

Role of Androgen in Mesenchymal Epithelial Interactions in Human Hair Follicle

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Human hair follicles, which are distributed in various and specific sites of the body, appear to have an inherited susceptibility for androgen-dependent growth. Beard, axillary, and frontal scalp dermal papilla cells (DPC) were recently shown to possess the characteristics of androgen target cells. These DPC show strong expression of androgen receptors, and the expression of type II 5 α reductase is restricted to beard and frontal scalp DPC. These findings suggest that DPC mediate the signals of androgen to follicular epithelial cells in a paracrine fashion. We developed an *in vitro* co-culture system using DPC and keratinocytes (KC) to characterize the mode of androgen action in human hair follicles. Androgen significantly stimulated the proliferation of KC co-cultured with beard DPC, indicating that beard DPC produce androgen-dependent diffusible growth factors. Insulin-like growth factor-I was identified as one of the androgen-dependent paracrine growth factors produced by beard DPC. We also identified the inhibitory role of androgen on the growth of KC co-cultured with DPC from androgenetic alopecia (AGA) when the DPC were transfected with an expression vector encoding the androgen receptor. This growth suppression of KC was mediated by transforming growth factor- β 1 (TGF- β 1) derived from DPC of AGA, suggesting that TGF- β 1 is a paracrine mediator for AGA.

Key words: hair follicle/androgen/dermal papilla cells/IGF-1/TGF- β 1/androgenetic alopecia

J Investig Dermatol Symp Proc 10:209–211, 2005

Hair follicles are composed primarily of epithelial and dermal components that develop from the embryonic ectoderm and mesoderm, respectively. The hair growth cycle is a coordinated and complex process that depends on the interactions of epithelial and dermal components. For the past 10 y, the developmental mechanism of hair follicles has been extensively studied using transgenic and knockout mice (Millar, 2002). Spatial and temporal expression of many molecules is required for hair morphogenesis. In particular, the Wnt- β -Cat-Lef1 signaling pathway plays a key role (Alonso and Fuchs, 2003). These molecules are also required for progression of the hair cycle, whereas several molecules, usually required for catagen progression, are not indispensable in the morphogenesis (Stenn and Paus, 2001). The androgen receptor, which is a ligand-dependent transcription factor, is also not involved in hair morphogenesis, but plays an important role in the human hair cycle. This is in contrast to the prostate, where the androgen receptor is indispensable in morphogenesis (Chang and Chung, 1989). After puberty, androgen stimulates the growth of beard, axillary, and pubic hair, whereas paradoxically it causes vellus transformation in the frontal scalp hair of individuals with an appropriate genetic background, and prepubertal castration is known to prevent this transformation (Hamilton, 1942, 1958). These biological and patholog-

ical observations indicate that human hair follicles have an inherited susceptibility for androgen-dependent growth. But the paradoxical action of androgen in human hair follicles has long been a mystery. The pathophysiology of androgenetic alopecia (AGA) is thought to involve the transformation of terminal hair follicles to vellus hair follicles by androgen via the shortening of the anagen phase in genetically predisposed individuals. Conversely, androgen extends the anagen phase in beard follicles. In other words, the same transcription factor can have opposite functions depending on the type of hair. The androgen receptor thus mediates positive signals to prolong the anagen phase in beard follicles, whereas the same receptor exerts a negative function to shorten the anagen phase in AGA.

Androgen Targets Dermal Papilla Cells (DPC) in Human Hair Follicles

Although the process of regulation of hair growth by hormones is still unclear, beard, axillary, and frontal scalp DPC, the mesenchymal components of hair follicles, were recently shown to possess the characteristics of androgen target cells. Androgen receptors were detected in beard, axillary, and frontal scalp DPC, but not in DPC of androgen-independent occipital scalp hair follicles (Choudhry *et al*, 1992; Randall *et al*, 1992; Itami *et al*, 1995a; Hibberts *et al*, 1998). Beard DPC possess higher levels of 5 α -reductase activity than do reticular dermal fibroblasts or DPC from the occipital scalp *in vitro* (Itami *et al*, 1990, 1991; Thornton

Abbreviations: AGA, androgenetic alopecia; DPC, dermal papilla cells; IGF-I, insulin-like growth factor I; KC, keratinocytes; ORSC, outer root sheath cells; TGF- β 1, transforming growth factor- β 1

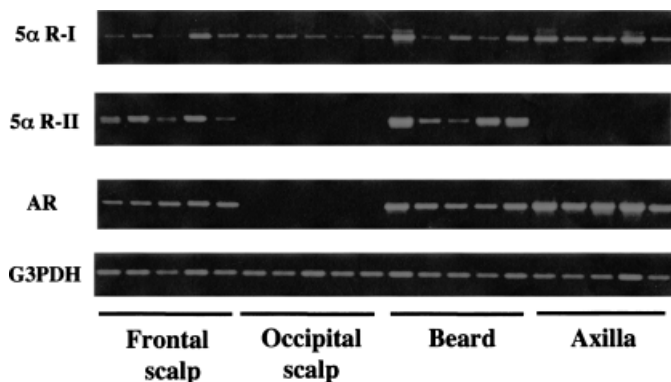


Figure 1
Expression of androgen receptor, type I, and type II 5 α reductase in human cultured dermal papilla cells from various body sites. AR, androgen receptor; 5 α R-I, type I 5 α -reductase; 5 α R-II, type II 5 α reductase.

et al, 1993). Further, the enzyme found in beard DPC appears to be type II 5 α reductase. In contrast, follicular epithelial cells do not have the characteristics of target cells except for medullary cells of sexual hairs (Jave-Suarez *et al*, 2004). When expression of mRNA encoding the androgen receptor, type I, and type II 5 α reductase in DPC from various body sites was examined, androgen receptor mRNA was strongly expressed in beard, frontal scalp, and axillary DPC (Fig 1) (Nakanishi *et al*, 1996; Asada *et al*, 2001). Although the expression of type I 5 α reductase is a ubiquitous property of DPC, expression of the type II 5 α -reductase gene is limited to beard and frontal scalp DPC. These data suggest that strong phenotypic expression in men, such as beard growth at puberty and genetically determined balding on the frontal scalp, requires type II 5 α reductase and androgen receptor in DPC. These findings also suggest that the primary target cells in human hair follicles that respond to the action of androgen are DPC, which mediate the signals to follicular epithelial cells in a paracrine fashion.

Beard Growth Regulation by Androgen

In order to know the mode of androgen action in human hair follicles, we developed an *in vitro* co-culture system using DPC and outer root sheath cells (ORSC). Androgen significantly stimulated the proliferation of ORSC co-cultured with beard or axillary DPC, suggesting that these DPC produce androgen-dependent diffusible growth factors (Itami *et al*, 1995a). Insulin-like growth factor-I (IGF-I) has been identified as one of the androgen-dependent paracrine growth factors in DPC (Itami *et al*, 1995b). IGF-I is a paracrine/autocrine growth factor found in many organs, and its expression is normally limited to mesenchymal cells. IGF-I has been found to stimulate human hair growth *in vitro* at physiologic concentrations and to prevent the premature entry of cultured hair follicles into catagen (Philpott *et al*, 1994).

Transforming Growth Factor- β 1 (TGF- β 1) Is a Paracrine Mediator for AGA

Coculture of ORSC and DPC isolated from the frontal scalp of stump-tailed macaques, a model animal for human

AGA, demonstrated androgen-induced growth inhibition of ORSC, suggesting that androgen-dependent soluble factors from DPC are involved in the hair growth suppression in AGA (Obana *et al*, 1997; Pan *et al*, 1999). Using the same co-culture system, we tried to identify the growth-suppressive role of frontal DPC derived from AGA for the growth of epithelial cells. In this study, we used epidermal keratinocytes (KC) instead of ORSC, because growth stimulation by testosterone was observed even in the case of inter-follicular epidermal KC in our co-culture system using axillary and beard DPC (Itami *et al*, 1995a). Androgen, however, had no significant influence on the proliferation of KC in these co-cultures. As the expression of androgen receptor decreases during the subcultivation of bald DPC, we re-introduced the androgen receptor by transfecting the expression vector into DPC, and then co-cultured these two kinds of cells in the presence or absence of androgen. Under this modified co-culture condition, we could reproduce the *in vivo* effects of androgen on human hair growth (Inui *et al*, 2002). Androgen stimulated the growth of KC when co-cultured with beard DPC, as already reported (Itami *et al*, 1995b). Paradoxically, androgen suppressed the growth of KC co-cultured with frontal DPC from AGA (Fig 2). Interestingly, androgen showed no significant effect on the growth of KC co-cultured with non-bald frontal or occipital DPC, even under conditions of androgen receptor overexpression. This suggests that not only the androgen receptor but also other factors are involved in the action of androgen on human hair follicles.

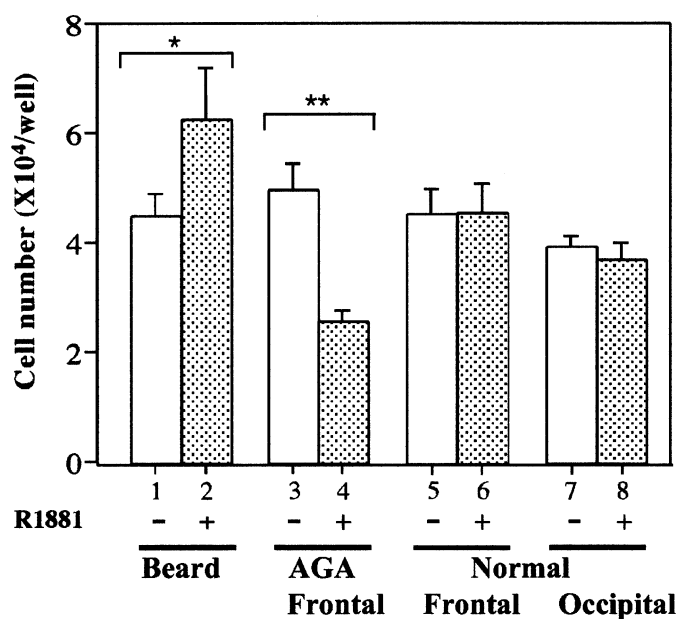


Figure 2
Effect of androgen on the proliferation of keratinocytes (KC) co-cultured with dermal papilla cells (DPC) from various body sites transiently transfected with pSG5-AR. KC were co-cultured with beard (lanes 1 and 2), bald frontal (lanes 3 and 4), non-bald frontal (lanes 5 and 6), or occipital DPC (lanes 7 and 8) transfected with pSG5-AR. The cells were cultured in the absence (lanes 1, 3, 5, and 7) or in the presence of 10^{-9} M R1881 (lanes 2, 4, 6, and 8) for 4 d. Each bar represents the mean \pm SD of three independent experiments using a different set of cell lines. * $p < 0.05$, ** $p < 0.01$ versus the respective controls (modified from figures in Inui *et al*, 2002).

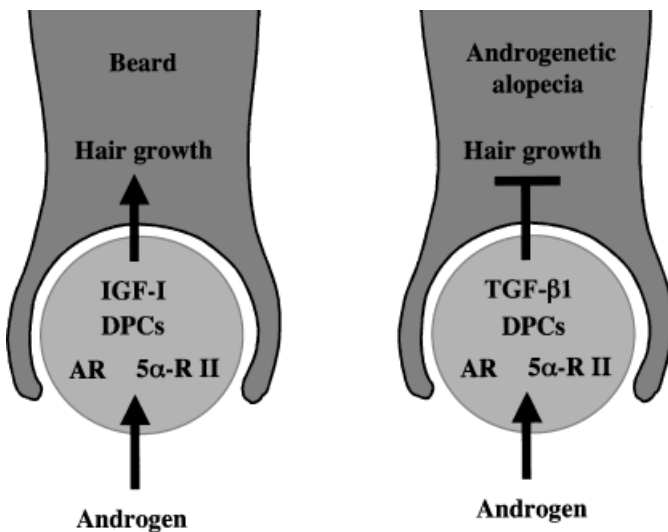


Figure 3
Schematic diagram of androgen action in human hair follicles. For the beard growth stimulation by androgen, insulin-like growth factor-I (IGF-I) acts as the paracrine mediator from dermal papilla cells (DPC) to epithelial cells. On the other hand, transforming growth factor- β 1 (TGF- β 1) is an androgen-dependent paracrine mediator for androgenetic alopecia.

To identify genes responsible for the androgen-induced growth inhibition of KC, we focused on TGF- β 1, because this growth factor is well known to inhibit the growth of KC and to induce catagen progression in mice (Shiple *et al*, 1986; Foitzik *et al*, 2000; Liu *et al*, 2001). We found that TGF- β 1 expression was upregulated by androgen in bald frontal DPC. Non-bald frontal DPC did not upregulate TGF- β 1 by androgen. Furthermore, secretion of TGF- β 1 into the medium was stimulated by androgen, indicating that TGF- β 1 mediates the signal from DPC to KC for their growth suppression (Inui *et al*, 2002). In the co-culture of non-bald frontal DPC, the secretion of TGF- β 1 is low and androgen did not stimulate the secretion.

We were unable to identify androgen-responsive elements in the TGF- β 1 promoter. TGF- β 1 promoter activation by androgen is bald frontal DPC specific and is not observed in non-bald frontal DPC, suggesting that some intrinsic factor(s) in bald frontal DPC are required (data not shown). Factor(s) that are genetically involved in AGA would be the true target of the pathomechanism of the disease. Our modified co-culture system will provide clues to further explore the role of androgens in the regulation of hair growth.

In conclusion, the long-standing paradox of the contrasting effects of androgen on human hair follicles is now partially elucidated (Fig 3), and TGF- β 1 is a new target to treat AGA.

DOI: 10.1111/j.1087-0024.2005.10107.x

Manuscript received September 20, 2004; revised December 13, 2004; accepted for publication January 6, 2005

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