ELISA

An IL-6 ELISA kit (R&D Systems, Minneapolis, MN) was used according to the manufacturer's protocol. For the measurement of IL-6 levels in conditioned medium of balding and non-balding DP cells, cells from passages 2–3 were plated overnight at a density of 300,000 cells per 100 mm culture dish, washed three times with phosphate-buffered saline (PBS), and then incubated in serum-free medium for 24 hours for the collection of conditioned medium. To examine IL-6 induction in response to DHT in balding DP cells, cells were treated with varying concentrations of DHT in serum-free medium for 24 hours and concentrations of IL-6 in conditioned medium were measured. Briefly, 50 μl of culture conditioned medium was loaded per well on titer plates coated with IL-6 antibody and incubated at 4 °C for 2 hours, washed, and incubated with IL-6 conjugate at 4 °C for 2 hours. Further, 200 μl of substrate solution was added and incubated for 30 minutes at room temperature. Optical density was measured by an ELISA reader at 450 nm. Serial dilutions of rhIL-6 were used to establish a standard curve.

Immunoblotting

Total cell lysates (5 μg per lane) were separated by 10% SDS-PAGE, and then transferred to nitrocellulose membranes. The membranes were blocked with 5% milk in PBS for 1 hour, and probed with rabbit polyclonal antibodies against IL-6R (1:1,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and gp130 (1:1,000 dilution; Santa Cruz Biotechnology) at 4 °C overnight. Horseradish peroxidase-conjugated donkey anti-rabbit Ig (Zymed Laboratories, San Francisco, CA) was used as the secondary antibody at a 1:7,000 dilution. The bands were visualized using ECL Plus (Amersham, Buckinghamshire, UK). The membranes were also probed with mouse mAb against actin (Chemicon, Temecula, CA).

Immunohistochemistry and immunofluorescence staining

Hair follicle specimens were placed in cryomolds using embedding medium (OCT compound, Tissue-Tek; Miles, Napierville, IL) in a freezer at −80 °C. The tissue block was cut into sections (7 μm thick) using a cryostat (Leica CM3050 S; Heidelberg, Germany) and sections were applied on glass slides. Tissue sections were fixed with 4% paraformaldehyde containing 0.1% Triton X-100 for 10 minutes and equilibrated in PBS for 15 minutes at room temperature. After blocking with 4% normal donkey serum (Jackson ImmunoResearch, West Grove, PA) for 1 hour at room temperature, sections were incubated with IL-6R (1:50 dilution) and gp130 (1:50 dilution) at 4 °C overnight, washed three times with PBS, incubated with secondary antibody-horseradish peroxidase-conjugated antibody for 1 hour, and rinsed again with PBS. AEC (DAKO, Glostrup, Denmark) was used as a color-developing reagent for horseradish peroxidase, and the slides were counterstained with hematoxylin.

For immunofluorescence staining of Ki-67, fixation and blocking procedures were performed as above. Sections were incubated with Ki-67 antibody (1:100 dilution; Becton Dickinson, Franklin, NJ) at 4 °C overnight, washed three times with PBS, and incubated with Alexa Flour 555-labeled donkey anti-mouse secondary antibody (Molecular Probes, Eugene, OR) for 1 hour. The slides were then washed with PBS and counterstained with 4,6-diamidino-2-phenylindole for 10 minutes.

In vivo study

Five-week-old female C57BL/6 mice in the anagen stage of the hair cycle (Müller-Röver *et al.*, 2001) were purchased from Orient Bio (Seongnam, Korea). After 1 day, the dorsal areas of each mouse were shaved with clippers and 500 ng ml^{-1} of rhIL-6 in 100 μl of PBS was administered subcutaneously for 3 days. Further, mice were killed and the treated region of the dorsal skin was stained with hematoxylin and eosin.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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REFERENCES

Ahmed AA, Nordlind K, Schultzberg M *et al.* (1996) Immunohistochemical studies of proinflammatory cytokines and their receptors in hair follicles of normal human skin. *Acta Derm Venereol* 76:348–52

Botchkarev VA, Kishimoto J (2003) Molecular control of epithelial-mesenchymal interactions during hair follicle cycling. *J Investig Dermatol Symp Proc* 8:46–55

Chiu HC, Chang CH, Chen JS *et al.* (1996) Human hair follicle dermal papilla cell, dermal sheath cell and interstitial dermal fibroblast characteristics. *J Formos Med Assoc* 95:667–74

Drake L, Hordinsky M, Fiedler V *et al.* (1999) The effects of finasteride on scalp skin and serum androgen levels in men with androgenetic alopecia. *J Am Acad Dermatol* 41:550–4

Hamada K, Randall VA (2006) Inhibitory autocrine factors produced by the mesenchyme-derived hair follicle dermal papilla may be a key to male pattern baldness. *Br J Dermatol* 154:609–18

Hardy MH (1992) The secret life of the hair follicle. *Trends Genet* 8:55–61

Hernández-Quintero M, Kuri-Harcuch W, González Robles A *et al.* (2006) Interleukin-6 promotes human epidermal keratinocyte proliferation and keratin cytoskeleton reorganization in culture. *Cell Tissue Res* 325:77–90

Hibino T, Nishiyama T (2004) Role of TGF-beta2 in the human hair cycle. *J Dermatol Sci* 35:9–18

Hoffmann R (2002) Male androgenetic alopecia. *Clin Exp Dermatol* 27:373–82

Imperato-McGinley J, Guerrero L, Gautier T *et al.* (1974) Steroid 5alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science* 186:1213–5

Inui S, Fukuzato Y, Nakajima T *et al.* (2002) Androgen-inducible TGF-beta1 from balding dermal papilla cells inhibits epithelial cell growth: a clue to understand paradoxical effects of androgen on human hair growth. *FASEB J* 16:1967–9

Inui S, Itami S (2011) Molecular basis of androgenetic alopecia: from androgen to paracrine mediators through dermal papilla. *J Dermatol Sci* 61:1–6

Jahoda CA (1998) Cellular and developmental aspects of androgenetic alopecia. *Exp Dermatol* 7:235–48

Kaufman KD (2002) Androgens and alopecia. *Mol Cell Endocrinol* 198: 89–95

Kuttenn F, Mowszowicz I, Wright F *et al.* (1979) Male pseudohermaphroditism: a comparative study of one patient with 5 alpha-reductase deficiency and three patients with the complete form of testicular feminization. *J Clin Endocrinol Metab* 49:861–5

Kwack MH, Sung YK, Chung EJ *et al.* (2008) Dihydrotestosterone inducible dickkopf 1 from balding dermal papilla cells causes apoptosis in follicular keratinocytes. *J Invest Dermatol* 128:262–9

Limat A, Hunziker T, Waelti ER *et al.* (1993) Soluble factors from human hair papilla cells and dermal fibroblasts dramatically increase the clonal growth of outer root sheath cells. *Arch Dermatol Res* 285: 205–10

Millar SE (2002) Molecular mechanisms regulating hair follicle development. *J Invest Dermatol* 118:216–25

Müller-Röver S, Handjiski B, van der Veen C *et al.* (2001) A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. *J Invest Dermatol* 117:3–15

Philpott MP, Sanders DA, Westgate GE *et al.* (1994) Effects of insulin and insulin-like growth factors on cultured human hair follicles: IGF-1 at physiologic concentrations is an important regulator of hair follicle. *J Invest Dermatol* 102:857–61

Randall VA (2008) Androgens and hair growth. *Dermatol Ther* 21:314–28

Randall VA, Thornton MJ, Hamada K *et al.* (1994) Androgen action in cultured dermal papilla cells from human hair follicles. *Skin Pharmacol* 7:20–6

Rathnayake D, Sinclair R (2010) Male androgenetic alopecia. *Expert Opin Pharmacother* 11:1295–304

Roh C, Tao Q, Lyle S (2004) Dermal papilla-induced hair differentiation of adult epithelial stem cells from human skin. *Physiol Genomics* 19:207–17

Rushton DH, Ramsay ID, Norris MJ *et al.* (1991) Natural progression of male pattern baldness in young men. *Clin Exp Dermatol* 16:188–92

Stenn KS, Paus R (2001) Controls of hair follicle cycling. *Physiol Rev* 81:449–94

Tanabe A, Ogawa Y, Takemoto T *et al.* (2006) Interleukin 6 induces the hair follicle growth phase (anagen). *J Dermatol Sci* 43:210–3

Turksen K, Kupper T, Degenstein L *et al.* (1992) Interleukin 6: insights to its function in skin by overexpression in transgenic mice. *Proc Natl Acad Sci USA* 89:5068–72

Wang Y, Yang J, Gao Y *et al.* (2007) Reciprocal regulation of 5alpha-dihydrotestosterone, interleukin-6 and interleukin-8 during proliferation of epithelial ovarian carcinoma. *Cancer Biol Ther* 6:864–71

Yu M, Kissling S, Freyschmidt-Paul P *et al.* (2008) Interleukin-6 cytokine family member oncostatin M is a hair-follicle-expressed factor with hair growth inhibitory properties. *Exp Dermatol* 17:12–9