Conventional and novel stem cell based therapies for androgenic alopecia

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Abstract: The prevalence of androgenic alopecia (AGA) increases with age and it affects both men and women. Patients diagnosed with AGA may experience decreased quality of life, depression, and feel self-conscious. There are a variety of therapeutic options ranging from prescription drugs to non-prescription medications. Currently, AGA involves an annual global market revenue of US$4 billion and a growth rate of 1.8%, indicating a growing consumer market. Although natural and synthetic ingredients can promote hair growth and, therefore, be useful to treat AGA, some of them have important adverse effects and unknown mechanisms of action that limit their use and benefits. Biologic factors that include signaling from stem cells, dermal papilla cells, and platelet-rich plasma are some of the current therapeutic agents being studied for hair restoration with milder side effects. However, most of the mechanisms exerted by these factors in hair restoration are still being researched. In this review, we analyze the therapeutic agents that have been used for AGA and emphasize the potential of new therapies based on advances in stem cell technologies and regenerative medicine.

Keywords: stem cells, stem cell therapies, hair follicle, dermal papilla, androgenic alopecia, laser, hair regeneration

Introduction

The prevalence of androgenic alopecia (AGA) increases with age, and is estimated to affect about 80% of Caucasian men.¹ Female AGA, also known as female pattern hair loss, affects 32% of women in the ninth decade of life.² The consumer market for products that promote hair growth has been increasing dramatically.³ These products promote hair regeneration based on the knowledge about the hair follicle (HF) cycle.⁴,⁵ However, in most cases, the mechanisms of action of these products are not well characterized and the results are variable or with undesirable side effects.⁶ At present, only two treatments for AGA have been approved by the US Food and Drug Administration (FDA): Minoxidil and Finasteride.⁷–¹⁰ Although these medications have proved to be effective in some cases, their use is limited by their side effects.¹¹,¹² With the emergence of stem cells (SCs), many mechanisms that lead to tissue regeneration have been discovered.¹³ Hair regeneration has become one of the targets for SC technologies to restore the hair in AGA.¹⁴ Several SC factors such as peptides exert essential signals to promote hair regrowth.¹⁵,¹⁶ Some of these signals stimulate differentiation of SCs to keratinocytes which are important for HF growth.¹⁷ Other signals can stimulate dermal papilla cells (DPCs) that promote SC proliferation in the HF.¹⁸,¹⁹ In this review, we describe HF characteristics and discuss different therapies used currently for AGA and possible novel agents for hair regeneration. These therapies include FDA-approved...
medications, non-prescription physical or chemical agents, natural ingredients, small molecules, biologic factors, and signals derived from SCs.

HF and SC niche

The HF undergoes biologic changes from an actively growing stage (anagen) to a quiescent stage (telogen) with an intermediate remodeling stage (catagen).4 HFSCs are located in the bulge region of the follicle and they interact with mesenchymal SCs (MSCs) located in the dermal papilla (DP).18 These signal exchanges promote activation of some cellular pathways that are essential for DPC growth, function, and survival, such as the activation of Wnt signaling pathway.19–21 Other signals, such as those from endothelial cells (ECs) located at the DP, are also essential for HF maintenance.22 EC dysfunction that impairs adequate blood supply may limit or inhibit hair growth.22 For instance, Minoxidil, a synthetic agent, is able to promote hair growth by increasing blood flow and the production of prostaglandin E2 (PGE2).7 It has been shown that proteins that belong to the transforming growth factor (TGF) superfamily, such as bone morphogenetic proteins (BMPs), also exert signals to maintain the capacity of DPCs to induce HF growing in vivo and in vitro.23 These BMPs may be released by several cells that compose the follicle, including ECs.24–26 ECs may provide signals for BMP receptor activation in DPCs similar to those signals that promote survival of MSCs in human embryoid bodies composed of multipotent cells.24,25 DPCs have been derived from pluripotent SCs in an attempt to study their potential for hair regeneration in vitro and in vivo.27 Together, dermal blood vessels and DPCs orchestrate a suitable microenvironment for the growth and survival of HFSCs.28,29 Interestingly, the expression of Forkhead box C1 regulates the quiescence of HFSCs located in the bulge region (Figure 1).30 HFSCs are quiescent during mid-anagen and maintain this stage until the next hair cycle.29,30 However, during early anagen stage, these cells undergo a short proliferative phase in which they self-renew and produce new hair.30 Therefore, the bulge region constitutes a SC niche that makes multiple signals toward quiescence or proliferation stages.30–34 It is known that fibroblasts and adipocyte signals are able to inhibit the proliferation of HFSCs.34 Additionally, BMP6 and fibroblast growth factor 18 (FGF18) from bulge cells exert inhibitory effects on HFSC proliferation.34 Dihydrotestosterone (DHT) also inhibits HF growth.35 Agents that reduce DHT, such as Finasteride, promote hair regrowth by inhibiting Type II 5α-reductase.6,14,36 In contrast to these inhibitory effects, DPCs located at the base of the HF provide activation signals (Figure 1).18,34 The crosstalk between DPCs and HFSCs leads to inhibition of inhibitory effects with the resultant cell

Figure 1. Diagram of the HF and factors involved in hair regeneration.

Notes: The HF is composed of different cell types including HFSCs, DPCs, and ECs, among others. HFSCs migrate from the bulge area after activation by growth factors released by DPCs. However, BMP6 and FGF18 from the bulge cells exert autocrine inhibitory effects in HFSC proliferation. Once the HFSCs are closer to DPCs and ECs, they differentiate and proliferate during anagen phase, forming new hair. Activation of Wnt signaling is essential for DPCs to release the factors that promote differentiation and proliferation of HFSCs. DHT interferes with this Wnt signaling and, in this way, inhibits hair growth and promotes hair miniaturization. Effective cell–cell interactions between HFSCs, DPCs, and ECs are essential for hair growth.

Abbreviations: BMP6, bone morphogenetic protein 6; DHT, dihydrotestosterone; DP, dermal papilla; DPCs, dermal papilla cells; ECs, endothelial cells; FGF18, fibroblast growth factor 18; HF, hair follicle; HFSCs, hair follicle stem cells.
proliferation toward hair regeneration (anagen).30,31,37 With the self-renewal of HFSCs, the outer root sheath (ORS) forms, and signals from DPCs to the bulge sheath diminish in a way that the bulge cells start again with their quiescent stage.4,34 As mentioned earlier, Forkhead box C1 transcription factor has an important role in maintaining the threshold for HFSC activation.30 The knockdown of these factors in bulge cells reduces the cells’ threshold for proliferation, and the anagen cycle starts more frequently due to promotion of HFSC proliferation in shorter periods of time.30

Prescribed and non-prescription products that promote hair growth and possible mechanisms of action

FDA-approved chemical agents

At present, the only therapeutic agents for AGA approved by the FDA in the USA are Finasteride and Minoxidil9,10 Minoxidil promotes hair growth by increasing the blood flow and by PGE2 production.7 Although Minoxidil is now a non-prescription medication, Finasteride and other drugs require a medical prescription for AGA treatment (Table 1). Dutasteride and Finasteride inhibit 5α-reductase, blocking the conversion of testosterone to DHT.36,38 While Finasteride is a selective inhibitor of type II 5α-reductase, Dutasteride inhibits type I and type II 5α-reductases. These medications have also been used to treat benign prostatic hyperplasia.39

Natural ingredients

In addition to prescribed medications, some natural ingredients have been used to promote hair growth (Table 2). For example, procyanidin B-2 (found in apples and in several plants) is able to inhibit the translocation of protein kinase C (PKC) in hair epithelial cells.40 PKC isozymes, such as PKC-βI and -βII, play an important role in hair cycle progression and inhibiting their translocation can promote hair growth.40 Procyanidin B-3 can promote hair growth by inhibiting TGF-β1.41 Another group of natural ingredients, such as saw palmetto, alfatradiol, and green tea (Epigallocatechin gallate), have the capacity to inhibit 5α-reductase and block DHT production.52-43 The natural ingredients and their proposed mechanisms of action are summarized in Table 2 (the commercial web page is included, since there are no formal studies about their mechanisms of action).

Laser therapy

Light amplification by stimulated emission of radiation (LASER) generates electromagnetic radiation which is uniform in polarization, phase, and wavelength.45 Low-level laser therapy (LLLT), also called “cold laser” therapy, since it utilizes lower power densities than those needed to produce heating of tissue. Transdermal LLLT has been used for therapeutic purposes via photobiomodulation.46,47 Several clinical conditions, such as rheumatoid arthritis, mucositis, pain, and other inflammatory diseases, have been treated with these laser devices.48-50 LLLT promotes cell proliferation by stimulating cellular production of adenosine triphosphate and creating a shift in overall cell redox potential toward greater intracellular oxidation.51 The redox state of the cell regulates activation of signaling pathways that ultimately promotes high transcription factor activity and gene expression of factors associated with the cell cycle.52 Physical agents such as lasers have been also used to prevent hair loss in a wavelength range in the red and near infrared (600–1,070 nm).53-55 Laser therapy emits light that penetrates the scalp and promotes hair growth by increasing the blood flow.54 This increase gives rise to EC proliferation and migration due to upregulation of vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase.55,56 In addition, the laser energy itself stimulates metabolism in catagen or telogen follicles, resulting in the production of anagen hair.57 A specific effect of LLLT has been demonstrated to promote proliferation of HFSCs, forcing the hair to start the anagen phase.57

Biologic agents that promote hair growth and their mechanisms of action

SC signaling

Recently, it has been found that SCs release factors that can promote hair growth.16 These factors and their mechanisms of action have been summarized in Table 3. These factors, known as “secretomes”, are able to promote skin regeneration, wound healing, and immunologic modulation, among other effects.58,59

Table 1 Prescribed products used for AGA

<table>
<thead>
<tr>
<th>Prescribed products</th>
<th>Source</th>
<th>Mechanism of action</th>
</tr>
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<tbody>
<tr>
<td>Finasteride/Dutasteride</td>
<td>Synthetic (small molecule)</td>
<td>Inhibits type II, 5α-reductase</td>
</tr>
<tr>
<td>Latanoprost and Bimatoprost</td>
<td>Synthetic prostaglandin analog of PGF2α (originally used to decrease ocular pressure in glaucoma)</td>
<td>Activates prostaglandin receptor</td>
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Abbreviation: AGA, androgenic alopecia; PGF2α, prostaglandin F2α.
Some of these factors, such as epidermal growth factor (EGF), basic fibroblast growth factor, hepatocyte growth factor (HGF) and HGF activator, VEGF, insulin-like growth factor (IGF), TGF-β, and platelet-derived growth factor (PDGF), are able to provide signals that promote hair growth. Enhancement in growth factor expression (except for EGF) has been reported when the adipose SCs are cultured in hypoxic conditions. Also, SCs increase their self-renewal capacity under these conditions. Low oxygen concentrations (1%–5%) increase the level of expression of SC factors that include VEGF, basic fibroblast growth factor, IGF binding protein 1 (IGFBP-1), IGF and PDGF. Some of these factors such as IGF play a prominent role in epidermal cell division in the hair shaft and bulb. 

Table 2 Non-prescription products used for AGA and their proposed mechanisms of action

<table>
<thead>
<tr>
<th>Non-prescription product</th>
<th>Source</th>
<th>Proposed mechanism of action</th>
</tr>
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</table>
| Minoxidyl (FDA approved) | Synthetic (small molecule) | Blocks upregulation of TGF-β1 induced by DHT. |}
| Apple Procyanidin B-2 (extract from apples) | Natural (apples and several plants) | Increases the strength of the hair (because of its great penetration) |
| Procerin (saw palmetto extract and other ingredients such as iodine, gotu kola, magnesium, grape seed extract, biotin, niacin, and vitamin B12) | Natural (small plant named saw palmetto) | It protects the hair bulb and shaft. It stimulates cell division in the hair shaft and bulb |
| Provillus (www.provillus.com) | Formulation (Minoxidil 2 or 5%, biotin, Zn, Fe, Mg, Ca, B6 complex) | Inhibits 5α-reductase and formation of DHT |
| Musol 20 (http://www.cosmeticingredients.co.uk/Ingredient/musol-20-pf) | Natural (yeast extract, mucoprotein) | It protects the hair bulb and shaft. It stimulates cell division in the hair shaft and bulb |
| EMortal Pep (http://www.revagain.co.kr/goods/catalog?code=0002) | Synthetic and natural | It protects the hair bulb and shaft. It stimulates cell division in the hair shaft and bulb |
| Planoxia-RG (https://www.ulproctor.com/en/na/PersonalCare/Detail/5314/195870/Planoxia-RG) | Natural | Inhibits 5α-reductase and formation of DHT |
| Trichostyl (http://dir.cosmeticsandtoiletries.com/PersonalCare/Detail/5314/195870/Planoxia-RG) | Natural (water, mannitol, Pterocarpus marsupium bark extract, disodium succinate, glutamic acid) | Inhibits 5α-reductase and formation of DHT |
| Seveov (http://www.naturex.asia/uk/1/markets/personal-care/natbeautytm/seveov.html) | Natural (formula that contains [200 mg] saw palmetto and [300 mg] β-sitosterol as the main ingredients) | Inhibits 5α-reductase and formation of DHT |
| Green tea (Epigallocatechin gallate) | Synthetic (small molecule) | Inhibits type II 5α-reductase |
| Nioxin (formulation of Coenzyme Q10 and other coenzymes) | Natural (flavonoid found in several non-citrus fruits, vegetables, leaves, and grains) | Inhibits PGD2 |
| Planoxia-RG | Natural (maca root extract) | It protects the hair bulb and shaft. It stimulates cell division in the hair shaft and bulb |
| Minoxidyl (FDA approved) | Synthetic (small molecule) | Inhibits type II 5α-reductase |
| Apple Procyanidin B-2 (extract from apples) | Natural (apples and several plants) | Inhibits PGD2 |
| Procerin (saw palmetto extract and other ingredients such as iodine, gotu kola, magnesium, grape seed extract, biotin, niacin, and vitamin B12) | Natural (small plant named saw palmetto) | Inhibits 5α-reductase and formation of DHT |
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| Hairomega (http://www.nioxin.com/nioxin/Search_Brand=Wejorgposureutm_medium=google&utm_source=othercoenzymeshttp://www.nioxin.com/othercoenzymes) | Natural (polyphenol antioxidant) | Inhibits type II 5α-reductase |
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Abbreviations: AGA, androgenic alopecia; DHT, dihydrotestosterone; ECM, extracellular matrix; FDA, US Food and Drug Administration; PGD2, prostaglandin D2; PKC, protein kinase C; TGF-β1, transforming growth factor β1.
binding protein 2 (IGFBP-2), macrophage colony-stimulating factor (M-CSF), M-CSF receptor (M-CSFR), and PDGF receptor β (PDGFR-β). While these groups of factors promote HF growth in intact skin, another group of factors, such as M-CSF, M-CSFR, and interleukin-6, are involved in wound-induced hair neogenesis. HGF and HGF activator stimulate DPCs to promote proliferation of epithelial follicular cells. Epidermal growth factor promotes cellular migration via the activation of Wnt/β-catenin signaling. VEGF promotes hair growth and increases the follicle size mainly by perifollicular angiogenesis. Blocking VEGF activity by neutralizing antibodies reduced the size and growth of the HF. PDGF and its receptor (PDGFR-α) are essential for follicular development by promoting upregulation of genes involved in HF differentiation and regulating the anagen phase in HFs. They are also expressed in neonatal skin cells that surround the HF. Monoclonal antibodies to PDGFR-α (APASA) produced failure in hair germ induction, supporting that PDGFR-α and its ligand have an essential role in hair differentiation and development. IGF-1 promotes proliferation, survival, and migration of HF cells. In addition, IGF binding proteins (IGFBPs) also promote hair growth and hair cell survival by regulating IGF-1 effects and its interaction with extracellular matrix proteins in the HF. Higher levels of IGF-1 and IGFBPs in beard DPCs suggest that IGF-1 levels are associated with androgens. Furthermore, DPCs from non-balding scalps showed significantly higher levels of IGF-1 and IGFBP-6, in contrast to DPCs from balding scalps.

### Small molecules

Small molecules with low molecular weight (<900 Da) and the size of 10^−9 m are organic compounds that are able to regulate some biologic processes. Some small molecules have been tested for their role in hair growth. Synthetic, non-peptidyl small molecules that act as agonists of the hedgehog pathway have the ability to promote follicular cycling in adult mouse skin. PGE2 and prostaglandin D2 (PGD2) have also been associated with the hair cycle (Table 3). PGD2 is elevated in the scalp of balding men and inhibits hair lengthening via GPR44 receptor. Also, it is known that PGE2 and PGF2α promote hair growth, while PGD2 inhibits this process. Prostaglandin analogs of PGF2α have been used originally to decrease ocular pressure in glaucoma with parallel effects in the growth of eyelashes, which suggests a
specific effect in HF activation. PGD2 receptors are located in the upper and lower ORS region and in the DP, suggesting that these prostaglandins play an important role in hair cycle. Molecules such as quercetin are able to inhibit PGD2 and, in this way, promote hair growth. Antagonists of PGD2 receptor (formally named chemoattractant receptor-homologous expressed in Th2 cells) such as setipiprant have been used to treat allergic diseases such as asthma, but they also have beneficial effects in AGA. Another small molecule l-ascorbic acid 2-phosphate promotes proliferation of ORS keratinocytes through the secretion of IGF-1 from DPCs via phosphatidylinositol 3-kinase. Recently, it has been described that small-molecule inhibitors of Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathway promote hair regrowth in humans. Janus kinase inhibitors are currently approved by the FDA for the treatment of some specific diseases such as psoriasis and other autoimmune-mediated diseases. Also, another group of small molecules such as iron and the amino acid l-Lysine are essential for hair growth (Table 3).

Cellular therapy

The multipotent SCs in the bulge region of the HF receive signals from DPCs in order to proliferate and survive. It has been shown that Wnt/β-catenin signaling is essential for the growth and maintenance of DPCs. These cells can be isolated and cultured in vitro with media supplemented with 10% fetal bovine serum and FGF-2. However, they lose versican expression which correlates with decrease in follicle-inducing activity in culture. Versican is the most abundant component of HF extracellular matrix. Inhibition of glucose synthase kinase-3 by (2’Z,3’E)-6-bromoindirubin-3’-oxime (BIO) promotes hair growth in mouse vibrissa follicles in culture by activation of Wnt signaling. Therefore, the increase of Wnt signaling in DPCs apparently is one of the main factors that promote hair growth. DPCs have been also generated from human embryonic SCs that induced HF formation after murine transplantation.

Platelet-rich plasma

Platelets are anucleate cells generated by fragmentation of megakaryocytes in the bone marrow. These cells are actively involved in the hemostatic process after releasing biologically active molecules (cytokines). Because of the platelets’ higher capacity to produce and release these factors, autologous platelet-rich plasma (PRP) has been used to treat chronic wounds. Therefore, PRP can be used as autologous therapy for regenerative purposes, for example, chondrogenic differentiation, wound healing, fat grafting, AGA, alopecia areata, facial scars, and dermal volume augmentation.

In search of novel therapies

In this paper, we reviewed and discussed the use of therapeutic agents for hair regeneration and the knowledge to promote the development of new therapies for AGA based on the advances in regenerative medicine. The HF is a complex structure that grows when adequate signaling is provided to the HFSCs. These cells are located in the follicle bulge and receive signals from MSCs located in the dermis that are called DPCs. The secretory phenotype of DPCs is determined by local and circulatory signals or hormones. Recent discoveries have demonstrated that SCs in culture are able to activate DPCs and HFSCs and, in this way, promote hair growth. The study of these cellular signals can provide the necessary knowledge for developing more effective therapeutic agents for the treatment of AGA with minimal side effects. Therefore, advancements in the field of regenerative medicine may generate novel therapeutic alternatives. However, further research and clinical studies are needed to evaluate their efficacy.

Disclosure

The authors report no conflicts of interest in this work.

References

Stem-cell therapies for androgenic alopecia


