CHAPTER

48

Hair Physiology (Hair Growth, Alopecia, Scalp Treatment, etc.)

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48.1 INTRODUCTION

The tissue responsible for human hair growth is the hair follicle, which is a skin appendage found inside the dermis. As a unique miniature organ that self-regenerates throughout almost the entire lifetime, the hair follicle has been frequently used as a research model not only in dermatology but also in developmental biology, molecular biology, and genetics, and a significant number of research reports on the physiology of the hair follicle have been published in top journals, such as *Nature*, *Science*, and *Cell*. We will let other reviews explain the entire aspect of the hair follicle; in this chapter, we will review its role mainly from a cosmetics or cosmeceutical point of view. We will cover sufficient basic knowledge and provide a concise overview of the history of research of the structure and function of the hair follicle and factors that affect its functions. Although we will not consider serious intractable hair diseases in dermatology, the scope of this chapter is hair thinning/hair loss with aging. We will introduce methods and challenges that should be overcome, mainly in the field of cosmetics/cosmeceutics, as well as updates on the latest treatments including cosmetic surgery and cell-based therapy, and we will conclude with a vision of what awaits us in the future.

48.2 BASIC CONCEPTS, HAIR BIOLOGY, CAUSE OF HAIR LOSS, AND TREATMENTS

48.2.1 Hair Follicle Structure and Hair Cycle

Many reviews and books have summarized the structure and functions of the hair follicle. By the 1990–2000s, most of the basic structure and functions of the hair follicle and mechanisms of the hair cycle had been revealed. However, rapid progress in molecular biology and genome-associated gene manipulation and analysis have led to the revelation of even more details about factors and mechanisms that are related to hair follicle formation and dynamics of the hair cycle.

The structure of the hair follicle can be roughly classified into the hair shaft (the hair fiber) that is exposed from the stratum corneum of the skin and the "hair follicle" structures that are found inside the dermis. The hair shaft is composed of the cortex, medulla, and cuticle structure and is made of terminally differentiated cornified cells that lack nuclei but have abundant keratin fibers, which are visually perceived as "hair." However, each "hair" is actually produced from a hair follicle, which is made of dermal sheath (DS) cells, the inner and outer root sheath (IRS, ORS), hair matrix cells, and dermal papilla (DP) cells that actively grow (proliferate). Hair follicles are also classified in the epithelial or dermal component based on their embryology into ectodermal origin, including hair matrix cells, IRS, ORS, and cells of mesodermal origin known as mesenchymal cells, such as DP and DS cells. Active cell components are concentrated within the bottom of the hair follicle, called the hair bulb, which is shaped in a bulbar structure like an "onion" (Fig. 48.1). Ultimately, the cornified epithelial tissue becomes the hair shaft that functions as "hair" so the hair epithelium is important, but the inner dermal components, DP cells, are the actual "control towers" that send out commands to balance the growth (proliferation) and division (differentiation), namely the cornification degree, of hair epithelial cells. Further, the hair follicle sends interactive signaling factors between the mesenchymal cells (DP and DS

Cosmetic Science and Technology: Theoretical Principles and Applications
http://dx.doi.org/10.1016/B978-0-12-802005-0.00048-3

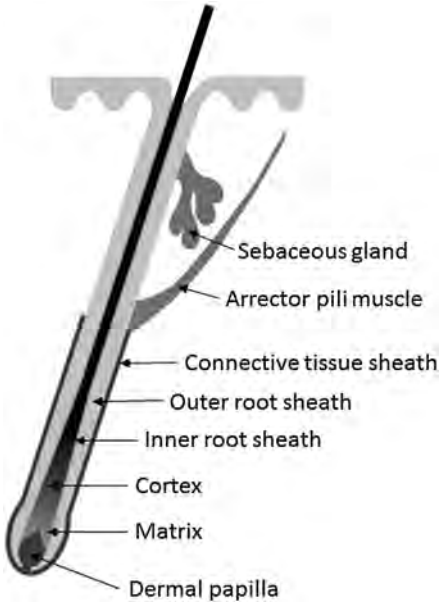

FIGURE 48.1 Structure of the hair follicle.

cells) and the hair follicle epithelial tissue, known as the epithelial–mesenchymal interaction (EMI). Through EMI, the origin of hair follicles (hair germ) forms at embryogenesis, and postnatally they continue the hair cycle for nearly the entire lifetime. EMI is not only a concept important in hair follicle development but also a clinically important mechanism that is common to the formation of many other organs, such as the heart, liver, kidney, and lungs.

The hair cycle has three physiological and morphological stages: growing, transitional, and dormant phases, called anagen, catagen, and telogen, respectively (Fig. 48.2). With certain stimulatory factors, many of which are still unknown, they re-enter the anagen phase, initiating active EMI between hair stem cells and DP cells, resulting in the induction of matrix cell proliferation. There are approximately 100,000 hairs in the human scalp, and although there are ethnic differences, the density is approximately 100–200 hairs/cm^2. The human hair cycle has a long 3- to 7-year cycle with 90% in the active anagen phase. Recent gene profiling analysis has revealed that each hair cycle has a unique genetic expression pattern.[19,34] Further, in addition to the classic three phases noted earlier, an extra phase has been proposed, namely an "exogen phase," in the telogen phase where the hair shaft sheds (hair shedding).[50]

As for seasonal changes, autumn shows the largest change in hair shedding, and spring shows a similar change in shedding, although it is smaller than that for autumn.

48.2.2 Target Cells and Tissues in the Hair Follicle

48.2.2.1 Secondary Hair Germ

Hair matrix cells are the original source of the hair shafts that become hair, and are actively growing epithelial cells found in the hair bulb at the base of each hair follicle. Since these cells start to grow after an early embryogenetic stage, they are also known as "secondary hair germs." Hair matrix cells grow and differentiate into the ORS, IRS, cortex, and medulla, which compose the multiple layers of the hair shaft and the hair follicle epithelium. As such, the hair matrix cells are the "mother" of hair and are believed to function as a switch for growth (proliferation) and differentiation, so most topical agents and cosmetic hair growth promoting reagents aim to work on the hair matrix cells in the hair follicle either directly or indirectly.

48.2.2.2 Hair Follicle Stem Cells

Many of the organs and tissues in the human body, including the brain and neurons, are known to have somatic stem cells, but the hair follicle is one of the first tissues in the body where stem cells were experimentally identified. In 1990, a historical article by Cotsarelis and Lavker clearly proved that stem cells of the hair epithelium were found in vivo and in vitro in an area called the bulge, an area in the upper one-third of the hair follicle where the arrector pili muscle associates.[7] Many biomarkers of hair follicle stem cells, such as keratin15 (K15[35]) and LRG5,[33] have been

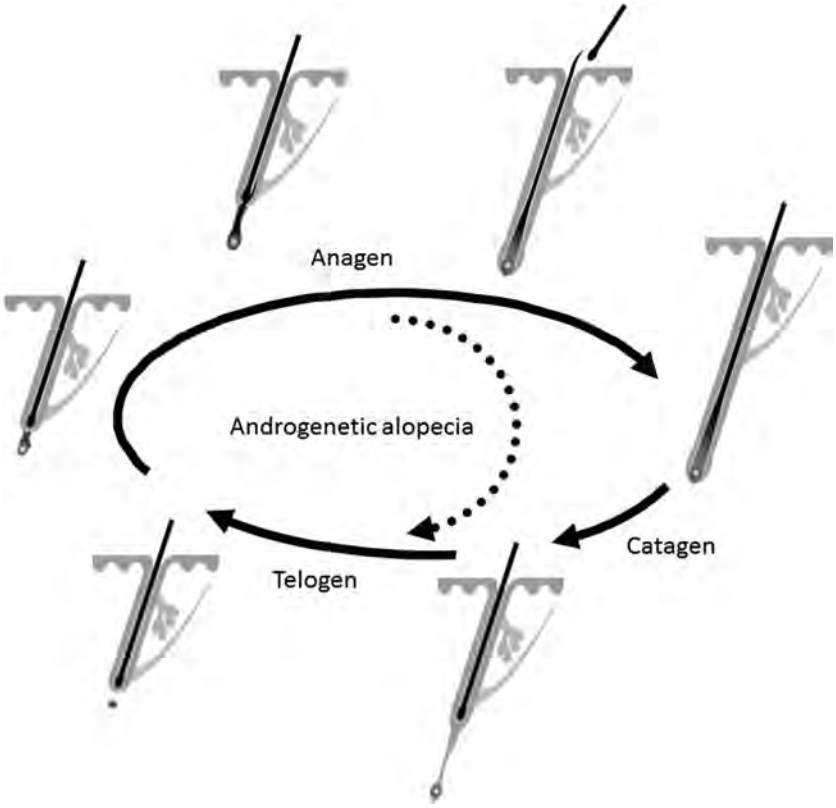

FIGURE 48.2 Human hair follicle growth cycle.

identified, and there are studies that have isolated stem cells by using those specific markers. Although hair stem cells define their stemness as their multipotency to differentiate into different cell types, they are also defined by their quiescent (or dormant) nature. Therefore, the concept of the simple activation of stem cells by external compounds does not always cause a positive biological and physiological effect on hair growth, and this makes applications in the field of cosmetics more difficult, as well as more complicated.

48.2.2.3 Dermal Papilla Cells

Dermal Papilla (DP) cells are found at the base of each hair bulb and are some of the few mesenchymal cells described in the hair follicle. Embryologically, the DP results in dermal condensations or compact structures of mesenchymal cells of the fetal dermis and are believed to be specifically differentiated fibroblasts that show different properties compared to normal dermal fibroblasts. Many studies have shown that they interact with the epithelium and work as the "control tower" through EMI to work reciprocally on the hair matrix to secrete tropic hair growth factors, and DP cells have been repeatedly claimed as the most important cell type in the hair follicle. Stimulating DP cells to initiate the release of tropic factors is regarded an effective approach to regulate hair growth, so DP cells have been targeted in cosmeticpproaches and many hair growth−promoting reagents claim to work on DP cells as well as on matrix cells.

Some reports have implied that the number of DP cells defines the size of the hair follicle (i.e., they contribute to the hair thickness[9]). Moreover, Chi et al. provided more direct evidence where the number of DP cells, rather than the size of individual cell volumes, contributes to the size of the DP and the resultant hair follicle size.[5] Since DP cells are known to grow slowly relative to dermal fibroblasts, and they do not seem to actively divide in vivo, many hair growth−promoting reagents aim to induce DP cell growth.

48.2.2.4 Dermal Sheath Cells

Until recently, DP cells were the main focus with mesenchymal hair follicle cells and DS cells being considered as only minor components without significant functions, but in the past decade, their roles have gradually been revealed to be more important than previously thought.[38] The DS, also called the connective tissue sheath, is the outermost layer of the hair follicle and is the border between the hair follicle and the interfollicular dermis. The

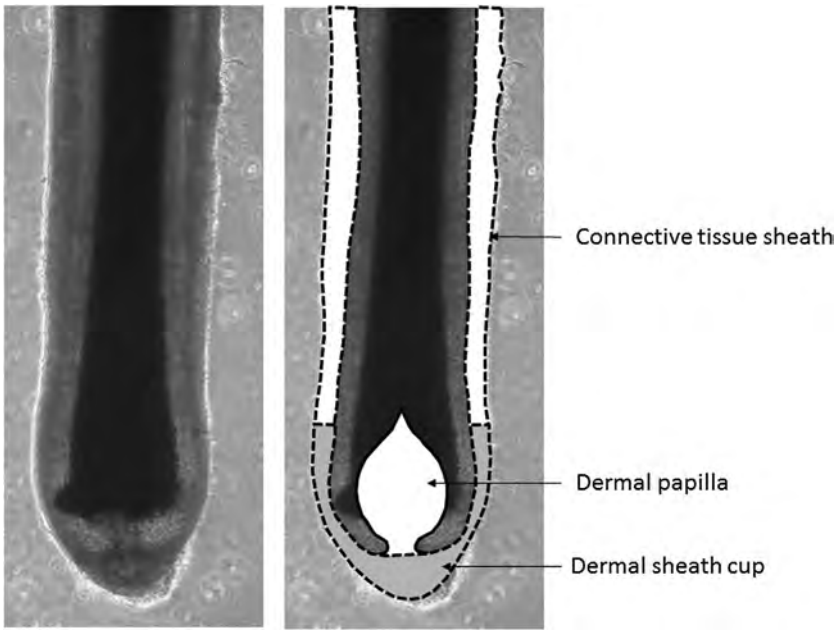

FIGURE 48.3 Dermal sheath cup.

DS is thought to be composed of fibroblast-like mesenchymal cells but includes blood vessels (pericytes) and nerves as well as collagen and elastic fibers.

DS cup (DSC) cells are DS cells located at the bottom of the hair bulb and have been found to have an especially high hair follicle induction potential (Fig. 48.3). For example, Reynolds et al. isolated DP cells and DSC cells, transplanted them individually to the upper arm, and reported that hair growth was seen only with the DSC cell transplants.[47] Additionally, recent reports have indicated that by using lineage analysis with genetically engineered mice, DSC cells are actually the precursor (or reservoir) of the DP.[46] DSC cells have gained much interest since their activation can be an alternative effective approach for hair growth from cosmetics.

48.2.2.5 Microvasculature/Vascular Endothelial Cells Surrounding the Hair Follicle

Capillaries associated with each hair follicle carry the nutrients that are required for hair follicle growth, and based on this concept, the circulatory system has always been suggested as a vital factor for hair growth. However, research on the relationship between skin/hair follicles and blood vessels was not significant until appropriate blood vessel markers were found. After 2000, many unique factors were found in the circulatory system, and this has led to many discoveries and reports on blood vessels around the hair follicle and their functions, proving the importance of the circulatory system. According to Yano et al., VEGF-1, a vascular endothelial growth factor, and its inhibitory factor, TSP-1, complementarily adjust the renewal of blood vessels around the hair follicle.[59,60] Blood vessels are one of the main targets for hair growth–promoting reagents, and indeed, there are two major such compounds, one a medical drug (minoxidil) and the other a quasi-drug (adenosine), that focus their proof of concept on the vasodilation of the microvasculature surrounding the hair follicle. However, it is important to remember that hair growth is ultimately caused by EMI between the DP and hair matrix cells through these vessel networks. Recent progress in image analysis technology has revealed that the capillary plexus is more complex than originally predicted, and it is also organized and spreads out around each hair follicle (Fig. 48.4).

48.2.3 Trophic Factors That Affect Hair Growth

Many protein nutrient factors and growth factors have been reported to play a role in regulating hair growth and the hair cycle. Many of those factors also play essential roles in the developmental phase of the hair follicle (folliculogenesis). Whenever possible, cosmetics should try to use chemical agents that have an effect only on the hair cycle and do not affect the initial hair development. However, in general, factors that work only on the hair cycle seem to be theoretically rare since hair development and the hair cycle share a similar mechanism of EMI. Table 48.1 shows potential factors categorized by mesenchymal and epithelial origin.[4] We consider in this section prominent growth

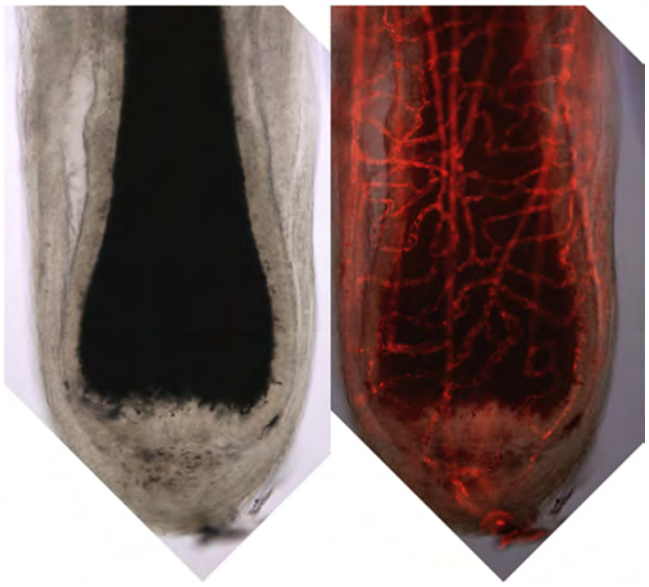

FIGURE 48.4 Capillary plexus around the hair follicle.

TABLE 48.1 List of Modulators Potential to Affect Hair Growth

Modulators	Origin
BMP2/BMP4/Noggin	Epithelium/Mesenchyme
EGF	Epithelium
Edar	Epithelium
FGF5/FGF18	Epithelium
FGF7	Mesenchyme
HGF	Mesenchyme
IGF-I	Mesenchyme
Shh	Epithelium
Wnt3a/Wnt7/Wnt10b	Epithelium
Wnt5	Mesenchyme

and tropic factors that are expected to be used as cosmetics along with their mechanisms of action and application approaches.

48.2.3.1 Fibroblast Growth Factor

Fibroblast growth factor (FGF) is a family of growth factors that have various functions such as in cell growth and in wound healing. In the hair follicle, FGF-7 [also known as KGF (keratinocyte growth factor)] has been reported to have a hair growth mechanism where it is produced by DP cells during the anagen phase of the hair cycle and helps DP cell growth through FGF receptors.[58] Adenosine is thought to induce hair growth via this mechanism.[18] FGF-5 increases during the latter half of the hair follicle anagen phase, and mice with an FGF-5 gene mutation have long hair and are called Angola.[51] Because the hair of FGF-5 knockout mice does not have a catagen phase and the mice have long hair, FGF-5 is believed to be a factor that stimulates the transition from anagen to catagen phase.[14] A plant extract from "Burnet" has an inhibitory activity on FGF-5 and is claimed to improve hair loss by extending the anagen phase.[37] Likewise, FGF-18 was found to be a factor that maintains the telogen phase of the hair cycle in studies using knockout mice.[29] Additionally, a factor called FGF-5s is another known factor that inhibits FGF-5 activity and induces hair growth.[23] The degree of efficacy among these FGF family members (FGF-5, FGF-5s, and FGF-18) has not

yet been clarified. Because FGF is a factor that works on DP cells and other mesenchymal cells, it is expected to be useful to extend the anagen phase through its effects.

48.2.3.2 Epidermal Growth Factor

Epidermal growth factor (EGF) is a factor that modulates cell growth through EGF receptors. Mice with knocked-out EGF receptor genes in their epithelial cells showed that hair follicle formation was relatively normal during development, but those hair follicles remained in anagen phase and did not transition to the catagen stage, indicating that EGF has an effect to induce the transition from the telogen phase to anagen phase and extends the anagen phase.[26] There is a substantial literature regarding EGF/EGF receptor and hair growth promotion. Most of those studies used mice, and although the compounds show an inhibitory effect on EGF receptors and have been developed as anticancer and anti-inflammatory drugs, there has been no hair-promoting reagent developed to date that affects EGF/EGF receptor activity.

48.2.3.3 Insulin-Like Growth Factor

Insulin-like growth factor (IGF) is a polypeptide that has a similar structure to that of insulin and is produced in the liver and genital organs. IGF is believed to be vital for folliculogenesis during the fetal period and for maintaining the anagen phase. Among members of the IGF family, IGF-1 is produced by DP cells during the anagen phase and is widely known as a hair growth induction factor from its lengthening induction action on hair follicles in cultured organs.[57] With hair follicle tissues that are stimulated by male hormones, such as facial hair, IGF-1 shows autocrine-like effects with receptors in DP cells, and it has been reported that they induce hair matrix cell growth and inhibit the catagen phase.[21]

48.2.3.4 Wnt

Wnt is a molecule that plays one of the most important roles in the development, proliferation, differentiation, and regeneration of hair follicles. Wnt is a family of glycoproteins with 350–400 amino acids, and they are secreted by cells and interact with the extracellular matrix to work on neighboring cells through their unique and highly sophisticated signaling pathways. The details of the Wnt signaling pathway have already been revealed, but, ultimately, Wnt targets an intracellular factor called beta-catenin that is normally located in the cytosol, but which then moves into the nucleus and works on the transcriptional control of its target genes. Although it may be difficult to directly use factors that modify the Wnt pathway, they have potential applications in methods such as screening procedures to search for compounds and extracts that modify the effects of Wnt on DP cells or hair epithelium cells. Wnt3a, Wnt7, and Wnt10b are subtypes that are known to form a family and have effects on the hair follicle. Wnt5 seems have inhibitory properties. Additionally, a group in China has recently reported that Wnt1 also has physiological effects similar to other inducing Wnt family members.[8]

48.2.3.5 Shh

Shh is a unique factor named after "Sonic the Hedgehog," a video game character that was popular when the factor was found, but around 2000, Shh became a popular research subject along with Wnt due to its relationship with the hair cycle. Sato et al. reported that Shh works by switching the telogen phase to anagen phase.[48] Shh was reported not to be vital in the early folliculogenetic stage,[6] and as mentioned earlier, it has attracted much attention since it may be a factor that does not affect the formation of the hair follicle and only works on hair growth. However, due to safety concerns with Shh-related compound agonists (pseudo-compounds that bind to Shh receptors), it appears that development of hair growth–promoting reagents using Shh has been discontinued. Although not related to hair growth effects, there are also reports that Shh is related to the mechanism of hair flow, or direction of hair.[44,48]

48.2.3.6 Bone Morphogenic Protein

Bone morphogenic protein (BMP) is known to be expressed and to function not only during bone development but also with other organs. Botchkarev et al. reported that BMP is an important factor in hair follicle development and in hair cycling.[3] BMP is thought to work with a competitive inhibition factor, Noggin, to control folliculogenesis. In cosmetic research, BMP has been studied through its relationship with a BMP-related substance called ephrin and has been reported to be related to blood vessel formation.[40] The application of BMP signal–related factors and extracts is actively being developed in cosmetic research, and it is expected that BMP will be a highly potent factor to promote hair growth. (See also "cytopurine" in Section 48.3.4.)

48.2.3.7 Edar

Edar is a factor that was recently discovered and has attracted much attention. Edar is a receptor protein molecule that mainly works specifically on the epidermis in the early stage of folliculogenesis. There are reports that Edar may have a direct causal relationship with heredity and hair loss.[10,31] Because Edar is a factor that works during early developmental stages, reagents that modulate Edar may be difficult to use as hair growth–promoting reagents.

48.2.4 Hair Loss Diseases and Their Causes

Serious hair thinning and hair loss are skin appendage disorders, and patients with those disorders should consult with a dermatologist. These disorders include patterned hair loss, alopecia areata, and telogen alopecia. In this chapter, we will focus on patterned hair loss, which is seen most frequently and is the disorder most often treated and can be prevented from worsening cosmeceutically as well as pharmaceutically. Patterned hair loss is thought to be caused both by inherited and by physiological backgrounds, in addition to yet undiscovered causes. Patterned hair loss gradually proceeds following a specific pattern in front and on top of the scalp. With males, hair shedding often begins after puberty due to the effect of the male hormone, androgen, and is named male pattern baldness, also known as androgenetic alopecia (AGA). The major cause of AGA in humans is thought to be as follows. The human hair follicle follows a cycle of a relatively long-lasting anagen phase and relatively short catagen and telogen phases. Each hair follicle is replaced with a revitalized newly generated hair (Fig. 48.2), but when AGA occurs, the anagen phase becomes shorter and the hair follicle does not fully grow and enter the next hair cycle, which results in increasing amounts of short and thin hairs (miniaturized hairs), and eventually the temporal or forehead scalp surface skin becomes visible.[20] Recently, the number of female patients with patterned hair loss has increased, and this disorder is sometimes called female AGA or female pattern hair loss (FPHL), but it is believed to be substantially the same disorder as male pattern hair loss.[2] FPHL proceeds independently of male hormones, and its pattern is characterized where the forehead hair line is maintained but all hair on the head becomes thinner with decreased numbers of hairs and thinning of each hair.[36,52,61]

48.3 HAIR GROWTH–PROMOTING COMPOUNDS

48.3.1 General View

The recent challenging competition for functional cosmetics has made the boundary between cosmetics and pharmaceuticals a thin line. However, even under such situations, the boundary between pharmaceuticals and cosmetics is clearer in the category of hair growth–promoting reagents compared with other fields in cosmetics, such as skin aging (antiwrinkle formation) or antipigmentation (whitening). Although the situation varies depending on each country, it seems that the development of hair growth ingredients in cosmetics has become discreet, especially in Europe and North America, since the first hair growth–promoting reagent was approved as a pharmaceutical drug by regulatory authorities (i.e., U.S. Food and Drug Administration [FDA] in the United States). Of course, many ingredients can be found online that claim to be effective, but there are only a few evidence-based hair growth–promoting ingredients that are proved to be effective based on fair scientific and clinical data. In this section, we will review hair growth–promoting ingredients that have been reported to be effective based on evidence from scientific publications with peer review.

48.3.2 Guidelines for the Management of Androgenetic Alopecia (2010)

Approved hair growth chemical agents include minoxidil, a topical agent that was approved in Canada in 1986, in 1988 in the United States, and in 1999 in Japan, and finasteride, an oral drug that was approved in 1997 in the United States and in 2005 in Japan. Medical products with those two reagents have been sold as hair growth–promoting products and have been widely accepted by consumers, thus widening the gap between previous hair growth–promoting cosmetic products and medical drugs. Thus, it has become difficult to objectively outline hair growth–promoting reagents other than those two chemical agents in the cosmetics field. In such situations, an important opinion and criteria were announced by the Japanese Dermatological Association in 2010, namely the Guidelines for the Management of Androgenetic Alopecia (Tsuboi 2010). In those guidelines, several products approved by the Ministry of Health, Labor, and Welfare of Japan as quasi-drugs were listed as suggested hair growth–promoting reagents along with minoxidil and finasteride. Although quasi-drugs are a standard by the

regulatory unique to Japan, clinical data that show the safety, effectiveness and effective mechanism must be substantially submitted for approval. Thus, they have a certain degree of credibility as scientifically tested evidence-based chemical agents. The guidelines do not classify whether the agents are approved as medical drugs or as quasi-drugs as a specific standard; rather, the standard is based on whether there is sufficient clinical data reported on the agents. In this section, we will briefly introduce minoxidil and finasteride and will focus on other chemical agents, mainly quasi-drugs.

48.3.3 Approved Drugs

48.3.3.1 Minoxidil

Minoxidil was first used as an oral drug to treat high blood pressure, but it was found to have hair growth–promoting effects and was then developed as a topical hair growth–promoting reagent. Minoxidil is transformed into minoxidil sulfate in the hair follicle, and works on the sulfonylurea receptor (SUR) to release adenosine triphosphate (ATP) from the cells. The released ATP is decomposed into adenosine by ATPase and works on adenosine receptors in DP cells to make them produce VEGF (vascular endothelial cells), which is the proven hair growth mechanism of minoxidil.[32] There have been numerous clinical tests with 1–5% minoxidil on both male and female patterned hair loss patients in many countries, and its safety and efficacy have been established. However, this does not mean that minoxidil is effective with all patients, and continuous use is required to maintain significant improvement.[27]

48.3.3.2 Finasteride

Finasteride is an antiandrogen agent (male hormone) that was developed as a drug for prostatitis. By inhibiting the enzyme 5α-reductase, finasteride blocks male hormone testosterone from transforming into dihydrotestosterone (DHT), a hormone that shows a much stronger activity than testosterone. 5α-Reductase is classified into type I and type II depending on the originating organ or optimal pH, but finasteride selectively blocks the type II 5α-reductase that is expressed by the prostate. Since type II 5α-reductase is expressed by DP cells in male pattern baldness,[22] finasteride was developed as an oral hair growth–promoting reagent. There have been numerous clinical tests in many countries that show the efficacy of finasteride, but like minoxidil, continuous use is required and hair loss may proceed again if intake is discontinued.[28] Since there are concerns with potential effects on fetuses, its use is limited to males. Finasteride is a prescription drug that must be used with strict management under medical consultation.

48.3.3.3 Dutasteride

Like finasteride, dutasteride is a drug that was used for prostatitis but was diverted as a hair growth–promoting reagent. Unlike finasteride, which blocks only type II 5α-reductase, dutasteride shows a stronger blocking action against both type I and type II 5α-reductase and is expected to be used for more effective treatment for male pattern baldness. International joint clinical trials have shown that dutasteride is superior to a placebo and is not inferior to finasteride.[12] Dutasteride has been approved in South Korea in 2009 and in Japan in 2015. This drug was not listed in the AGA Management Guidelines (2010 Edition).

48.3.4 Quasi-Drugs

Quasi-drugs such as adenosine, t-flavanone, citopurine, pentadecane, and the pharmaceutical drug carpronium chloride, are categorized as Suggested Rate C1 (use may be considered) in the guidelines and provide an alternative option to FDA-approved drugs for mild hair loss symptoms with reduced undesired side effects (Fig. 48.5).

48.3.4.1 Carpronium Chloride

Carpronium chloride was synthesized as a γ-amino acid derivative and was approved as a pharmaceutical ingredient in Japan in 1968. Carpronium chloride shows hair growth–promoting activity through its vasodilatory effect on local blood vessels and stimulates the transition from telogen phase to anagen phase. With both male and female subjects with patterned hair loss, carpronium chloride mixed with other herbal medicine ingredients reportedly showed some efficacy as a hair growth–promoting reagent.[13]

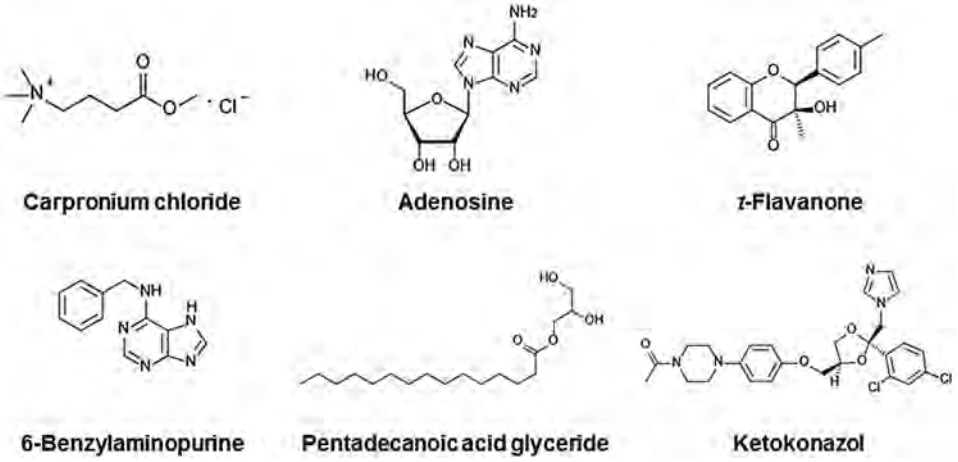

FIGURE 48.5 Chemical structures of hair growth quasi-drug.

48.3.4.2 *Adenosine*

Adenosine is known as a part of ribonucleotide (RNA) or ATP and shows various physiological effects. In the hair follicle, it has been reported that adenosine works through A2b receptors on DP cells and increases cyclic adenosine monophosphate levels, resulting in the transcriptional activation on fibroblast growth factor-7 (FGF-7), which is weakened in patterned hair loss.[18] The hair growth mechanism of minoxidil has also been reported to induce the production of adenosine via SUR,[32] so adenosine potentially has a more direct effect on DP cells than minoxidil. It is believed that the FGF-7 produced by DP cells promotes hair growth by inducing cell proliferation through FGF receptors in hair matrix cells (Li 2001). In a 6-month double-blind clinical test of 102 Japanese male subjects with patterned hair loss, it was found that the adenosine-added hair growth–promoting reagent group showed a significant improvement based on global assessment by physicians compared with the control group. The improvement rate was 32.0% in the control group and 80.4% in the adenosine-treated group. Local evaluation using a phototrichogram also showed that the percentage of thick hair (with a hair radius larger than 60 μm) in the adenosine-treated group was significantly higher than that in the placebo-treated group.[56] In a double-blind clinical test of 30 female subjects with patterned hair loss, the visual improvement after 12 months was significantly higher compared with the placebo-treated group, and the hair growth rate of the anagen phase and the rate of thick hair (with a hair diameter larger than 80 μm) also increased.[45] Additionally, in a 6-month test of 40 white male subjects, the results showed a significant increase in the percentage of thick hair (with a hair diameter larger than 60 μm).[25] These results show that adenosine is effective for thickening hair regardless of ethnicity or sex and suggest that it is effective for improving patterned hair loss.

48.3.4.3 *t-Flavanone*

t-Flavanone is a chemical compound that induces the growth of cultured hair epithelium cells and dermal fibroblasts. t-Flavanone is synthesized from taxifolin which is astilbin analog. Astilbin was initially identified as a derivative of the active component of *Hypericum perforatum*, which was identified in a screen of compounds that promote the proliferation of hair epithelial cells from more than 2000 products in a natural crude extract library. t-Flavanone promotes hair growth by inhibiting transforming growth factor-β (TGF-β), a signaling molecule that transits anagen hair to the catagen phase, and by inducing the growth of hair matrix cells in the hair bulb. Paired testing of t-flavanone and a placebo on 14 subjects showed that after 6 months of topical application, the hair diameter increased, especially with new-grown hair. A 6-month application test of 197 male subjects with patterned hair loss using t-flavanone, a placebo, and market hair growth–promoting reagents, with global assessment by doctors, revealed an improvement rate above slight improvement with t-flavanone being significantly higher than the placebo and increased firm hair (with a diameter larger than 40 μm). The overall improvement, including the amount of firm hair, was 19.4% and 75.0% with the placebo and t-flavanone agents, respectively.[16]

48.3.4.4 *Cytopurine*

Cytopurine (6-benzylaminopurine) is expected to inhibit hair loss due to its effects on increasing bone morphogenetic protein and ephrin in DP cells, which reportedly decreases with hair loss and inducing ORS cell growth.

Cytopurine has been found to have a significantly higher improvement compared with placebo-treated subjects in an efficacy test of patterned hair loss subjects.[42]

48.3.4.5 Pentadecan

Pentadecan is known to improve the energy metabolism of the hair follicle, such as by inducing ATP growth, and has been found to have a significantly higher improvement compared with placebo-treated subjects in an efficacy test of male subjects with patterned hair loss.[54]

48.3.4.6 Ketoconazole

Ketoconazole is a synthetic antifungal drug used for the treatment of ringworm and seborrheic eczema, but it has also been reported to show a 5α-reductase–blocking activity.[17] Ketoconazole has been reported to be an effective material for treating patterned hair loss as a topical agent and as a shampoo, and it is categorized as C1 in the guidelines.

48.3.4.7 Cepharanthine

Alkaloids (organic compounds) extracted from the root of a plant called *Stephania cepharantha hayata* are generally called cepharanthine and are categorized as natural ingredients. As a pharmaceutical component, they have been reported to show effects and functions on alopecia areata and on alopecia pityroides, but since there are not enough reports on their effectiveness on patterned hair loss, it is categorized as C2 (use not suggested).

48.3.5 Other Chemical Compounds

First, we will consider chemical agents that have a history as active components of quasi-drug hair growth–promoting reagents in Japan. β-Glycyrrhetinic acid is derived by the hydrolysis of an extract of glycyrrhizin in the legume family and has been expected to promote hair growth with its anti-inflammatory activity and 5α-reductase inhibitory action. Nicotinamide, vitamin E derivatives, and pantothenyl ethyl ether have been formulated in many hair growth products with expectations to promote hair growth from their cell activator action and blood circulation–promoting activity.[24]

Stemoxydine has an effect to maintain a hypoxic local environment to optimize the functionality of hair follicle stem cells, and it has shown an effect to increase hair density in a 3-month clinical test.

An amino acid, 5-aminolevulinic acid, has an enhancement action on the cytochromes of mitochondria and has been reported to show hair growth–promoting actions on animals and humans (Orokuma 2008). Development of 5-aminolevulinic acid in hair growth–promoting reagents is now being considered.

Epimorphin is a membrane protein found on the surface of mesenchyme cells and is known as an organ formation factor. Epimorphin has been expected to be used as a hair growth–promoting reagent due to its growth inducing action on hair, but its development was reportedly discontinued due to safety concerns.[15]

48.3.6 Natural Plant Extracts

Many plant extracts have been known to have hair growth–promoting activities from traditional folk therapies such as Chinese medicine and from advanced screening methods, and they are used in hair growth–promoting products mainly as cell activators. Here, we review the functions of prominent natural plant extracts. Ginseng extract is a long-used herbal medicine known to have an immunostimulatory action. A *Sophora* extract, extracted from the root of *Sophora*, has growth-inhibiting effects on cultured hair epithelium cells and hair lengthening effects on organ-cultured hair follicles. The *Sophora* extract has been found to be effective on human hair[53] and is used widely in Japan and in South Korea. Coriander is a commonly used spice, but coriander fruit extract has growth-stimulating effects on cultured hair epithelium cells and is used in hair growth products.[55] Mulberry root bark extract has an action to shorten the telogen phase and extend the anagen phase of hair and has been found effective on human hair.[30] A Cuachalalate extract from the bark of a tree that originated in Mexico inhibits the hair follicle's transition to catagen. The Cuachalalate extract has a three-step action: inhibiting DHT production with a 5α-reductase–blocking action,[43] inhibiting the activity of TGF-β2, a catagen-inhibiting factor that increases with DHT, and blocking caspase that causes apoptosis.[49] A hydrolyzed yeast extract has been reported to have a mechanism of inducing hair growth by growing the primary cilium on the surface of DP cells to promote various cell growth signals.[41]

The following methods require operations at medical institutions, but since some procedures of cosmetic surgery have overlapped the line between cosmetics and cosmetic surgery, relatively safe methods operated in medical clinics are becoming alternatives to cosmetics to consumers. As such, we will review some of these operations in this section of the chapter.

48.4 LIGHT-EMITTING DIODES, LASERS, AND OTHER COSMETIC SURGERIES

Laser surgery has been widely used in cosmetic surgery for hair removal. However, light-emitting diodes (LEDs) with a specific spectrum, also called low-level laser therapy (LLLT), have been reported to be effective in stimulating hair growth. Inui et al. studied hair growth promotion by activating hair matrix cells with a specific-spectrum red LED,[11] and Avci et al. reported the results of their tests in clinical research.[1]

48.5 GROWTH FACTOR COCKTAIL, CELL CULTURE MEDIA INJECTION, AND PLATELET-RICH PLASMA

Injecting biologically derived components directly into the scalp is an operation that has been actively performed in recent years. Platelet-rich plasma (PRP) therapy is a method that uses autologous components, whereas so-called HARG therapy and culture supernatant injection therapy use nonautologous, allogenic components. PRP therapy is a method used in clinics where the patient's platelets are isolated from their blood and are injected into their scalp to promote hair growth. Reports have shown that PRP therapy has a certain effect. To improve those effects, there is a method called W-PRP, where the components are modified with additional factors, such as including leukocytes. Although they do not have nuclei, platelets are perceived as "cells," and in Japan they are subject to new medical regulations for regenerative medicine. On the other hand, HARG therapy is an operation that uses a nutrient cocktail extracted from adipose tissues, which is believed to include stem cells. Although some medical institutions claim that HARG therapy has a substantial efficacy, it seems that objective clinical data are still insufficient. Further, the potential risks of injecting components derived from other persons cannot be ruled out. Culture supernatant therapy is a method that injects the supernatant from fibroblasts cultured under low-oxygen conditions into the scalp and has been reported to induce hair growth.[62]

48.6 HAIR TRANSPLANTATION

Various surgical treatments for male pattern baldness have been developed, such as the flap method, scalp resection, and hair transplantation, but the most common method seen globally now is hair transplantation of autologous hair follicles. Since symptoms of male pattern baldness start from the forehead or temporal area and do not usually occur at the side or occipital areas, this method takes advantage of this characteristic and uses donor hair follicles (whole hair follicles including the hair bulbs) from the occipital area and transplants them to the balding areas. Since this method requires a surgical operation, it must be done at a medical institution so it is a method that is contrary to cosmetics. In Europe and in the United States, hair transplantation has grown into a market larger than hair growth–promoting reagents and new operation methods such as follicular transplant units (FTUs) have been developed, and currently the invasiveness and stability of FTU are being improved. Further, automated grafting transplantation using robots has reached a practical level. This is a surgical method with conclusive results and has been widely adopted mainly due to the high engraftment rate and because of transplanting their own, autologous hair follicle tissues. Additionally, hair transplantation has an advantage over other surgical methods where the hair line and hair style can be designed according to the patient's preference.

48.7 FUTURE TREATMENT IN COSMETICS: REGENERATION OF HAIR FOLLICLES BY AUTOLOGOUS CELL-BASED THERAPY FOR HAIR LOSS

The technology of regenerative medicine, as well as the development of related legal regulations, is accelerating. Under such social and environmental circumstances, it is assumed that the category of regenerative medicine treatment is considered safe, such as the injection of autologous, differentiated somatic cells to specific areas locally with

homologous use, and will overlap with the field of cosmetics. When applying cells to humans, choosing between autologous or allogenic cells is an issue, but in the hair transplantation method introduced earlier, there have been issues during the development process where intense inflammatory reactions or rejection reactions occurred with allogenic hair follicles, which did not engraft and new hair did not grow. Based on these experiences with hair transplantation, autologous cells are planned to be used with clinical research in cell transplantation therapy.

48.7.1 Autologous Cell-Based Therapy for Hair Loss Treatment

Clinical trials on hair loss treatment with the injection of autologous hair follicle derived cells is advancing mainly in Europe and in North America and two ventures based in the United Kingdom and in the United States have passed their safety issue in phase 1 studies. They were proceeding to phase 2 trials for efficacy and safety with small patient groups. Although details for the specification of cells and the preparation procedures, including cell culture conditions, were not disclosed, they seem to use either DP cells or dermal fibroblasts together with epithelial components, and some trophic factors as well. However, these trials were discontinued in phase 2, and the final results have not been announced.

Although DP cells are a common primary target for hair growth promotion in the search for compounds and natural extracts, for cell-based therapy more premature cells may be appropriate such as DSC cells, which are thought to be precursors of DP cells. McElwee et al.[39] used hair follicles from the whiskers of mice to isolate DP cells, DSC cells, and nonhair bulb DS cells. After a few passages of cultivation, they were each injected into the auricle ear of immunodeficient mice to test the hair growth−induction effects. These preclinical tests showed that areas injected with DP cells or DSC cells had longer hair shafts compared with the noninjected areas after 4 weeks. DS cell−injected areas did not show any change. The DSC cell−injected areas showed an especially natural hair distribution and direction (angle) compared with the DP cell−injected areas, and observation using florescent-labeled cells showed that the DSC cells were incorporated into the DP. Further, there have been studies on humans, where DSC cells and DP cells were taken from male donors and transplanted to the inner forearm of female subjects. The results showed that the DSC cells had hair growth with male Y chromosomes, but hair regeneration was not found with DP cells.[47] These studies indicate that DSC cells work as precursor cells of DP cells and have the potential to derive hair follicles and induce hair growth. A clinical trial conducted in Europe with cultured autologous human DSC cells showed that in its phase 1 safety test, they have been confirmed to be safe as well as to show a certain degree of efficacy. These challenging and exciting approaches with cell-based therapies may take time to develop and be ready to use for hair loss patients, but very promising new treatments are being developed to be widely used together with cosmetic pretreatments and posttreatments.

48.8 SUMMARY AND FUTURE DIRECTIONS

In this chapter, we have considered hair, mainly from the viewpoint of hair growth promotion, including pharmaceutical, cosmeceutical, and some cosmetic surgery methods. We also looked at the future possibilities of hair growth with cell-based therapies and regenerative medicine. Hair strongly influences the esthetics of an individual and is undoubtedly important to improve our quality of life. Regardless of gender, there are desires or dissatisfaction with our hair, especially with women. Depending on the culture, the range of social acceptance is smaller in women compared with in men, and the desires and distress tend to be more serious. We hope that new and improved approaches can help meet such wishes. Further, as a long-time researcher in this field, there seems to be a large potential need for areas other than hair growth, such as the prevention and/or improvement of gray hair or fundamentally fixing curled hair. There have been some interesting studies on gray hair and curled hair. Most of those studies are fundamental studies using genetics or mouse models, and none of them propose solutions comparable to the studies on hair growth. We hope that in the future, the continued use of cosmetic/cosmeceutical products, clinical methods such as cell-based therapy, and the development of safe genetic manipulations can be combined to offer various effective options for users to choose from, and that people will be able to live more actively with vivid hair throughout their lifetime.

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