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Perspectives of Alopecia behind the Regulation of Foxn1 Gene Exposes the Human Nude Phenotype

Shahnaz Begum, Md Jamil Hossain, Li Juan Gu and
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Additional information is available at the end of the chapter

Abstract

The hair follicle (HF) is remarkable for its dynamic structure and one of the most prominent mini organs of the skin. The most visible end product formed by the hair follicle known as "hair shaft"- a tissue with a highly keratinized protein. Therefore, alopecia or baldness issue largely depends on the equilibrium between keratinocyte growth and differentiation of the HF. However, molecular nature in mice, rats, and humans, loss of transcription factor Foxn1 the keratinization processes is significantly impaired. Hence, this nude gene lack of function makes very similar hair pattern baldness in both human and nude mice (Foxn1nu/Foxn1nu). Thus highlighting the usefulness of mouse mutants and mouse genomics as a research tool for better molecular controls of human hair biology. To enhance research efforts of Foxn1 target gene regulation and the pharmacological manipulation of the nude phenotype are important open questions for promising research strategies related with Foxn1 biology. Taken together these issues may open the discovery of the investigative dermatology that promises the controls of epithelial differentiation in mammalian skin.

Keywords: hair follicle, nude mouse, alopecia, Foxn1, T-cell immunodeficiency, keratinocytes

1. Introduction

The hair follicle (HF) is the most prominent mini organ of the skin and it undergoes repeated cycles of regression and regeneration throughout the lifetime of a mammal. Each phase of the hair cycle is distinctively characterized. During the anagen phase hair is actively growing

with a progression of tissue proliferation, a short resting phase with a massive apoptosis of hair follicle (catagen) and the relatively quiescence of telogen follicle, thus maintaining hairy phenotype in mice, rats, and humans [1–3].

The natural hair cycle in human poses a unique paradox involves many signaling molecules, transcription factors, and structural components are differentially and sequentially expressed and generate this organ. The differential expression of receptor and enzyme also provides basics for the variable responsive into the active hair follicle. On the other hand, genetically acquired disorders (nude gene) also inhibit to generate well differentiated hair follicles related with abnormal keratinization of hair fiber; taken together all these phenomena may cause baldness in human (alopecia, hair loss/balding); is a serious psychological distress in human society [4–7]. All these regulatory phenomena of hair cycle are highly conserved between rodents and humans. Although, some clinical differences might be present at the molecular level between the human and animal models; but it may not fully represent the morphological presentation of disease in humans. Consequently, especially when it comes up with the pathogenesis of human hair growth and disorder, it is essentially required to carry out research on human hair follicles in to animal models studies in the parallel lines.

Mammalian models, especially the development of genetically engineered mice which is role model to study for hair biology and hair disorder research those includes (1) failure in hair follicle formation and consequently abnormally low number of hair follicle in epidermis, (2) disorder of hair morphogenesis causing to fail the hair shaft to penetrate the epidermis, (3) hair follicle structure disorder leading to hair shaft defects and alopecia and also (4) immunological abnormalities resulting in alopecia [8]. Moreover, rodents those are genetic manipulation has been conducted to produce knockout (gene inactivation) mice for specific gene of interest the regulatory events of hair follicle development and hair growth, directly relevant to the hair follicle biology in humans [9].

During embryogenesis the development of the hair follicle appendage formation involves a complex sequence of signals interacting with the ecoderm and mesenchyme to form a mature hair follicle [10]. Once the hair follicle is generated, it displays dynamic cell kinetics: anagen (growth phase), catagen (regression phase), and telogen (resting phase), throughout postnatal file. Among the skin appendages, the hair follicle has the most complicated structure, composed of several distinct cell types that produce highly specialized protein. The anagen hair shaft has a common structural organization, in which a multicellular cortex is encased in a cuticular layer of flattened cell, often with a medulla layer centrally placed in the cortex. The hair is surrounded and supported by the inner root sheath (IRS), companion layer, and outer root sheath (ORS). The IRS consists of three distinct layers: IRS cuticle, Huxley layer, and Henle layer. The matrix cells in the hair bulb, which originally derive from the stem cells located in the bulge region, actively proliferate and differentiate into these cell layers except for ORS [11]. Like the epidermis, the hair follicle is also a highly keratinized tissue forms a rigid structure and also the end-product of the hair follicle. Recently, more of the genes that control the expression of hair keratins have been defined, including Foxn1 (FOXN1 in humans), which was first to be found using linkage analysis and an autosomal recessive mouse mutant, "nude". This gene encodes a member of the Forked/Winged-helix domain family designated as Whn (Winged-helix-nude)

or Hfh11 (hepatocyte nuclear factor 3/forkhead homolog 11) but was later renamed Foxn1 (Forkhead box n1) [12–15]. In the anagen hair follicle, Foxn1 is strongly expressed in the upper matrix, precortex and cortex of the hair fiber [12, 16] suggesting that Foxn1 might activate genes essential for hair fiber differentiation. Several lines of evidence support this suggestion, specially about the involvement of Foxn1 in the expression of hair keratin genes.

It is known that nude mice have disrupted postnatal hair growth and show T-cell immunodeficiency due to thymic aplasia [17]. Hairs of nude mice are very thin and easily forms coils within the hair follicle, indicating a defect in hair keratinization. Also, nude mice possess homozygous mutant Foxn1 alleles, which results in the trunked protein lacking both the DNA- binding and transcriptional activation domains as pointed as the need for DNA-binding of Whn to fulfill its function [12]. Finally, in 1999, Frank et al. [18] reported the crucial finding that the nude phenotype is by no means just a peculiarity of the nonhuman animal kingdom, but also occurs in humans equivalent of the "nude" murine phenotype was first described in two sisters in 1996 and also described by Lin et al. [19] in consanguineous Chinese family affected by PHNED and identified a homozygous nonsense mutation. This made the nude gene the second gene to be defined after hairless [20] whose lack of function generates a very similar hair phenotype (alopecia) in both human and mouse, and it underscored the usefulness of mouse mutants and mouse genomics as a research tool for better understanding the molecular controls of human hair biology [21].

2. Morphological characteristics expressed by Foxn1 gene in the skin

2.1. Imperfect haircoat leading to immunodeficiency

The role of Foxn1 gene expression unambiguously expressed in the skin of nude mice with a lack of haircoat compared with the wildtype and heterozygous animals. As this phenomenon can also be selectively expressed in the thymus [22] and the epidermis shows the sign of abnormal differentiation and the nails of nude animals are severely malformed [16, 23]. In 1966, S.P. Flanagan from the Institute of Animal Genetics in Edinburgh, UK, nude mouse Foxn1nu/Foxn1nu (hereafter called nu/nu) established a classical mouse model with a lack of fur coat and an increased rate of postnatal mortality [17]. On that time, Flanagan failed to notice that nude mice suffer from agenesis of thymus and as a results leads to severe immunodeficiency [24]. It caused by lack of mature T cells thus makes nude mice also an ideal model system for immunological research and experimental [25] oncology. Later advanced studies have demonstrated that both defects are the pleiotropic effects of the same gene [22, 26]. Thus, restoration of a thymus gland may not govern the hair growth of nude mice. Hereafter, it becomes an attractive mouse model for immunodeficiency caused by congenital athymia and also for great research tool for congenital alopecia.

According to Flanagan's observation, at birth nude mouse found no histological abnormalities. The impaired differentiation of nude follicles exhibits structural imperfections (**Figure 1**) of the cortex, hair cuticle and inner root sheath (IRS) [27]. The most remarkable phenotypic characteristic
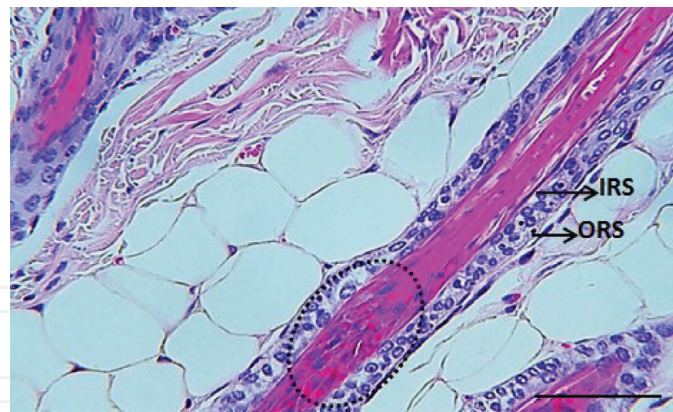
Figure 1. Cortex formation is severely injured in nude follicle and exhibits structural imperfections of hair cuticle and inner root sheath. Scale bar: 100 μm.
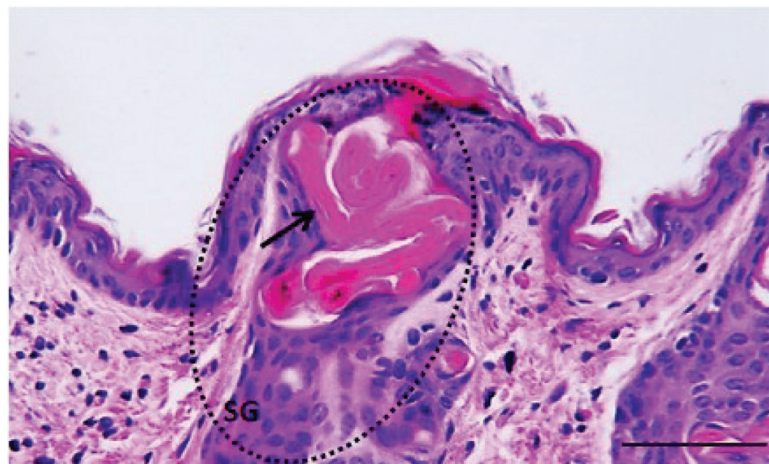

Figure 2. The follicular infundibulum, hair shaft twist and coil (arrow) at the level of sebaceous gland that does not penetrate the epidermis. Scale bars: 50 μm.

of nude mice skin is the disintegrated IRS leads to bend and coiling inside the sebaceous gland thus fails to achieve to penetrate the epidermis on postnatal day 8 [28, 29]. Subsequently, the follicular infundibulum, lined by a hyperplastic epithelium, becomes dilated (**Figure 2**) by keratinaceous debris and a small and curly hair shaft that does not penetrate the epidermis [30].

Finally, inadequate sparse hairs are visible at the head, neck, and the front extremities of nu/nu mice by approximately postnatal day 10 [17, 30–32], and later on spread it over the trunk area. Hair shafts those are able to enter the epidermis are often rigorously twisted or locally thickened and often breakdown before achieving a substantial length [30]. However, these are the molecular consequences of the nude phenotype cause by the mutations in the Whn gene [12, 13, 18, 33–35].

2.2. Nude mouse hair follicle undergoes normally cycling and usual hair bulb number

In general, *Foxn1*[nu] nude mice exhibit the same number of hair bulbs as normally mouse at day 42 postnatal [32], although marked defects observed within the nude mouse hair shaft, as which is mainly responsible for synthesizing the active hair shaft [36].

The *Foxn1*[nu] nude mouse hair follicle, it passes through a regular cycle of hair growth (anagen), regression (catagen), and a resting period (telogen) and it does not directly interrupt with well-differentiated the three phase (**Figure 3**) of the hair growth cycle [17]. Flanagan observed that during the third week postnatal hair follicles undergoes rapid organ **involution**, corresponding to the catagen stage of normal skin. During catagen phase the follicles in nude mice also shorten and build a club hair (**Figure 4**) that stopped growing any more as also observed in normally haired mouse skin [17].

Therefore, hair follicles in nude mice go through a normal cyclic transformation, as was already mentioned by Flanagan in 1966 and later on validated by some other researcher [25, 37, 38]. According to the typical morphological criteria during hair follicle formation events [39], no profound weakness were formed by the nu/nu mice, comparing to normally haired littermates [40]. Only the peculiarities found in the sebaceous glands as also been assumed by Flanagan [17], but sebaceous glands are simply a little displaced by the bending of developing
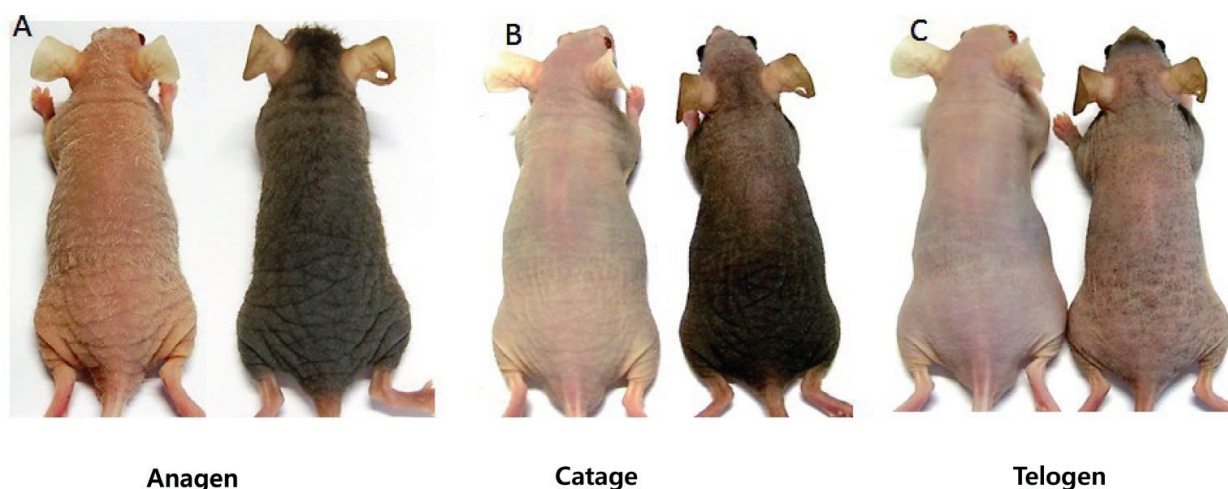

Anagen　　　　　　　　　**Catage**　　　　　　　　　**Telogen**

Figure 3. The Foxn1nu nude mouse (albino and pigmented) hair follicle passes through a regular cycle of hair growth A (anagen), regression B (catagen), and a resting period C (telogen). Inadequate sparse hairs (A) are visible at the head, neck, and the front extremities of nu/nu mice by approximately postnatal day 10.
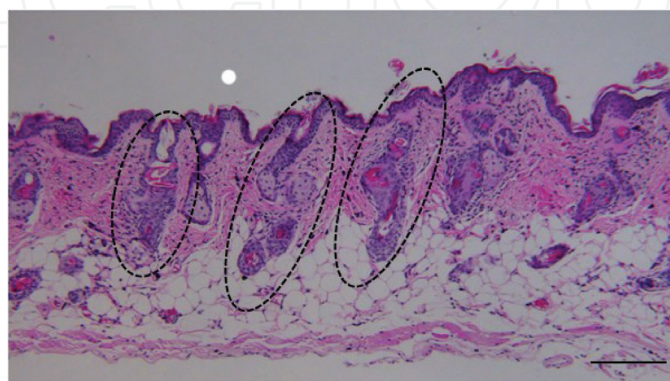

Figure 4. Catagen phase of HF in nude mice become shorten and fragmented hair shafts with keratinized debris that are heavily twisted. Scale bar: 100 μm.
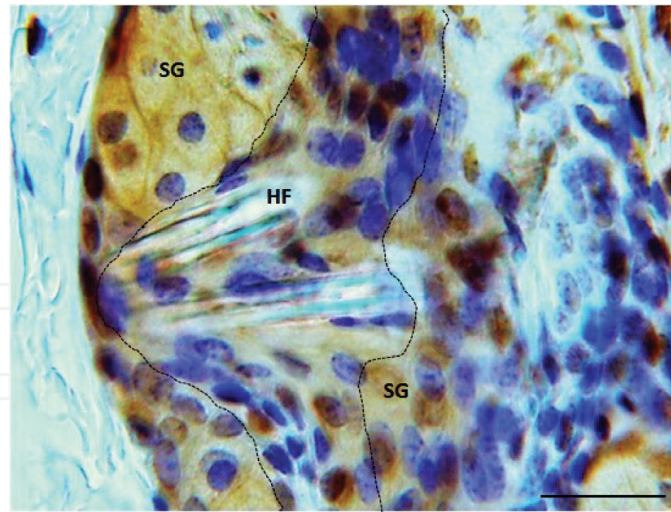
Figure 5. Sebaceous glands are simply a little displaced by the bending of developing hair shaft. BrdU labeling. Scale bars: 50 μm.

hair shaft and the dilated (become wider or larger) infundibulum is formed as like as funnel like structure (**Figure 5**), but without any morphological and structural abnormalities in the sebocytes themselves [30].

2.3. Expression of Foxn1 gene in epidermis

Foxn1 gene expression expressed in typical regions of developing skin, first in the nasal region as early as day 13 of gestation, subsequently *Foxn1* mRNA, the FOXN1 protein, and keratinocytes can be detected in the developing suprabasal epidermis but not found in the hair bulb as the first stage of hair follicle development on day 16 as it confirmed by using a reporter marker Beta-Galactosidase [16]. In addition, an increased proliferation and an impaired keratinocyte differentiation have been found in interfollicular epidermis, and the hair follicle of the nude mice, which overexpress Foxn1 in terminally differentiating cells [41]. Within the mature hair follicle and all anagen stage hair follicles, Foxn1 is preferentially transcribed in the supramatrical region, in the hair shaft, and in the inner and outer root sheath [16].

For the formation of an active hair follicle, interaction between the epithelial and dermal cell plays a vital role. The dermal papilla cell delivers signaling activators to induce follicle development [42–44]. Now the link between a mutated nude gene and its changes in molecular level in keratinocytes was presented by [45] as follows: While the reconstitution grafting assays was conducted with the nude keratinocytes recombined with wildtype follicular papilla cells, the resultant hair follicles trait expressed the nude phenotype [45]. Hence, functional defect of nude keratinocytes cannot be restored by wild-type dermal cells. Consistently, primary keratinocyte cultures derived resulting Whn gene is expressed either from the epidermis or developing hair follicles [45].

Taking these studies together, whn activity appears specific to epithelial cells, but the location and timing of whn expression in the skin is not clear at all. The epithelial cells of the epidermis

and hair follicles are similarly divided into proliferative and postmitotic compartments. During epidermal or follicular self-renewal, postmitotic cells originate from the proliferative populations, and the loss of the ability to multiply is accompanied by the initiation of terminal differentiation. While the precise characteristics of differentiating cells may vary depending on the location, the differentiation programs of the epidermis and hair follicles share certain features, including keratin accumulation and the eventual death of the cell [16]. The whn expression is present in many tissues during development and is also confined to the epithelial cells at different stages of maturation. In the epidermis and hair follicles, whn expression is associated primarily with the early stages of terminal differentiation but is also induced in a small subset of multiplying cells. Given this expression pattern as well as the effects of nude mutations, it is likely that whn influences the conversion of proliferative epithelial cells to postmitotic, differentiating cell resulting epithelial cell proliferation is enhanced and their terminal differentiation is disrupted in the absence of FOXN1, leading to epidermal thickening persistent anagen [16, 41].

2.4. The structural defects of nude hair shaft but hair bulb region entirely unaltered

According to hypothesis from Flanagan 1966 [17] and also supported by Mecklenburg et al. [30] defect of keratinization as the cause for hair shaft abnormalities, it could be found in nude mice exhibited with multiple fractures and locally twisted or thickened (**Figure 6**).

Indeed, in nude hair fibers, contains a reduced sulfur concentration was confirmed by elemental X-ray microanalysis as also been supported by the concept of impaired keratinization as observed for the hair shaft abnormalities [46]. Hair follicle ultrastructural analyses exposed that the cuticle of the IRS and the cuticle of the hair shaft are filled up by abnormal globular aggregates, that the hair cortex is fragmented into irregular cornified material, and that the hair medulla is partially lacking [32]. Whereas the Henle and Huxley layers of the IRS are normally keratinized in both nu/nu and wildtype mice, both the IRS cuticle and the cuticle of the hair shaft are fragmented into globular amorphous structures [29, 32]. A similar fragmentation is observed within the hair shaft cortex [32].

Figure 6. Short and sparse hair bending and exhibited with multiple fractures and locally twisted or thickened. Digital images were acquired using Kong, Bom-Viewer Plus software at 80×, 300× magnifications.

This possibly cause of this results is a complete lack of hair follicle keratin gene in nu/nu mice, particularly mHa3 a mouse ortholog of human acidic hair Keratin gene, which is normally expresses in the IRS and the hair shaft cortex of [47, 48]. Whereas previously thought that the hair shaft medulla is normal in nu/nu mice [30, 32], this observation has recently been challenged based on new experimental evidence [49]. According to Johns et al., the medulla of nu/nu hair shafts is less septulated than in heterozygous or wildtype animals, possibly due to a reduced expression of the adhesion molecule Desmocollin-2 in keratinocytes of the hair shaft medulla [49]. Taken together, these investigations support the concept of an impaired keratinization, both within the hair shaft and its cuticle and within the IRS, although the precise underlying molecular defects are still ill defined.

In spite of the great morphological changes of the hair shaft infundibulum, but the hair bulb area still remains entirely unaltered. Although the in some areas of the body the number of hair bulbs may be decreased [50], but in general, nude mice hold on the equal number of hair bulbs as normally hairy mice [51]. Dermal papilla fibroblasts and keratinocytes of the hair follicle matrix are also basically unaltered [51].

A loss-of-function mutated single gene, designated Whn nude gene expression analysis was conducted by using the Beta-Galactosidase activity in the morphogenesis of hair follicle and cycle: According to [16] Lee et al., during the anagen stage Whn expression was profoundly expressed in the precursor cells, hair cortex, ORS; in catagen follicle expression was terminated in the degenerating parts of the follicles, but remained and expressed surrounding the club; in telogen Beta-Galactosidase was expression level was very low and it was in the isthmus. Subsequently following the next hair cycles no significant differences was observed compared to the first hair cycle [16]. The nude hair follicle generally lacks a hair cortex containing the most of the hair's pigment melanin [32]. Therefore only little Beta-Galactosidase activity was detected in the differentiating hair shaft. In all other expression domains gene transcription was not affected significantly [16].

3. The physiological and genetic processes associated with nude hair follicle disorder

The hair follicle abnormalities in nude mice phenotype in the skin results from abnormal keratinization, possibly reduced synthesis of keratin protein. As the hair is the highly keratinized tissue and the final product is postnatally observed both in mouse and human. Research found that several kinds of hormonal changes may also as a cause of the nude phenotype; as this evident found in female nude mice with decreased concentration of progesterone, estradiol, prolactin, and thyroxin [51, 52].

The FOXN1 protein is evolutionarily highly conserved [53] and its analogue in *Drosophila* spp. possesses essential functions throughout the development [54, 55]. Foxn1 like transcription factor genes have been conserved in single copy throughout an animal of the large phylum *Chordata* [56]. Naturally occurring mutations in human and mouse Foxn1 genes shows very identical genomic structures with the location, phase, number, and sizes of introns as well as

also shows similarity index 85% for Foxn1 proteins [14]. DNA-binding domain and transactivation domain are similar. The mouse Foxn1 gene is localized on chromosome 11 [13, 14, 57–60] and is composed of nine exons, of which exon 1 is non-coding [14]. Altogether, six different spontaneously arisen allelic mutations in the mouse nude gene, all characterized by the lack of fur development and thymic agenesis. The original nude mouse phenotype (Foxn1nu) is caused by a single base pair deletion in exon 3. This deletion leads to a frameshift mutation and a premature stop codon, resulting in a protein that is predicted to lack the DNA-binding domain of the Foxn1 protein [12]. The 'winged-helix' structure of the Forkhead protein domain contains N-terminal DNA-binding domain and a C-terminal transcription activating domain [33]. Separation of both domains leads to a loss of function, while function is regained after they are linked noncovalently, representing that structural integrity and physical proximity of both domains are necessary for transactivation [43]. Human and mouse FOXN1 proteins have 85% sequence homology [53]. Very recently a human nude mutation was described [18]. Phenotypically, the affected person resembles the murine defect, i.e. she lacks hair and a thymic shadow upon X-ray examination, has dystrophic nails, and showed immunological abnormalities that have been overcome by bone-marrow transplantation. On the molecular level the defect is characterized by a homozygous nonsense mutation (R255X) in exon 5 of the WHN gene resulting in the absence of DNA-binding and transactivation domains. Forkhead factors mostly bind to DNA as monomers [61], however cases of homodimers [62] and heterodimers [63] have also been documented. Forkhead proteins also interact with non-transcription factor proteins such as coactivators, co-repressors, enzymes and other proteins. Furthermore, some Forkhead proteins are also subject to many posttranslational modifications such as phosphorylation, acetylation, methylation, and ubiquitination [64]. These post-translational modifications affect binding affinity and specificity of their target Forkhead proteins, their nuclear localization and even stability of some of these transcription factors. Finally, Forkhead proteins act as effector molecules for several signaling pathways, converting extra-cellular signals to changes in gene expression [64].

The FOXN1 protein is expressed exclusively in epithelial cells. This is in line with observations from hair reconstitution grafting assays: If wildtype dermal papilla cells are recombined with nude keratinocytes, hair follicles of the nude phenotype develop, suggesting that *Foxn1* activity is specific to epithelial cells [45]. Expression of the *Foxn1* express gene, the subsequent *Foxn1* mRNA, and the FOXN1 protein can be detected as early as day 13 of gestation in the developing nasal region [16]. On the 16th day of gestation, *Foxn1* is expressed in the suprabasal epidermis. It cannot be found in the hair bud, the first stage of hair express follicle development, but becomes detectable in a conical region above the bulbar matrix. Within the more mature hair follicle and in all anagen hair follicles, *Foxn1* is transcribed in the supramatrical region, in the hair shaft, and in the inner and outer root sheath [16]. FOXN1 is possibly involved in regulating the balance between epithelial cell growth and differentiation [16, 45]. This is in line with several observations that keratinocytes from nude mice have an increased propensity/tendency to differentiate abnormally and that the FOXN1 protein can specifically suppress the expression of differentiation-responsive genes in keratinocytes [25, 45]. Even in the hair follicle, expression of the *Foxn1* gene and its subsequent translation appear to correlate with the onset of terminal differentiation, although (FOXN1) has occasionally been found in some proliferating cells of the basal epidermis, the outer root sheath, and the hair

follicle matrix [16]. During hair follicle regression (catagen), *Foxn1* expression is lacking in the regressing epithelial compartment but remains in keratinocytes surrounding the developing club hair and is retained in some cells of the isthmus region during telogen [16]. Epithelial cell proliferation is enhanced and their terminal differentiation is disrupted in the absence of FOXN1, leading to epidermal thickening and persistent anagen [41]. *Foxn1* targeted genes are believed to (i) promote the differentiation of *Foxn1*-expressing keratinocytes and (ii) stimulate cell proliferation of neighboring cells via a paracrine mechanism [41]. Changes in gene expression keratinization are indeed associated with FOXN1 malfunction. Recently, the gene for a novel serine protease was shown to be overexpressed in nude mouse skin. However, its upregulation is probably an indirect consequence of the differentiation defect in the nude mouse hair follicle rather than a direct effect of FOXN1 signaling. The role of this novel gene in skin physiology and pathology has not been clarified to date [65]. *Foxn1* mRNA and the mouse ortholog of human acidic hair Keratin gene 3 (*KRTHA3*, hereafter *mHA3*) mRNA are coexpressed in hair follicles, nails, and papillae of the tongue, as it is critical for reliable prediction of gene function. In nude mice *mHa3* expression is completely absent in pelage hair follicles, indicating that *Foxn1* malfunction leads to a loss of expression of keratin genes [48, 65].

Although the transcriptional control of Foxn1 expression has not yet been completely elucidated, however Wnt glycoproteins regulatory signal is critical to regulate thymic function in the epithelial Foxn1 expression in both autocrine and paracrine fashions [66]. Therefore, genes including WNT, SHH, signal transducer and activator of transcription 3 that may be able to initiate anagen onset. Also some other genes that can extend to maintain the anagen stage, such as FGF7 and WNT are the potential candidates. In addition, activating genes that can increase the size of the hair follicle, such as SHH, or inhibiting genes that control anagen catagen transformation: such as FGF5 might be the key to success for future gene therapy research. Finally, it would be very useful if there is a complete loss of hair follicles, restoring the formation of entirely new follicles as it is similar to the occurrence during hair follicular embryogenesis [67].

4. Past, present and future therapeutic approach: from gene therapy to pharmacology

To design therapeutic approaches, the most effective way to restore hair would be to reactivate the miniaturized hair follicles and set back to normal cycle. As our understanding gene therapies might be designed to improve alopecia on the basis of polygenic approach.

In 1995, Hoffman et al. first demonstrated topically applied selective gene therapy on targeted mouse hair matrix cells with a liposome-entrapped lacZ reporter gene [68]. Topical administration of highly selective nature liposomes composition (containing the vector) for targeting the hair follicle are very effective and safe way at anagen onset and to increase the number of follicles [69, 70]. On the other hand, intradermal injection can also be considered to introduce plasmid DNA or viral vectors into the superficial dermis of hair-bearing skin. In a study, adenovirus vector was transfer the murine SHH DNA into the skin of postnatal mice and the authors concluded that localized overexpression of SHH in postnatal skin initiates the onset of anagen and thus acts similar to a biologic switch with no evidence of any pathologic abnormalities [71].

Based on its therapeutic effect the effectively deliver of a gene to the hair follicle, it must be either by an in vivo or an ex vivo method. The in vivo method is simple and direct, but has been shown to have only transient expression, usually; a plasmid or viral vector delivers the gene directly into the follicular keratinocytes. This can be done using a topical application of lipoplexed deoxyribonucleic acid (DNA), a liposome mixture containing the vector, or intradermal injection of vectors.

In contrast, in the ex vivo method, genes are introduced during the tissue culture. This method could give long-term gene expression because keratinocyte stem cells and progenitor cells can be manipulated and targeted. However, this is more technically demanding [72].

Various studies been shown that uncontrolled activation of SHH in the epidermis can cause basal cell carcinoma, and overexpression of Beta-catenin can cause either trichofolliculomas or pilomatrixomas [73]. Considering that hair restoration is a cosmetic procedure, therefore, highest diligence must be taken for the patient, to ensure any means of hair restoration is safe both locally on the scalp and systemically.

Certain clinically relevant pharmacological agents and drugs may also moderately overcome the absence of functional Foxn1 gene. As these agents may play active role for the development of new therapeutic tools to patients with hair growth disorders due to the absence of functional Foxn1.

Nude mice is very useful model system for studying a range of biological processes of skin and hair biology and various research reported that; cyclosporin A (CsA) [74, 75], keratinocyte growth factor (KGF) [76] and AS101 [77] are prospective therapeutic implements. Cyclosporin A (CsA), an immunosuppressive metabolite [78] induces macroscopically visible hair growth in nu/nu mice and isolated cultured Foxn1nu/nu mice vibrissae. Topical, oral, or subcutaneous administration of Cyclosporine induced hair growth, which was dose-dependent [74, 75, 79]. The underlying mechanism of Cyclosporin A on nu/nu hair follicles is still obscure. Since CsA stimulates hair growth in nu/nu speculate that, CsA partially restoring the inherited structural hair shaft disorders based on abnormal keratinization in absence of functional Foxn1. Recombinant keratinocyte growth factor (KGF) also known as FGF7 [80] been reported to stimulates hair growth in nude mice while injected intraperitoneally or subcutaneously. An increase in keratinocyte proliferation and normalization the morphology of nude hair follicle was observer under KGF treatment, probably by upregulating the expression of certain hair keratins [76, 81, 82]. It has been reported that the synthetic tellurium immunomodulator AS101 also activates the expression of KGF and able to induce hair growth in nu/nu mice via activation of the ras-signaling [77] pathway. Consequently, synthetic analogs of vitamin D3 (calcitrolis), which is also known to stimulate keratinocyte terminal differentiation, also stimulate hair growth in nu/nu mice associated with increased mRNA levels of hair keratin gene mHa1, mHa7, mHa8, and mHb3 [83]. The above mentioned pharmaceutical agents underlying mechanisms still remain obscure, however a putative molecular regulation of hair shaft keratinization have been shown to rescue of the nude phenotype with a proposed schematic presentation by Mecklenburg et al. [40] with the modification from Botchkarev [84].

Now new hair research has motivated on ethnopharmacognosy, a plant-derived natural product or their derivatives deliver tremendous prospects to discover novel therapeutic agents to replace synthetic drugs. As the chemically synthesized drugs and synthetic compounds is

known for their adverse side effects on human body. Various study reported that hundreds of plants or natural substances are prospective to stimulate hair growth. In particular, only little candidate plant plays vital role to enhance hair growth whose efficacy are now under investigation. Therefore, some plants those were traditionally acclaimed and purported in oriental medicine such as *Asiasari radix* [85], *Chrysanthemum zawadskii* [86], *Panax ginseng* [87, 88], and *Eclipta alba* [89, 90] exert to promote hair growth on C57BL/6 mice and rat.

Study has demonstrated that, *E. alba* might be considered an effective modulator to overcome defects in keratinocyte differentiation in the hair follicle of nude mice by stimulating the proliferation of epidermal basal cells and cells in the hair matrix [1, 91]. Based on these fruitful findings, the use of such a stimulatory agent may deliver a novel approach for the management of various forms of alopecia and may have clinical implications for hair loss.

5. Conclusions

For intractable alopecia, until now lot of research attempts have been made to develop or improve therapeutic approaches. While the prevalence of hair loss is ever increasing in the society but their effective treatment options are still limited. Therefore, future research endeavor is challenged for the modulation of the nude phenotype and need to definitively illuminate the target genes and regulate the function of Foxn1; a novel insights for the control of keratinocyte differentiation in epidermis. Researchers need to pay their attention to investigate the genes and signaling pathways underlying hair follicles to develop targeted therapies through comprehensive transcriptome analysis by using next-generation sequencing (NGS). At last, future research it is advised to exploit to correspondence all the novel findings for the development of innovative strategies and for the management of keratinization disorders by regulating Foxn1 targeted genes.

Conflict of interest

No conflict of interest.

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