



REVIEW ARTICLE

Role of TGF- β 2 in the human hair cycle

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KEYWORDS

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Caspase

Summary Male pattern baldness is the result of premature entry into catagen due to androgens. In order to prevent hair loss, it is important to understand two critical steps, i.e., the induction mechanism of premature entry and the regression process of catagen. At the initiation, dihydrotestosterone (DHT) stimulates synthesis of transforming growth factor- β 2 (TGF- β 2) in dermal papilla cells. TGF- β 2 suppresses proliferation of epithelial cells and stimulates synthesis of certain caspases. Then TGF- β 2 triggers the intrinsic caspase network and subsequently epithelial cells are eliminated through apoptotic cell death. TGF- β antagonists are effective in preventing catagen-like morphological changes and in promoting elongation of hair follicles in vivo and in vitro. These lines of evidence strongly suggest the presence of a ‘‘catagen cascade’’ in male pattern baldness, involving: (1) the conversion of testosterone to DHT by type II 5- α -reductase; (2) the synthesis of TGF- β 2 in dermal papilla cells; and (3) the activation of the intrinsic caspase network. These sequential events contribute to the shortening of the human hair cycle.

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1. Introduction

The hair follicle is a very complicated organ, composed of many layers of epithelial cells and a mass of mesenchymal cells called the dermal papilla (DP). It is well known that each hair follicle repeats a cyclical process consisting of three phases: a growing phase (anagen), a regressing phase (catagen), and a resting phase (telogen). The reg-

ulatory mechanisms of the hair cycle are largely unknown. Since hair loss is the result of premature entry into catagen, caused by a variety of factors [1–3], an understanding of the mechanism of induction of the regressing phase is critical for the prevention of hair loss.

We still do not know what kind of molecule(s) has a critical role in the initiation of catagen or what kind of signal transduction system mediates the signal from androgen to the executioner of catagen. However, recent studies have revealed significant information about possible regulatory factors of hair follicle catagen transformation. They include thyroid hormone and Vitamin D [4], hairless gene product [5], thrombospondin-1 [6], neurotrophin-3 [7], neurotrophin-4 [8], glial cell line-derived neurotrophic factor (GDNF) [9], fibroblast growth factor-5 (FGF-5) [10–12] and transforming growth factor- β 1 (TGF- β 1) [13]. These putative regulators have been extensively studied in mouse models.

Abbreviations: TGF- β , transforming growth factor- β ; TUNEL, terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate-biotin nick end labeling

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However, it is pivotal to analyze the catagen transition in human scalp hair follicles, since there is a distinct mechanism based on androgen action in the human hair cycle.

In 1992, Hardy described the hair follicle as “a treasure waiting to be discovered by more molecular geneticists” [14]. It is certainly true that molecular genetic approaches have yielded unexpected insights into hair biology. The recent completion of the human genome project has provided all the sequence data of our own genome, providing a huge database for fundamental studies on the biology of *Homo sapiens*. This is especially valuable for such disorders as androgenetic alopecia, for which no animal model has yet been found. In this review, we will discuss recent progress in hair biology, focusing on the genes controlling the hair cycle, especially key players involved in the regulation of catagen.

2. Catagen is an apoptotic process

2.1. Molecular mechanism of apoptosis

In catagen, epithelial components are eliminated without affecting surrounding tissues. This is indeed a typical apoptotic process. Molecular mechanisms

of apoptotic cell death have been well characterized in the past 10 years. Fig. 1 illustrates representative apoptotic pathways, in which cell death enzymes, caspases, play a central role. Caspases are a family of cysteine proteinases that cleave the carboxyl terminal of aspartic acid; 14 members are known so far in mammals.

The caspase family consists of three groups [15]. The initiator caspases are relatively large molecules such as caspase-8 and -9, and have domains interacting with regulatory molecules. Caspase-8 is associated with TNF- α receptor and Fas, which possess death domains and are activated by TNF- α and Fas ligand, respectively [16]. This is called the extrinsic or death receptor pathway. Caspase-9 is activated by apaf-1 apoptosome, whose formation is dependent on the release of cytochrome c from mitochondria [17]. This is called the “intrinsic pathway”. The effector caspases, such as caspase-3, -6 and -7, are located downstream of the above enzymes. They are activated by the initiator caspases and are responsible for the degradation of various substrates, leading inevitably to cell death. A representative of the third group is caspase-1, which has been known as interleukin-1 β converting enzyme. This group is considered to have a role in inflammatory processes, which are distinct from the cell death

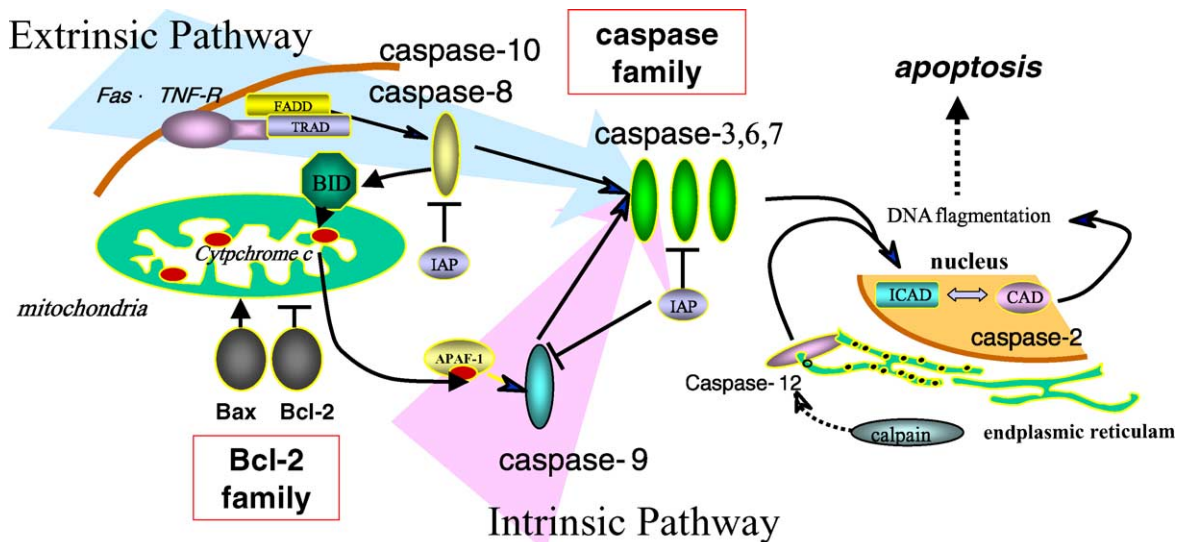


Fig. 1 Molecular mechanism of apoptotic cell death. The death pathway leading to apoptosis is regulated by various factors. Proapoptotic factors include many Bcl2 family members such as Bax, Bad, Bak, Bid, Bim, etc. Caspases are also called death enzymes and 14 are currently known. There are two major death pathways, namely the extrinsic pathway and the intrinsic pathway. The former is associated with death receptors, and activation of caspase-8 via ligand binding. The latter is closely related to mitochondrial function: upon release of cytochrome c, apaf-1 is recruited to form apoptosome, resulting in caspase-9 activation. These initiator caspases then cleave executioner caspases through limited proteolysis. The activated executioner caspases destroy various death substrates, leading inevitably to cell death.

pathway. In addition, the latest member of this family to be discovered, caspase-14, is specifically localized in mouse and human skin and has been suggested as a participant in terminal differentiation of keratinocytes [18–21].

It is crucial to understand the precise pathway(s) leading to the apoptosis of follicular epithelial cells.

2.2. Localization of TUNEL-positive cells during the anagen-catagen transition

Precise analysis of apoptotic cell death during the transition phase has revealed that sequential cell death occurs from the lower bulb to the upper outer root sheath (ORS) until the formation of club hair [22]. In late anagen to very early catagen follicles, some cells in the lower part of Henri's layer and the middle of Huxley's layer are TUNEL-positive. The positive reaction seems to be related to keratinizing areas. In the very early catagen hair follicles, there is a slight increase of TUNEL-positive cells around the dermal papilla. In early catagen hair, indentation of the epithelial component just above the DP becomes obvious and this portion is strongly positive for TUNEL reaction, showing broad and profound apoptotic cell death at this stage. In mid-catagen, when the long epithelial strand is formed, strongly TUNEL-posi-

tive cells appear in the upper area of the epithelial component and a part of the lower regressing strand. These features clearly show the elimination of follicular keratinocytes by apoptotic cell death.

3. TGF- β plays essential roles

3.1. Mechanism of TGF- β signal transduction

The TGF- β family contains two subfamilies, the TGF- β /activin/nodal subfamily and the BMP/GDF/MIS subfamily [23]. They are potent cytokines, which control a plethora of biological reactions [24–26]. They are also known to be inducers of apoptosis in certain cell types [27]. The biological roles of TGF- β and its signaling mechanisms have been extensively studied and reviewed elsewhere. Fig. 2 shows a schematic diagram of the signal transduction pathway for TGF- β . Briefly, a TGF- β ligand binds to TGF- β receptors type I and type II, and type II receptor phosphorylates type I receptor, leading to its activation [28]. TGF- β signals are mediated via two pathways, Smad [26] and/or TAK1 [29]. Three classes of Smads, the receptor-regulated Smad (R-smad), the co-mediator Smad

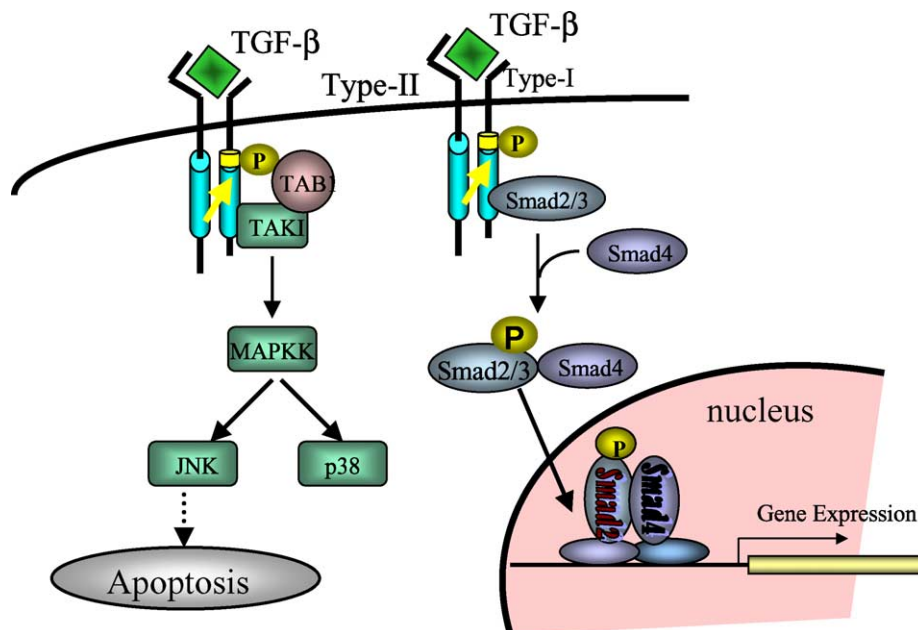


Fig. 2 TGF- β signal transduction. There are two distinct signaling pathways from cell surface receptors to the nucleus. Types I and II receptors are necessary for ligand binding. Type II receptor kinase phosphorylates type I receptor upon ligand binding and then type I receptor kinase activates R-Smad, Smad2 or Smad3. The phosphorylated R-Smad becomes associated with Co-Smad, Smad4, and the active Smad complex recruits transcription machinery. Another cascade includes a MAPKKK, TAK1. TAK1, in association with the activator TAB1, phosphorylates MAPKKs, including MKK4 and MKK7, leading to the activation of MAPK, JNK and/or p38 kinase.

(Co-Smad) and the inhibitory Smad (I-Smad) are present in mammals [30]. R-Smads are directly phosphorylated and activated by type I receptor. Among R-Smads (Smad1–3, 5, and 8), Smad2 and Smad3 are involved in TGF- β subfamily signal transduction. Phosphorylated Smad2 (and Smad3) is translocated into the nucleus where it associates with Smad4, and the Smad complex leads to activation or suppression of the target genes. TAK1 is a member of the mitogen activated kinase kinase kinase (MAPKKK) family and TAB1 is required for its activation. It is also activated by various cytokines including interleukin 1 [31]. TAK1 then phosphorylates MAPKK, MEKK4 and MEKK7 [32,33], leading to the activation of MAPK, c-Jun N-terminal kinase (JNK) [34]. It is not clear how these two pathways interact in various physiological reactions.

3.2. TGF- β induces catagen-like morphological changes

What is responsible for inducing catagen or, in other words, apoptotic cell death of epithelial cells? Proapoptotic factors, growth factors, deprivation of growth factors, cytokines or hormones could be involved in this process. However, catagen is not a simple death pathway process. For example, TNF- α ,

well known as a strong inducer of apoptosis, causes unusual morphological changes of whole hair follicles [22]: in addition to epithelial cells, there are many TUNEL-positive cells among dermal papilla cells, and, interestingly, some ORS cells are proliferating, as judged on the basis of BrdU incorporation. These features of TNF- α -treated hair follicles are markedly different from those of catagen hair follicles *in vivo*.

On the other hand, TGF- β induces catagen-like morphological changes. In the presence of TGF- β , germinative matrix cells cease to grow and initiate apoptotic cell death, resulting in club hair formation. Caspase-3 is activated and TUNEL-positive cells are observed in the epithelial component of TGF- β -treated hair follicles [22]. As described above, the initiation factor of catagen is not merely an apoptotic factor, but orchestrates complex reactions with the construction of telogen hair follicles as a final goal. TGF- β seems to be an excellent candidate for this factor.

3.3. Localization of TGF- β isoforms and TGF- β receptor in anagen and catagen hair follicles

Fig. 3 illustrates the localization of three types of TGF- β isoforms in anagen and catagen hair follicles

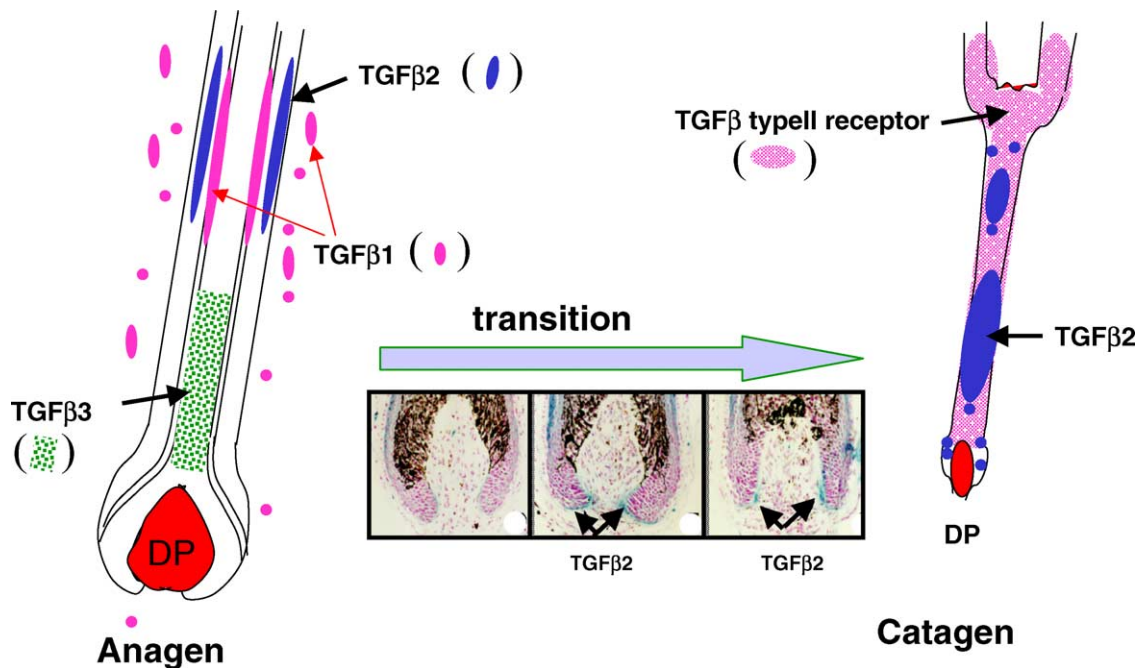


Fig. 3 Localization of TGF- β isoforms and TGF- β type II receptor in anagen and catagen hair follicles. In anagen hair follicles, TGF- β 1, TGF- β 2, and TGF- β 3 exhibit segregated localization. During anagen–catagen transition, deposition of TGF- β 2 occurs around the boundary area between germinative matrix cells and DP. On the regressing epithelial strand, TGF- β type II receptor is strongly positive.

[35]. TGF- β 1 was detected in the cuticle and the cells of the connective tissue sheath. TGF- β 2 was localized at the outermost layer of ORS cells, whereas TGF- β 3 was seen at the hair cortex and the hair cuticle in the keratogenous zone (KZ) of the upper hair bulb. Interestingly, TGF- β 2 shows specific localization during the anagen–catagen transition phase. Coincident with entry into the catagen phase, strong TGF- β 2 deposition was demonstrated in the lower part of the boundary area between the DPC and the germinative matrix cells. Deposition of TGF- β 2 was only observed in the early catagen hair follicles, characterized by the upward removal of hair shaft from the DP, decrease of hair color due to the down-regulation of melanogenesis, and the increased thickness of the connective tissue sheath. This staining pattern was hardly found in any anagen hair follicles. In an organ culture system, similar TGF- β 2 deposition in the bulb area was detected when hair follicles started catagen-like morphological changes. As catagen stages proceed, only TGF- β 2 could be detected in the regressing epithelial strand. In addition, TGF- β type II receptor was strongly positive in the epithelial strand, suggesting that the TGF- β signal is active and transmitted to these cells during the transition phase.

3.4. TGF- β induces activation of certain caspases in epithelial component

When hair follicles were cultured in the presence of TGF- β , activation of caspases occurred in the bulb area and the middle of ORS cells [36]. Appearance of active forms of both initiator caspase-9 and effector caspase-3 suggested that TGF- β is capable of triggering the caspase network in the epithelial cells. Active caspases were detected mainly in two areas, the middle part of ORS cells and germinative matrix cells near the bottom of the hair bulb. Interestingly, these areas correspond to the proliferating zone where BrdU incorporation is always observed (Fig. 4). TGF- β -induced morphological changes and appearance of active caspase-9 and caspase-3 in the convoluted area of the lower bulb were clearly demonstrated [36]. Precise observation of hair follicles in late anagen to catagen revealed removal of follicular keratinocytes by continuous apoptotic processes.

3.5. Molecular mechanism of TGF- β -induced apoptosis

The molecular mechanisms of TGF- β -induced apoptotic cell death are not fully understood. However,

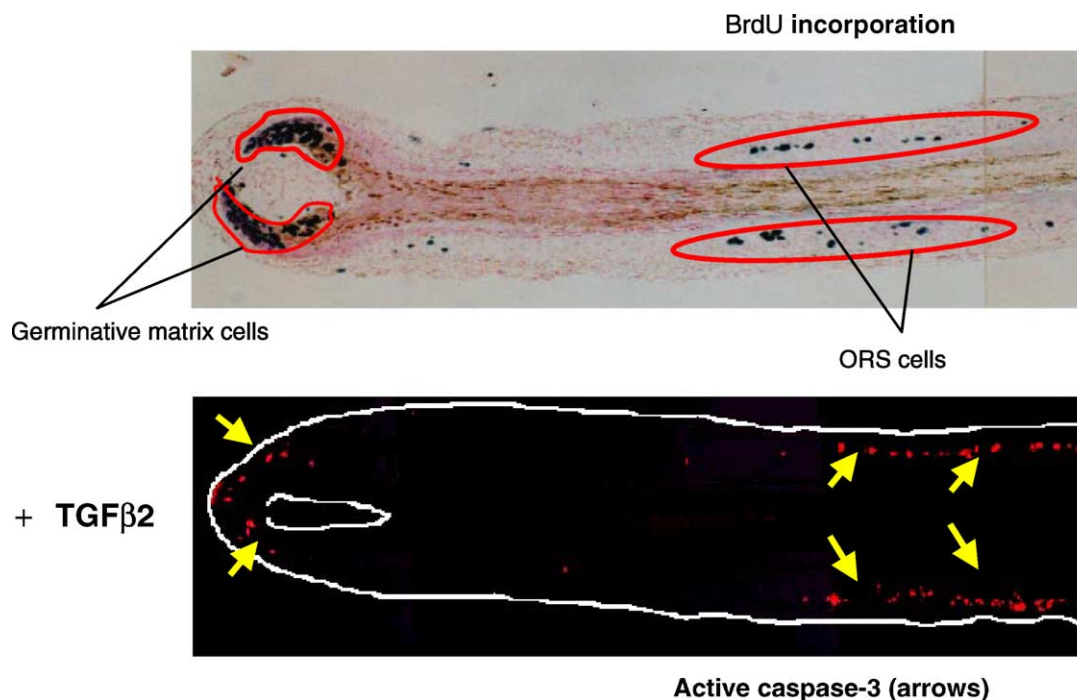


Fig. 4 Proliferating zone in the anagen hair follicle and effect of TGF- β 2 on caspase activation. BrdU incorporation studies showed that proliferating cells were mainly found in two areas, the lower part of the hair bulb and the middle of ORS cells. In the presence of TGF- β 2, BrdU incorporation immediately stopped and caspase-3 activation in those areas were clearly demonstrated using a cleavage-site directed antibody to caspase-3.

JNK may play a role in this signaling pathway [37,38]. JNK is also known as stress-activated protein kinase (SAPK), which is activated in cellular responses to cytokines and stress-inducing agents [39,40]. Recently, the missing link between JNK and caspase activation has been discovered. UV-induced apoptosis in murine fibroblasts requires functional JNK for cytochrome *c* release from mitochondria [39]. Oxidative stress caused JNK activation, cytochrome *c* release and apoptosis in rat cardiac myocytes [41]. Once cytochrome *c* is released, it forms a complex with Apaf1 and pro-caspase-9, resulting in caspase-9 activation in the presence of ATP [42,43].

In order to investigate further the molecular basis of this event, we performed a Clontech cDNA microarray analysis using follicular keratinocytes. Of the >8000 genes queried, expression of over 400 was affected in the presence of TGF- β 2. We found the following: (1) expression of many kinases and phosphatases was altered; (2) components of proteasomes and ubiquitins were up-regulated; and (3) caspase-9 was up-regulated, whereas caspase-8 was down-regulated (Table 1). Real-time PCR analysis using the LightCycler system confirmed that mRNAs of caspase-9 and caspase-3 were increased three-fold, whereas that of caspase-8 was decreased slightly. Our results revealed an additional function of TGF- β 2, that it stimulates synthesis of caspase-9 and caspase-3, members of the intrinsic caspase network.

Table 1 Summary of microarray analysis

	TGF- β 2+/control
(1) Caspases	
Caspase-9	Up
Caspase-8	Down
Caspase-3	Up
(2) Metabolic proteinases	
Cathepsin D	Up
Proteasome 26S-8	Up
Proteasome 26S-4	Up
Proteasome 26S-3	Up
MMP-9	Down
MMP-2	Down
(3) Structural proteins	
Laminin α 5	Down
Collagen type IV	Down
Loricrin	Down
(4) Kinases and phosphatases	
MAPKKK2	Up
Protein phosphatase 1A	Up
Protein phosphatase 2A	Up

Up: >2.0; down: <1/2.

These lines of evidence provided new insights into the mechanism of TGF- β -induced cell death, showing that TGF- β contributes to the synthesis of certain caspases and activation of the intrinsic caspase network, which are essential steps leading to catagen progression.

4. TGF- β antagonists promote hair growth

4.1. Suppression of TGF- β is effective for preventing catagen progression

If TGF- β is responsible for the induction of catagen, suppression of TGF- β could prevent entry into catagen or progression of catagen.

First of all, the effect of TGF- β 2 on elongation was obvious in cultured hair follicles. Hair elongation was suppressed in the presence of TGF- β 2, in a dose-dependent fashion [35]. When anti-TGF- β neutralizing antibody was applied to the organ culture system, hair growth was significantly stimulated. In addition, fetuin, which has a TGF- β receptor-like ligand binding domain and works as an antagonist of TGF- β activity [44], had a similar effect to the neutralizing antibody. In the presence of fetuin, hair growth was markedly and significantly stimulated [35].

A screening method for TGF- β suppression can be developed by monitoring the level of plasminogen activator inhibitor-1 (PAI-1), which is an early response gene for TGF- β in many cell types [45]. Extensive screening of plant extracts showed that hydrangea extract was able to suppress TGF- β action. Using an organ culture system, the effect of hydrangea extract on hair growth was examined [36]. The extract resulted in prolonged hair growth compared to the control, suggesting suppression of catagen entry. Caspase activation was also suppressed in the presence of the extract.

4.2. Effect of hydrangea extract on catagen progression in vivo

In mouse, morphological changes of catagen hair follicles are well characterized [46]. Since synchronized hair growth is easily achieved by hair removal and the hair cycle is strictly controlled, the mouse is a convenient model to examine catagen entry. Topical application of hydrangea extract on the back of mice during late anagen phase delayed catagen progression [36]. Histological analysis demonstrated that hair follicles of the extract-treated skin showed late anagen to early catagen appearance, in marked contrast to late catagen

to telogen follicles seen in the vehicle-treated control.

5. Androgen and its action

5.1. 5α-Reductase type II plays a critical role

Approximately 60 years ago Hamilton demonstrated that the male pattern baldness was due to androgen action. It is now clear that dihydrotestosterone (DHT) plays a central role in this process [47]. DHT is produced from testosterone by 5α-reductase. Two 5α-reductase isozymes (types I and II) are present in mammals. Fig. 5 illustrates factors involved in steroid hormone metabolism. Type II enzyme (5αR-II) is localized at sex-related target organs, such as testis and hair follicles. Recent investigations suggested that 5αR-II is present in axillary, pubic and beard hair, and in the case of scalp hair, it is present dominantly at the forehead [48,49]. Indeed inhibition of 5αR-II activity in vivo was effective in preventing hair loss [47,50].

5.2. Physiological roles of androgen in hair follicles

Long-unanswered questions include: (1) what is the function of DHT in hair follicles? and (2) why does DHT work in opposite ways in beard and

Effect of TGF-β2 on the production of DHT

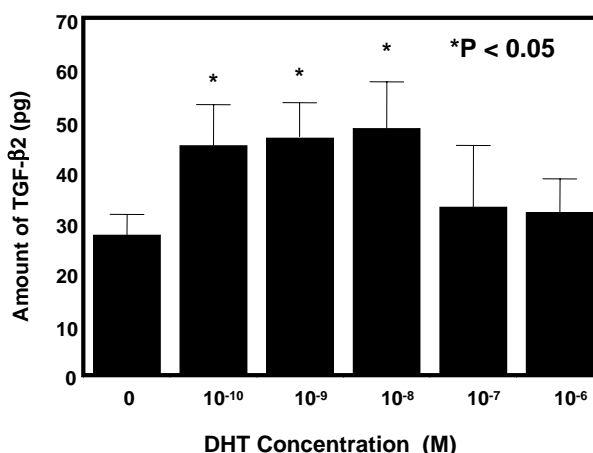
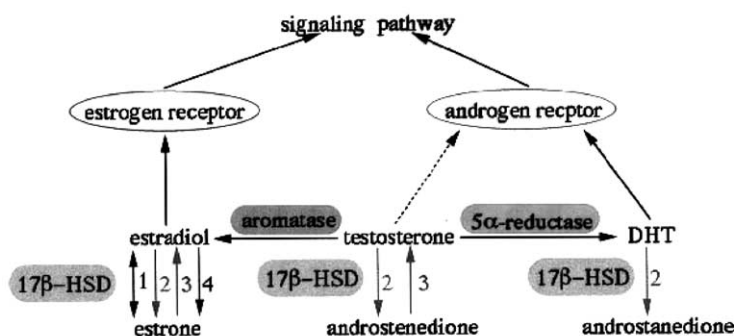


Fig. 5 Metabolism of steroid hormones. Testosterone is converted to the potent androgen, DHT, by 5α-reductase, and to estrogen by aromatase. 17β-HSD isoforms metabolize these active steroid hormones to related derivatives in both directions.

frontal hair? It is well known that DHT supports beard hair growth, whereas it promotes hair loss over the frontal scalp. So far genetic approaches have failed to give clear answers. However, there are some clues. First, we found that DHT works on dermal papilla cells and stimulates synthesis of TGF-β2 (Fig. 6). DHT has a very high affinity for the androgen receptor (AR) and AR resides distinctly

Metabolism of steroid hormones



Tissue distribution of 17β-HSD

- Type 1 placenta
- 2 prostate, ovary, placenta, liver, skin,
- 3 testis, dermal papilla cell
- 4 widely expressed

Fig. 6 DHT stimulates synthesis of TGF-β2. In the presence of physiological concentrations of DHT (10⁻⁶ to 10⁻⁸ M), the amount of TGF-β2 in the medium is clearly increased. This was observed in the 50% of DP cultures (2/4) obtained from different individuals with male pattern baldness. None of the fibroblasts (n = 4), or keratinocytes (n = 4) showed any significant changes in response to DHT.

Catagen Cascade

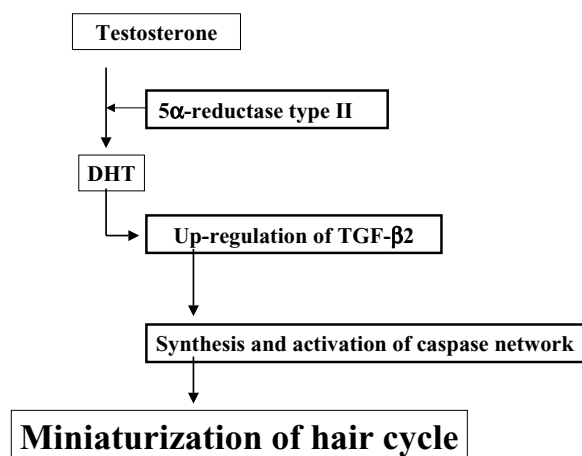


Fig. 7 Catagen cascade: (1) Testosterone is converted to DHT by 5- α -reductase type II, (2) DHT up-regulates synthesis of TGF- β 2 in DP cells, and (3) TGF- β 2 induces synthesis and activation of the intrinsic caspase network.

in the DP cells of anagen hair follicles [51]. This strongly suggests that DP cells are the target of androgen action. Using DP cells obtained from frontal scalp hair follicles, the effect of DHT on the production of TGF- β 2 was examined. In the presence of physiological concentrations of DHT (10^{-6} to 10^{-8} M), the amount of TGF- β 2 in the medium was clearly increased. Fibroblasts or keratinocytes did not show any significant changes with DHT. Inui, et al. also showed that DHT up-regulated TGF- β 1 in a co-culture system [52]. Concerning the second question, it is becoming clear that hormone action requires many receptor-associated cofactors and these regulate the receptor function in both positive and negative ways, depending on the combination with cofactors [53–56].

It is also important to consider the metabolism of androgens. As shown in Fig. 5, synthesis of DHT is not a simple one-way process. Further, the half lives of the products would be different depending on the presence of metabolic enzymes such as aromatase and 17 β -hydroxysteroid dehydrogenase (17 β -HSD) [57]. We still do not know the precise distribution and expression of these enzymes in hair follicles. Thus, it is important to know how the steroid metabolism is operating, and in what direction.

6. Closing remarks

In male pattern baldness, the following sequence of reactions is expected to occur (Fig. 7): testosterone delivered to hair follicles is converted to DHT by type II 5 α -reductase; DHT then stimulates the

synthesis of TGF- β 2 in dermal papilla cells; TGF- β 2 induces epithelial cells to promote up-regulation and activation of caspase-9 and caspase-3 in matrix cells, resulting in the removal of epithelial cells by apoptotic cell death. We named this series of reactions the ‘‘catagen cascade’’. We believe this is the main pathway of male pattern baldness. Our recent investigations further suggest that this cascade involves a feedback mechanism, in which TGF- β 2 plays a central role. In addition, other signal mechanisms influence this cascade. Therefore, it is difficult to envisage a simple inhibition strategy to stop the cascade. In order to have an effective countermeasure against male pattern baldness, it is important to suppress these complicated reactions at multiple stages. For example, the combination of a type II 5 α -reductase inhibitor, a TGF- β 2 antagonist and a caspase inhibitor might be effective to prevent male pattern baldness.

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