PATHOBIOLOGY OF ANDROGENETIC ALOPECIA

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Human hair morphogenesis is a dynamic process caused by the remodelling of the skin. Hair growth is cyclic in mammals consisting of three distinct stages: an active stage (anagen), a regressive stage (catagen), and a resting stage (telogen). One disorder in this process is gradual balding of the scalp called androgenetic alopecia. Little is known about the cell biological or molecular mechanisms involved and thus very little treatment is currently available. In this review we focus on the most significant parameters affecting hair growth which participate in baldness.

INTRODUCTION

The skin of almost entire the human body contains hairs. Only the palms of the hands, the soles of the feet and the penis are hairless. The hair follicle is a highly organized complex biologic system consisting of epithelial components, which include the inner and outer root sheath, hair shaft, and mesenchymal components, namely the dermal papilla, the follicular matrix and connective tissue sheath (Fig. 1) (ref.^{1,2}).

Fig. 1. The structure of the hair follicle.

Hair morphogenesis is dependent upon a series of mesenchymal-epithelial interactions in the embryonic skin^{3,4}. In embryogenesis the establishment of a dermal papilla (DP) is vital to the development of all hair

follicles and associated modified structures like sebaceous glands. For humans this initial aggregation begins when the embryo is approximately 60 days old⁵. The first hair follicles are formed from the ectoderm, an epithelial layer that will give rise to the epidermis, and the underlying mesoderm, a mesenchymal layer that will form the dermis. The hair placodes are established by local activators and inhibitors that act over different ranges. Additional epidermal and dermal signals organize the final follicle structure and orientation⁶. The basic hair follicle structures are complete throughout the skin of an embryo by 160 days.

HAIR-GROWTH CYCLE

In mammals including humans, lower portions of hair do not persist but are periodically shed and re-grown in a “hair cycle” (Fig. 2) (ref.⁷). It is believed that the hair follicle cycle originally evolved as an adaptation to the seasons, which is why mammalian follicles are usually synchronized, leading to seasonal moults. But

Fig. 2. The hair cycle.

the human has largely retained hair as a social or secondary sexual characteristic, and individual follicles' cycling is largely asynchronous². The human hair follicle totally regenerates itself every 3–5 years⁷. An active growth stage is **anagen** (from the Greek *ana*, again and *genein*, to produce), followed by a regressive, **catagen**, stage (from the Greek *kata*, down and *genein*, to produce) which gives rise to a resting, **telogen**, follicle (from the Greek *telos*, end, and *genein*, to produce)^{2,9}. Continuous self-renewal and cycling depend on pluripotent stem cells that reside in the bulge of the hair follicles, a portion of the outer root sheath⁶. At the initiation of a new anagen stage, follicular stem cells appear to respond to signals from the dermal papilla, and give rise to the next generation of proliferating matrix cells. Matrix cells undergo lineage restricted differentiation to form the component structure of the hair fiber and its root sheaths; during catagen the follicles undergo apoptosis, resulting in the formation of much smaller resting telogen follicles that after activation can again start the anagen phase^{9–11}. In human scalp hairs, the follicle spends some 90 % of the time in anagen¹². On average, the amount of new scalp hair formation essentially matches the amount that is lost due to shedding (approximately 100/day), thereby maintaining a consistent covering.

ANDROGENETIC ALOPECIA

Androgenetic alopecia (AGA) (synonyms: calvities hippocratica, male pattern baldness, androgenetic effluvium) is progressive thinning of the scalp hair that follows a defined pattern^{13, 14}. As baldness progresses, the hair follicles go through several hair cycles faster and with each one the follicles become shorter, finer, and less pigmented until the initially large terminal hair follicle has become a small vellus hair follicle, thin non-pigmented hair with much shorter growth period (Fig. 3). It follows a characteristic pattern, with the hair initially receding bilaterally backwards from the frontal-temporal region: in severe cases, regression of the hair line continue, denuding the crown region of the scalp¹⁵. At present, it is reasoned that there are three main reasons for this: genetic determination, age and androgens¹⁶. The tendency to balding of the male type is autosomal dominant heredity. Both homozygous (AA) and heterozygous (Aa) males show changes, but only when a certain

Fig. 3. The hair follicle miniaturization.

age is reached, and the androgen level is normal. The frequency of AGA depends on ethnic and familiar factors. It is very common in the white races, accounting for about 95 % of all types of alopecia. As AGA progresses, seven degrees of male baldness can be distinguished according to Hamilton-Norwood classification (Fig. 4). This was initially described by Hamilton in 1951 and then modified by Norwood in 1975 who divided androgenetic hair loss in men into two common patterns: the **regular type**, characterized by hair loss that begins in two different areas (at the temples and in the crown); and less common **type A**, that is characterized by “front-to-back” hair loss¹⁷.

Fig. 4. Hamilton-Norwood scale

(Type 1: No recession, Type 2: Temporal recession. Mild recession along frontal hairline; Type 2A: Entire frontal hairline recedes; Type 3: Further frontal recession. Deeper recession at corners; Type 3V: Hairloss predominantly in vertex (crown); Frontal hairline recession may be present; Type 3A: Hairline recedes back further; Type 4: Further frontal hair loss and temporal recession. Enlargement of vertex (crown). Solid band of hair across top separating front from vertex; Type 4A: Hairloss moves past „mid-coronal“ line; Type 5: Frontal bald and temporal areas enlarge further. Band separating the two areas becomes narrower and sparser; Type 5A: Hair loss extends towards the vertex; Type 6: Frontal and vertex balding areas merge into one and increase in size; Type 7: Narrow horseshoe band of hair. Low hairline in permanent zone may be sparse.) (adopted from the ref.¹⁷).

MOLECULAR MECHANISMS OF AGA

The pathophysiology of both male and female hair loss is not yet fully understood. It is, nevertheless apparent that the most influential factor is the effect of the androgenic hormones (AH) on the scalp hair follicles^{18, 19}. Androgens are regulated by several factors, including

pituitary ACTH (adrenocorticotrophin), immune cells, cytokines and neuroendocrine factors²⁰. Androgens exert their biological effects on their target cells as testosterone (T) or dihydrotestosterone (DHT).

DHT, the most potent circulating androgen hormone, is produced by the NADPH-dependent, stereoselective reduction of testosterone under catalysis of the enzyme steroid 5 α -reductase (5 α R)²¹. This enzyme plays a central role in human sexual physiology and is responsible for the differentiation of male external genitalia and prostate²². There are two isoforms of human 5 α R, namely, type 1 (5 α R1) and type 2 (5 α R2). The genes encoding type 1 and type 2 isozymes are found in chromosomes 5p and 2p, respectively, and each consists of 5 exons and 4 introns²³. 5 α R2 is found predominantly in androgen-dependent organs, such as the epididymis and prostate^{1, 21}. Thus 5 α R2, rather than 5 α R1, is thought to be important in the development of male pattern baldness. 5 α R2, which is encoded by the SRD5A2 gene at chromosome 2p and composed of 254 amino acids needs pH 5.5 for optimal function. It was found that the activities of 5 α -reductase are higher in cultured beard dermal papilla cells than in cells from the occipital scalp²⁴. During the last decade, several steroid analogues and non-steroid agents have been developed that interfere with 5 α -reductase activity²³.

Dihydrotestosterone is thought to be responsible for the gradual miniaturization of genetically marked hair follicles by shortening the duration of the anagen growth phase and reducing the cellular hair matrix volume^{25–27}. Previous work in young balding men has shown a substantial increase in the production of DHT in frontal anagen hair follicles compared to frontal hair follicles in nonbalding men^{28, 29}. It is not yet explained why AHs, with increasing age, switch from promoting growth of the scalp hair to its loss, inducing conditions known as AGA. Androgens seem to act on hair follicles via dermal papilla cells, presumably by affecting the transcription of regulating factors by these cells³⁰. A member of studies have shown that dermal papilla cells appear to offer a useful model for studying the action of androgens^{14, 28, 31–33}. Response to androgen is mediated through its receptor (AR), which is a transcriptional factor and a member of steroid/nuclear receptor superfamily, in which all members share basic structural and functional homology³⁴. Members of the superfamily are ligand dependent nuclear transcription factors, and consists of three basic functional domains: the DNA binding domain, the ligand binding domain and the amino terminal domain^{35, 36}. Many different naturally occurring mutations have been identified in the AR, and the study of these has allowed the localization of amino acids required for different receptor functions³⁷. These investigations have revealed that genetic variations in the AR may be associated with the risk of specific diseases, such as prostate cancer, and androgen-related skin disorders such as AGA, hirsutism, and acne³⁵. One of the most frequent androgen receptor mutation is characterized by an in-frame tandem duplication of exon 3 that

encodes the second zinc finger of the AR DNA-binding domain. Skewed X-chromosome inactivation may also play a role in the hypothetical hypersensitivity of the AR to androgens in women with idiopathic hirsutism. The expansion of a simple CAG trinucleotide repeat (C = cytosin, A = adenin, G = guanin) within the coding region of the androgen receptor gene leads to the motor neuropathy spinal and bulbar muscular atrophy. Shorter CAG-repeat lengths in AR may affect androgen mediated gene expression in hair follicles and sebaceous glands in men and women with androgenic skin disorders.

Futhermore we would like to discuss the role of two potential molecular candidates, β -catenin and p63, that may be important regulators of hair follicles development. The β -catenin protein has a dual role in the cells³⁸. In adult epithelial cells, it participates in binding neighbouring cells together to facilitate cell-cell communication and during embryogenesis, β -catenin appears to have another role as a part of the Wnt signaling pathway. It reacts with a molecule called LEF-1, which is expressed in cells predisposed to be hair follicles. The complex of β -catenin and LEF-1 forms a transcription factor that binds to the cellular DNA and activates the genes instructing the cell to become a hair follicle^{39–42}. In the case that β -catenin is mutated, formation of placods that generate hair follicles is blocked. Molecule of β -catenin is essential for the fate skin stem cells: in the absence of β -catenin, stem cells fail to differentiate into follicular keratinocytes and adopt an epidermal phenotype⁶.

The p63 gene, a homologue of tumour-suppressor p53, plays an essential role in the morphogenesis of epidermis^{43, 44}. This gene is expressed into at least six protein isoforms which are divided in two groups; those containing the transcription activation domain (TA isoforms) and those that do not (Δ N isoforms)⁴⁵. Histological analysis of neonatal p63-deficient mouse skin revealed the absence of normal epidermal structure and complete lack of hair follicles and other related structures depending on epidermal-mesenchymal interaction during embryonic development⁴². Thus we can expect involvement of the p63 molecule in AGA development because of its necessity for several aspects of ectodermal differentiation during embryogenesis.

THERAPEUTIC CHALLENGES

A logical approach for neutralizing androgen is the use of **antiandrogens** or compounds which prevent the interaction of T and DHT with the AR. The mechanism by which antiandrogens act may be either directly by interaction with the AR or indirectly through some nonreceptor-mediated action or nonspecific antimetabolite activity.

Another way for the development of new and improved hair-loss therapies could be connected with understanding how hair follicles form. Two above men-

tioned molecules, β -catenin and p63 in particular along with their associated partner proteins, may suggest possible treatments for premature baldness.

Currently there are two approved treatments for androgenetic alopecia **minoxidil** and **finasteride**^{13, 19}. Both minoxidil and finasteride require living hair follicles in order to work. Topical application of minoxidil is the most widely recommended treatment, but offers little practical benefit only. It was originally used to treat high blood pressure, but the drug was also found to stimulate hair growth. Minoxidil appears to work by gradually enlarging and lengthening hair follicles that had been shrinking due to the action of excessive amounts of the hormone DHT. Minoxidil may also extend the growth phase of the hair follicle, but the efficacy of minoxidil is variable and temporary. Finasteride is an oral prescription medication. It is approved for use in men only, because of its high potential for causing birth defects. Finasteride is an inhibitor of the enzyme 5 α -reductase. By inhibiting this enzyme, finasteride decreases DHT synthesis and thus reduces the destructive effects of excessive amounts of DHT on the hair follicle. However, it has considerable side effects.

Finally, it has been proposed that gene expression or apoptosis modulators may offer another approach for AGA treatment.

CONCLUSIONS

We are only beginning to understand precise mechanism of the development of AGA. The combination of molecular and histological approaches supplemented with more knowledge about hair follicle morphogenesis and organ culture techniques which test the influence of various antiandrogens, may suggest new therapeutic approaches, as the treatment options so far available, such as local minoxidil therapy, oral supplementation with finasteride, hair transplantation, and other surgical techniques, are not very successful.

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REFERENCES

- Asada Y, Sonoda T, Ojio M, Kurata S, Sato T, Ezaki T, Takayasu S. (2001) 5 α -reductase type 2 is constitutively expressed in the dermal papilla and connective tissue sheath of the hair follicle in vivo but not during culture in vitro. *J Clin Endocrinol Metab* 86, 2875–80.
- Kealey T, Philpott M, Guy R. (1997) The regulatory biology of the human pilosebaceous unit. *Bailliere's Clin Obst Gynaecol* 11, 205–27.
- Hardy MH. (1992) The secret life of the hair follicle. *Trends Genet* 8, 55–61.
- Gat U, DasGupta R, Degenstein L, Fuchs E. (1998) De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. *Cell* 95, 605–14.
- Holbrook KA, Minami SI. (1991) Hair follicle embryogenesis in the human. Characterization of events in vivo and in vitro. *Ann N Y Acad Sci* 642, 167–96.
- Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. (2001) β -catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. *Cell* 105, 533–45.
- Panteleyev AA, Jahoda CAB, Christiano AM. (2001) Hair follicle predetermination. *J Cell Sci* 114, 3419–31.
- Jarrousse F, Boissic S, Branchet MC, Beranger JY, Godeau G, Breton L, Bernard BA, Mahé Y. (2001) Identification of clustered cells in human hair follicle responsible for MMP-9 gelatinolytic activity: consequences for the regulation of hair growth. *Int J Dermatol* 4, 385–92.
- Bull JJ, Müller-Röver S, Patel SV, Chronnell CMT, McKay IA, Philpott MP. (2001) Contrasting localization of c-Myc with other Myc superfamily transcription factors in the human hair follicle and during the hair growth cycle. *J Invest Dermatol* 116, 617–22.
- Polakowska RR, Piacentini M, Bartlett R, Goldsmith LA, Haake AR. (1994) Apoptosis in human skin development: morphogenesis, periderm, and stem cells. *Dev Dyn* 3, 176–88.
- Soma T, Ogo M. (1998) Analysis of apoptotic cell death in human hair follicles in vivo and in vitro. *J Invest Dermatol* 111, 948–54.
- Kligman AM. (1961) Pathological dynamics of human hair loss. 1. Telogen effluvium. *Arch Dermatol* 83, 175–98.
- Trieb RM. (2002) Molecular mechanisms of androgenetic alopecia. *Exp Gerontol* 37, 981–90.
- Randall VA, Hibberts NA, Hamada K. (1996) A comparison of the culture and growth of dermal papilla cells from hair follicles from non-balding and balding (androgenetic alopecia) scalp. *Brit J Dermatol* 134, 437–44.
- Hamilton JB. (1951) Patterned loss of human hair. *Ann N Y Acad Sci* 53, 708–28.
- Braun O, Plewig G, Wolf HH, Winkelmann RK. Androgenetic Male Alopecia in: *Dermatology* (ed.). Springer Verlag, Berlin, 1991. p. 772–3.
- Norwood OT. (1975) Male pattern baldness: classification and incidence. *South Med J* 11, 1359–65.
- Thornton MJ, Hamada K, Messenger AG, Randall VA. (1998) Androgen-dependent beard dermal papilla cells secrete autocrine growth factor(s) in response to testosterone unlike scalp cells. *J Invest Dermatol* 11, 727–32.
- Sovak M. (2000) BP-766, a novel cosmetic agent for treatment of androgenic effluvium and alopecia. BP-766 Summary
- Alesci S, Bornstein SR. (2000) Neuroimmunoregulation of androgens in the adrenal gland and the skin. *Horm Res* 54, 281–6.
- Guarna A, Machetti F, Occhiato EG, Scarpi D. (2000) Benzoc[quinoxalin]-3-ones: A novel class of potent and selective non-steroidal inhibitors of human steroid 5 α -reductase 1. *J Med Chem* 43, 3718–35.
- Wilson JD, Griffin JE, Russell DW. (1993) Steroid 5-reductase 2 deficiency. *Endocr Rev* 14, 577–92.
- Chen W, Zouboulis CC, Orfanos CE. (1996) The 5 alpha-reductase system and its inhibitors. Recent development and its perspective in treating androgen-dependent skin disorders. *Dermatology* 3, 177–84.
- Itami S, Kurata S, Takayasu S. (1990) 5 α -reductase activity in cultured human dermal papilla from beard compared with reticular dermal fibroblasts. *J Invest Dermatol* 1, 150–2.
- Cowper SE, Rosenberg AS, Morgan MB. (2002) An investigation of apoptosis in androgenetic alopecia. *Am J Dermatopathol* 3, 204–8.
- Messenger AG. (1993): The control of hair growth: an overview. *J Invest Dermatol* 101, 45–95.
- Sawaya ME, Keane RW, Blume-Peytavi U, Mullins DL, Nusbaum BP, Whiting D, Nicholson DW. (2001) Androgen responsive genes as they affect hair growth. *Eur J Dermatol* 11, 304–8.
- Schweikert HU, Wilson JD. (1974) Regulation of human hair growth by steroid hormones II. Androstenedione metabolism in isolated hairs. *J Clin Endocrinol Metab* 6, 1012–19.
- Hibberts NA, Howell AE, Randall VA. (1998) Balding hair follicle dermal papilla cells contain higher levels of androgen receptors than those from non-balding scalp. *J Endocrinol* 156, 59–65.

30. Lachgar S, Charveton M, Sarraute J, Mourard M, Gall Y. (1999) In vitro main pathways of steroid action in cultured hair follicle cells: vascular approach. *J Invest Dermatol Symp Proc* 4, 290–5.
31. Ando Y, Yamaguchi Y, Hamada K, Yoshikawa K, Itami S. (1999) Expression of mRNA for androgen receptor, 5 α -reductase and 17 β -hydroxysteroid dehydrogenase in human dermal papilla cells. *Br J Dermatol* 141, 840–5.
32. Sato T, Tadokoro T, Sonoda T, Asada Y, Itami S, Takayasu S. (1999) Minoxidil increases 17 beta-hydroxysteroid dehydrogenase and 5 alpha-reductase activity of cultured human dermal papilla cells from balding scalp. *J Derm Sci* 19, 123–5.
33. Obana A, Chang Ch, Uno H. (1997) Inhibition of hair growth by testosterone in the presence of dermal papilla cells from the frontal bald scalp of the postpubertal stump-tailed macaque. *Endocrinology* 138, 356–61.
34. Sawaya ME, Price VH. (1997) Different levels of 5 α -reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenic alopecia. *J Invest Dermatol* 109, 296–300.
35. Sawaya ME, Shalita AR. (1998) Androgen receptor polymorphisms (CAG repeat lengths) in androgenic alopecia, hirsutism, and acne. *J Cutaneous Med Surg* 3, 9–15.
36. Calvo RM, Asuntion M, Sancho J, San Millan JL, Escobar-Morreale HF. (2000) The role of the CAG repeat polymorphism in the androgen receptor gene and of skewed X-chromosome inactivation, in the pathogenesis of hirsutism. *J Clin Endocrinol Metabol* 85, 1735–40.
37. Singh SM, Gauthier S, Labrie F. (2000) Androgen receptor antagonists (antiandrogens): Structure-activity relationships. *Curr Med Chem* 2, 211–47.
38. Ridanpää M, Fodde R, Kielman M. (2001) Dynamic expression and nuclear accumulation of β -catenin during the development of hair follicle-derived structures. *Mech Dev* 109, 173–181.
39. Gat U, DasGupta R, Degenstein L, Fuchs E. (1998) De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. *Cell* 95, 605–14.
40. Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W. (1996) Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* 382, 638–42.
41. Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destree O, Clevers H. (1996) XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos. *Cell* 86, 391–9.
42. Testu O, McCormick F. (1999) Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398, 422–6.
43. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. (1999) p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature* 398, 708–13.
44. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT, Tabin C, Sharpe A, Caput D, Crum C, McKeon F. (1999) p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature* 398, 714–8.
45. Little NA, Jochemsen AG. (2001) p63. *Int. J. Biochem. Cell Biol.* 34, 6–9.