An insight into hair follicle via epigenetic modification and molecular docking: A review

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***Abstract*: Hair follicle, a mini-organ developed in the epithelial layer is one of the complex structural and functional unit of the hair growth. In this review, we discuss about the Androgenetic alopecia, where miniature of the hair takes place converting testosterone to dihydrotestosterone by 5-alpha reductase. We also discuss about LHX2, which is responsible for the initiation of anagen phase of hair growth. Prostaglandin D2 synthase converts PGH_2 to PGD_2, which leads to the hair loss. Few key players in the epigenetic modification process like DNMT1, DNMT3a, DNMT3b, DNMT3L and TETs group of proteins, which gives structural stability to the gene and expression, play an important role in hair growth. We review the previous and future possibilities of drug design for preventing hair loss with the help of molecular docking performed on these players as well as on Prostaglandin D2 synthase.**

Keywords: Androgenetic alopecia, hair follicle, LHX2

Functional Hair Follicle Anatomy

From structural point of view hair starts in the hair follicle, which is situated in the dermal layer of the skin. Papilla, hair matrix, root sheath and hair bulb are the anatomical constituents of the hair follicle. The mature anagen hair follicle is composed of a multicylindric stem that contains the hair shaft in its center and originates as an oval hair bulb proximally [1]. Hair bulb lies an onion-like structure, called the dermal papilla (DP) (sometimes referred to as the "follicular papilla"). The DP functions as the main part of the hair follicle and determines thickness, length, and hair cycle [2]. Each hair follicle consists of epithelial and Mesenchymal parts. The epithelium is divided into an upper permanent region, distal to the arrector pili muscle (APM) and an inferior region (including the hair bulb).

Apart from serving as hair shaft factory, the anagen hair bulb also provides the hair shaft's trichocytes with melanin granules. Within the hair bulb is a population of cells with the highest proliferation rate in the human body: the keratinocytes of the hair matrix. These differentiate into trichocytes, or cells of the inner root sheath (IRS). The outer root sheath (ORS), hair matrix, and hair shaft derive from epithelial stem cells in the bulge area, functioning as a pluripotent epithelial stem cell population for the skin [3]. The bulge stem cells not only form the secondary hair germ, which is involved in the generation of the new hair, but they can even be reconstituted by dedifferentiating keratinocytes in response to wounding of the bulge area. The size of the anagen hair bulb, the duration of anagen, and the hair shaft diameter are determined by the volume, the number of cells, and the secretory activity of the DP [4][5].

Epithelial and Mesenchymal portions are needed to maintain the cyclic hair follicle growth [6]. Mesenchymal stem cells within the tissue sheath serve as a recruitment pool for new DP cells. Apart from Mesenchymal stem cells, the hair follicle also contains mast cell precursors [7] and neuronal stem cells, the latter of which can develop into neurons and blood vessels. The large numbers of stem cells make the hair follicle a fascinating organ in the field of stem cell biology.

Androgenetic alopecia

Androgenetic alopecia, or male-pattern hair loss, is a hair loss disorder mediated by dihydrotestosterone, the potent form of testosterone. Dihydrotestosterone induces miniaturization of hair follicles, causing transformation of terminal hair into vellus hair [8]. Without treatment, patients undergo progressive hair loss. Androgenetic alopecia is common, and its incidence increases with age.

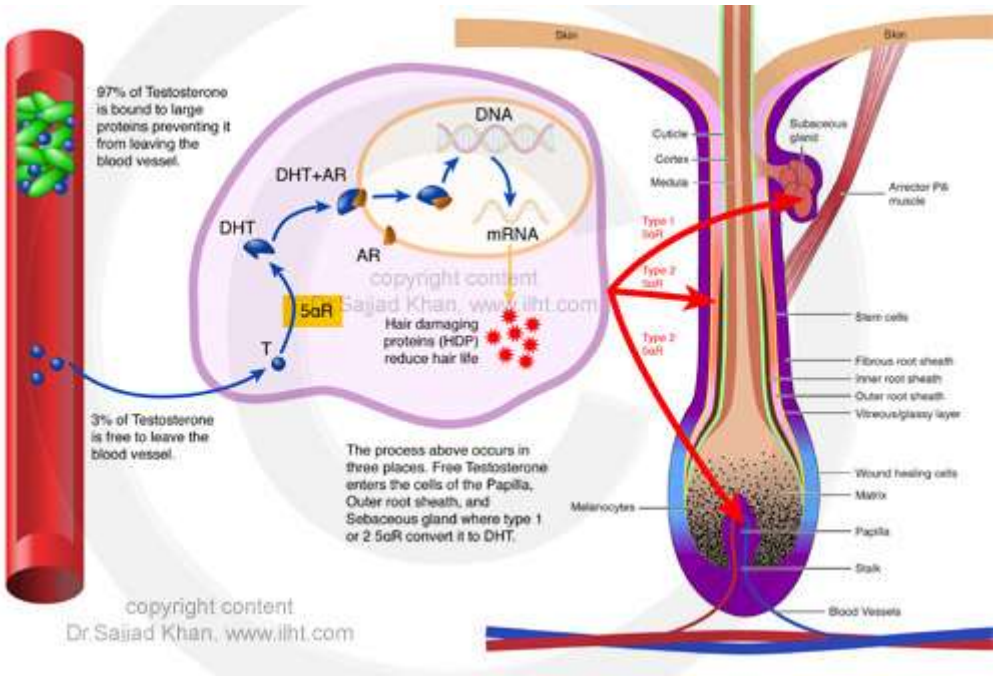

Figure 2: Depicting the role of DHT in Hair follicle (http://hair-problems.esy.es/testosterone-conversion-thinning-hair/)

AGA is caused by genetic and hormonal factors. While some genetic studies suggest an autosomal dominant inheritance with incomplete penetrance [9], other studies describe a unique polymorphism of the androgen receptor (X-chromosome) in these patients [10]. Early studies demonstrated that AGA hair loss does not occur in the absence of androgens or the androgen receptor [8]; moreover, if androgens are administered to genetically predispose but androgen deficient males, the non bald androgen recipients will now develop AGA.

It is a biologically benign, appearance-altering trait that may significantly affect a variety of psychological and social experiences and the individual's quality of life [11]. After briefly considering the meaning of hair within socio-cultural contexts, the present review critically examines the extant scientific evidence regarding:

(i) The influence of androgenetic hair loss on social perceptions and behavior;

(ii) Its effects on self-image and well-being; and

(iii) The impact of medical treatments of AGA on psychosocial functioning.

Currently, Minoxidil and finasteride are the only Food and Drug Administration (FDA) approved drugs and low-level laser light therapy (LLLLT) the only FDA-cleared device for

the treatment of androgenetic alopecia. Studies have been conducted on these treatments, but, to our knowledge, a meta-analysis summarizing the efficacy of these treatments for androgenetic alopecia has not been conducted [12].

Lim-Homeodomain Gene

Hair is important for thermoregulation, physical protection, sensory activity, seasonal camouflage, and social interactions. Hair is generated in hair follicles (HFs) and, following morphogenesis, HFs undergoes cyclic phases of active growth (anagen), regression (catagen), and inactivity (telogen) throughout life. The transcriptional regulation of this process is not well understood. We show that the transcription factor LHX2 is expressed in cells of the outer root sheath and a subpopulation of matrix cells during both morphogenesis and anagen. As the HFs enters telogen, expression becomes undetectable and reappears prior to initiation of anagen in the secondary hair germ [13].

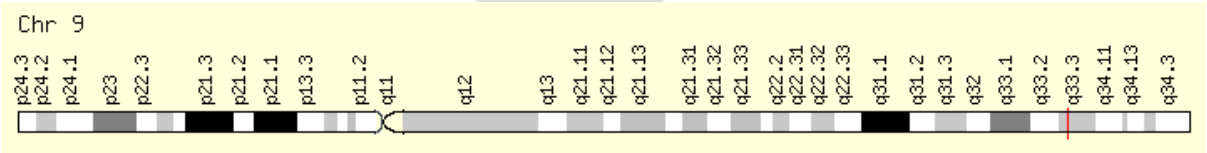

Figure 3: LHX2 gene on chromosome 9 in Human (https://www.genecards.org/cgi-bin/carddisp.pl?gene=LHX2).

In contrast to previously published results, we find that LHX2 is primarily expressed by precursor cells outside of the bulge region where the HF stem cells are located. This developmental, stage- and cell-specific expression suggests that LHX2 regulates the generation and regeneration of hair. In support of this hypothesis, we show that LHX2 is required for anagen progression and HF morphogenesis. Moreover, transgenic expression of LHX2 in postnatal HFs is sufficient to induce anagen. Thus, our results reveal an alternative interpretation of LHX2 function in HFs compared to previously published results, since LHX2 is periodically expressed, primarily in precursor cells distinct from those in the bulge region, and is an essential positive regulator of hair formation. [13].

Lim-homeodomain transcription factor LHX2 was particularly interesting because *LHX2* null mutant animals display defects in patterning and cell fate determination during brain development [14][15].
LHX2 concentrated in the upper outer root sheath (ORS) at a presumptive site (bulge) of the developing postnatal follicle stem cell compartment. Concomitantly, expression diminished at the base of the follicle, where highly proliferative matrix cells give rise to the differentiating inner root sheath and hair shaft. In adult follicles, LHX2 concentrated in the bulge, and as the new hair cycle began, LHX2 extended to the emerging secondary hair germs. Based on these patterns, we posit that LHX2 functions in specifying the embryonic hair follicle progenitor cells that then persist as bulge stem cells in adult follicles [16].
LHX2 as a transcription factor positioned downstream of signals necessary to specify hair follicle stem cells, but upstream from signals required to drive activated stem cells to terminally differentiate [16]. Transcriptionally profile embryonic hair placodes and interfollicular epidermis has enabled us to substantiate genes previously implicated in hair and epidermal development and to uncover differences that could be important in orchestrating lineage specification of multipotent skin progenitors. LHX2 has served as a paradigm for testing this premise, and our studies reveal that it functions as a molecular brake in regulating the switch between hair follicle stem cell maintenance and activation. Although follicles can be specified embryonically without LHX2, their overall numbers are reduced, and *LHX2* null follicles that do form are not proficient in maintaining the resting state and precociously activate. Once committed, cells no longer require or express LHX2 and progress along a normal program of terminal differentiation. Finally, LHX2 is the first identified marker expressed specifically by both embryonic hair placodes and postnatal follicle stem cells of the bulge. LHX2 now provides a means to dissect the transcriptional mechanisms that underlie stem cell maintenance within the hair follicle [16].
TGFβ2 signaling is impaired in NF-κB-deficient and LHX2 knockout embryos and that exogenous TGFβ2 rescues the HF phenotypes in LHX2 knockout skin explants, indicating that it operates downstream of LHX2. NF-κB/LHX2/TGFβ2 signaling axis that is crucial for primary HF morphogenesis [17].

Prostaglandin D2 Synthase

PGD2 inhibits hair growth and thus represents a negative counterbalance to the positive effects on hair growth shown for PGE2 and PGF2α. There is precedence for the opposing functions of individual prostaglandins that are downstream from the PTGS enzymes [18]. In AGA, large "terminal" hair follicles forming thick hair shafts miniaturize over time to small follicles that generate microscopic effete hairs. Follicle miniaturization is accompanied by a decrease in the duration of the growing phase of the follicle (anagen), which normally lasts several years to produce hair more than 1 m long, but which decreases to only days or weeks in AGA. This results in an increase in the percentage of resting (telogen) hair follicles containing microscopic hairs in bald scalp [4]. In addition to these intrinsic changes to the hair follicle, infiltrating lymphocytes and mast cells have been identified around the miniaturizing follicle, especially in the area of the stem cell–rich bulge area [3]. Sebaceous glands, which attach to each follicle, hypertrophy in bald scalp . In balding scalp, the number of hair follicle stem cells remains intact, whereas the number of more actively proliferating progenitor cells markedly decreases [18]. This suggests that balding scalp either lacks an activator or has an inhibitor of hair follicle growth.

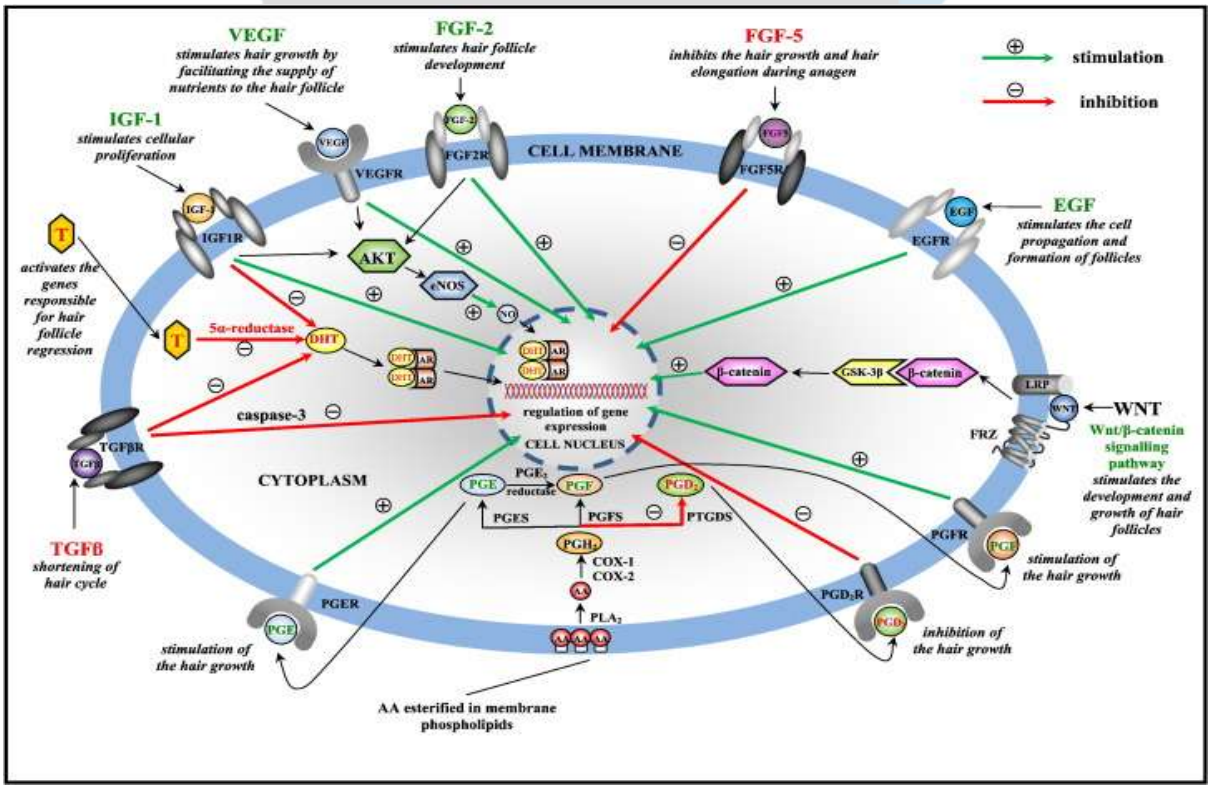

Figure 4: Pathway of different regulators in Hair follicles (Anna *et al.*, 2016).

3.4 Epigenetic

The term epigenetic was introduced by Conard Waddington in 1942 as a concept of environmental affect in inducing phenotype modification [19]. Epigenetic is the study of mitotically heritable alterations in gene expression potential that are not mediated by changes in DNA sequence. Genetics is the study of heritable changes in gene activity or function due to the direct alteration of the DNA sequence. Such alterations include point mutations, deletions, insertions, and translocation. In contrast, epigenetic is the study of heritable changes in gene activity or function that is not associated with any change of the DNA sequence itself [20].

Modern definition, which was the study of heritable changes in gene expression that to occur without a change in DNA sequence. Although there is no uniform definition of epigenetics, it has been described as heritable changes in gene function that occur without a change in the nucleotide sequence [21]. The term epigenome has emerged to describe the epigenetic modifications all over the epigenome, thus the epigenome controls the genome in both normal and abnormal cellular processes and events.

Epigenetic regulation is critical for mammalian development and cellular differentiation, and epigenetic dysregulation causes human developmental diseases [22]. Epigenetic factors include DNA methylation, Histone modifications, and microRNAs, and they can help to explain how cells with identical DNA can differentiate into different cell types with different phenotypes.

The DNA methyltransferase Dnmt1 is expressed in the IFE basal layer and HF outer root sheath (ORS) and matrix cells in humans and mice. Deletion of Dnmt1 specifically from skin and HFs showed striking similarities with PRC2 loss-of-function, with defects in SC maintenance and proliferation, at least in part attributed to INK4A/Arf locus up regulation [23].

DNMTs play an important role in genomic integrity, disruption of which may result in chromosome instability and tumor progression. It is well established that DNMTs are required for transcriptional silencing of a number of sequence classes, including imprinted genes, genes on the inactive X chromosome and transposable elements [24] and silencing of these sequences is essential for maintaining chromosome stability. Much compelling evidence has come from targeted deletion experiments showing that all three DNA methyltransferase are involved in stabilization of the genome, particularly repetitive sequences. Either single knockout of Dnmt1 or double knockout of Dnmt3a and Dnmt3b, enhances telomere recombination. MD simulations have advanced to a point where the atomic level information of biological macromolecule (protein or DNA-protein or protein-protein) can easily be advantageous to predict the functionality. xanthomicrol and galloyl compounds to investigate potential compounds for the inhibition of DNMT1, and the results of these two compounds are compared with drug decitabine.

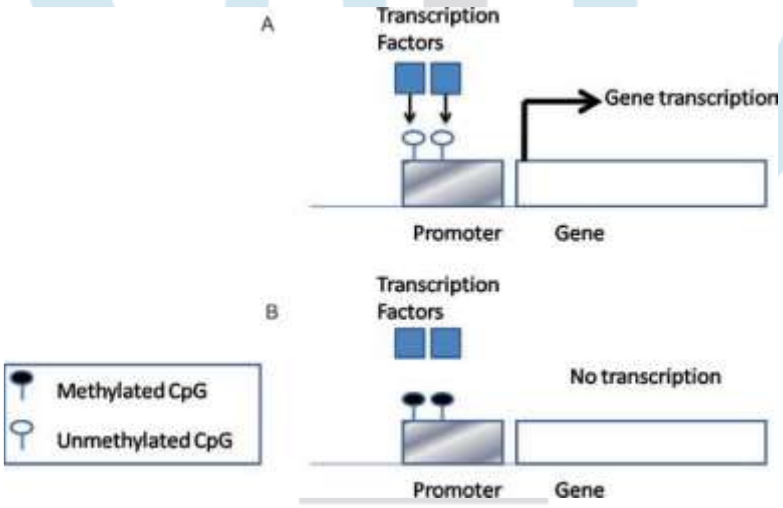

Figure 5: DNA methylation regulating gene expression. (A) The CpG island promoter is unmethylated and allows binding of transcription factors, which is required for transcription initiation. (B) The CpG island promoter methylation prevents binding of transcription factors and results in gene silencing (Lim and Maher 2010).

Molecular Docking

Molecular docking is a kind of bioinformatics modeling which involves the interaction of two or more molecules to give the stable adduct. Depending upon binding properties of ligand and target, it predicts the three-dimensional structure of any complex. Docking studies were performed on commercial software like GOLD from GLIDE from and free-wares like AutoDock Vina etc. Structures of different protein crystal structures were retrieved from the PDB. Molecular docking is a well-established computational technique which predicts the interaction energy between two molecules [25].

"Molecular docking tries to predict the structure of the intermolecular compound formed between two or more constitute molecule" Molecular docking studies are used to determine the interaction of two molecules and to find the best orientation of ligand which would form a complex with overall minimum energy. The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small

molecule. Hence docking plays an important role in the rational drug design. Significance of molecular docking, docking technique was used to suggest the binding energy, free energy and stability of complexes [25].

Main aim of molecular docking to predict the biological activity of a particular ligand- The net predicted binding free energy (ΔGbind) is revealed in terms of various parameters, hydrogen bond (ΔGhbond), electrostatic (ΔGelec), torsional free energy (ΔGtor), dispersion and repulsion (ΔGvdw), desolvation (ΔGdesolv), total internal energy (ΔGtotal) and unbound system's energy (ΔGunb). Therefore, good understanding of the general ethics that govern predicted binding free energy (ΔGbind) provides additional clues about the nature of various kinds of interactions leading to the molecular docking. The 3D pose of the bound ligand visualize using different visualizing tools like Pymol, Rasmol etc which could help in inference of the best fit of ligand. Predicting the mode of protein ligand interaction can assume the active site of the protein molecule and further help in protein annotation. Moreover molecular docking has major application in drug designing and discovery.

3.5.1 Different types of Interactions

Interactions between particles can be defined as a consequence of forces between the molecules contained by the particles. The pose score is a measure of the fit of a ligand into the active site. Scoring during the posing phase usually involves energy calculation.

o **Electrostatic forces** - Forces with electrostatic origin due to the charges residing in the matter.

o **Electrodynamics forces** - The most widely known is the van-der waals interactions.

o **Steric forces** – atoms in different molecules come into very close contact. The resulting forces can affect chemical reactions and the free energy of a system.

o **Solvent-related forces** - These are forces generated due to chemical reactions between the solvent and the protein or ligand. Examples are Hydrogen bonds (hydrophilic interactions) and hydrophobic interactions.

Other physical factors - Conformational changes in the protein and the ligand Molecular docking can be divided into two separate sections.

a) **Search algorithm** – These algorithms determine all possible optimal conformations for a given complex (protein-protein, protein-ligand). They can also calculate the energy of the resulting complex and of each individual interaction.

b) **Scoring function** – These are mathematical methods used to predict the strength of the noncovalent interaction called as binding affinity, between two molecules after they have been docked. Scoring functions have also been developed to predict the strength of other types of intermolecular interactions [26].

Binding Energy; ΔG bind = ΔGvdw + ΔGhbond + ΔGelect + ΔGconform + ΔG tor + ΔG sol

Types of Docking- There are various kinds of molecular docking procedures involving either ligand/target flexible or rigid based upon the objectives of docking simulations.

a) Lock and Key or Rigid Docking – In rigid docking, both the internal geometry of the receptor and ligand is kept fixed during docking

b) Induced fit or Flexible Docking - In this model, both the ligand and side chain of the protein is kept flexible and the energy for different conformations of the ligand fitting into the protein is calculated.

Applications of Molecular Docking

A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme. Docking is most commonly used in the field of drug design most drugs are small organic molecules, and docking may be applied to:

1. Hit identification – docking combined with a scoring function can be used to quickly screen large databases of potential drugs *in silico* to identify molecules that are likely to bind to protein target of interest.
2. Lead optimization – docking can be used to predict the relative orientation of a ligand that binds to a protein (also referred to as the binding mode or pose).
3. Bioremediation – Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes.

Areas, of molecular docking have revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or drug's binding properties to nucleic acid. Medicinal chemists are doing *in silico* observations where their main finding is to predict whether the compound/drug is interacting with the protein/DNA. Detection of those structural modifications in a drug that could result in sequence/structure specific binding to their target.

The cytidine nucleoside analogs azacitidine (AZA) and decitabine (DAC) are used for the treatment of patients with myelodysplastic syndromes and acute myeloid leukemia (AML). Few non-clinical studies have directly compared the mechanisms of action of these agents in a head-to-head fashion, and the agents are often viewed as mechanistically similar DNA hypomethylating agents [26]. Some of these drugs that are binding to the protein molecules effect the methylation pattern, so by docking the these ligand molecules with the protein for which the epigenetic regulation takes place. Also the hair fall can be controlled by docking the PTGDS protein molecules with various molecular compounds. For docking some of the supporting free version tools are available such as AutoDock Vina, swiss dock etc.

Bioinformatics done for the analysis of molecular docking, ligand-protein interaction would be studied. LHX2 gene is expressed low in the hair follicle, epigenetic modification is done to DNMT1 that unregulated LHX2 gene expression promoting hair growth. In another study where the Prostaglandins D2 synthase helps to convert PGH_2 to PGD_2 that leads to the hair loss [18]. By molecular

modification to the Prostaglandin D2 synthase we could inhibit the conversion and reduce the hair fall. Epigenetic plays important role for the modification of the gene to reduce its risk from mutation, it also regulate the expression of the gene by adding methyl group to its promoter region. DNA methyltransferase enzyme helps to add methyl group and gives stability to its structure. So by targeting the methyltransferase enzyme we could help our gene to regulate its expression [22].

Conclusion

Since the LHX2 gene has been identified as one of the gene responsible for the hair growth that leads to the development of the hair follicular stage anagen. LHX2 plays an important role in hair generation and regeneration. During HF morphogenesis *LHX2* has a widespread expression in the epidermal portion of the HF and decreased levels of the protein results in fewer HFs that in addition are arrested or delayed in development. Thus, LHX2 is essential for HF morphogenesis. Furthermore, transgenic expression of *LHX2* in HFs results in premature anagen initiation and thus *LHX2* expression is sufficient to induce anagen. Taken together, LHX2 plays a role in differentiation and patterning of HFs [13].

Epigenetic plays important role for the modification of the gene to reduce its risk from mutation, it also regulate the expression of the gene by adding methyl group to its promoter region. DNA methyltransferase enzyme helps to add methyl group and gives stability to its structure. So by targeting the methyltransferase enzyme we could help our gene to regulate [22].

As per the study conducted LHX2 gene is expressed low in the hair follicle, epigenetic modification is done to DNMT1 that upregulated LHX2 gene expression promoting hair growth. In another study where the Prostaglandins D2 synthase helps to convert PGH_2 to PGD_2 that leads to the hair loss [18]. By molecular modification to the Prostaglandin D2 synthase we could inhibit the conversion and reduce the hair fall.

References

[1] Whiting DA (2004) The Structure of the Human Hair Follicle. Light Microscopy of Vertical and Horizontal Sections of Scalp Biopsies. Pfizer, Canfield Publishing.

[2] Paus R, Foitzik K (2004). In search of the "hair cycle clock": A guided tour. Differentiation 72:489-511

[3] Cotsarelis G, Sun TT, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell. 61:1329-37.

[4] Paus R, Cotsarelis G (1999). The biology of hair follicles. N Engl J Med 341:491-497

[5] Jahoda CA, Reynolds AJ (1996). Dermal-epidermal interactions. Adult follicle derived cell populations and hair growth. Dermatol Clin 14:573-583.

[6] Stenn KS, Paus R (2001) Controls of hair follicle cycling. Physiol Rev. 81:449-94.

[7] Jahoda CA (2003). Cell movement in the hair follicle dermis—more than a two-way street?. J Invest Dermatol 121:1267-1275.

[8] Imperato-McGinley J (2002). 5alpha-reductase-2 deficiency and complete androgen insensitivity: Lessons from nature. Adv Exp Med Biol; 511:121-31.

[9] Bergfeld WF(1995). Androgenetic alopecia: An autosomal dominant disorder. Amer J Med; 98:95S-8S.

[10] Hillmer AM, Hannekin S, Ritzmann S (2005), et al. Genetic variation in the human androgen receptor gene is the major determinant of common early-onset androgenetic alopecia. Amer J Human Genet; 77:140-8.

[11] Olsen EA (1994). Androgenetic alopecia. In: Disorders of Hair Growth: Diagnosis and Treatment (Olsen EA, ed.). New York: McGraw-Hill: 257-83.

[12] Adil, A., & Godwin, M. (2017). The effectiveness of treatments for androgenetic alopecia: A systematic review and meta-analysis. *Journal of the American Academy of Dermatology*, *77*(1), 136-141.

[13] Törnqvist, G., Sandberg, A., Hägglund, A. C., & Carlsson, L. (2010). Cyclic expression of lhx2 regulates hair formation. *PLoS genetics*, *6*(4), e1000904.

[14] Porter FD, Drago J, Xu Y, Cheema SS, Wassif C, Huang SP, Lee E, Grinberg A, Massalas JS, Bodine D, Alt F, Westphal H (1997) Lhx2, a LIM homeobox gene, is required for eye, forebrain, and definitive erythrocyte development. Development. 124:2935-44.

[15] Bulchand S, Grove EA, Porter FD, Tole S (2001) LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem. Mech Dev. 100:165-75.

[16] Rhee H, Polak L, Fuchs E (2006) Lhx2 maintains stem cell character in hair follicles. Science. 312:1946-9.

[17] Tomann, P., Paus, R., Millar, S. E., Scheidereit, C., & Schmidt-Ullrich, R. (2016). LHX2 is a direct NF-κB target gene that promotes primary hair follicle placode down-growth. *Development*, *143*(9), 1512-1522.

[18] Garza LA, Yang CC, Zhao T, Blatt HB, Lee M, He H, Stanton DC, Carrasco L, Spiegel JH, Tobias JW, Cotsarelis G (2011). Bald scalp in men with androgenetic alopecia retains hair follicle stem cells but lacks CD200-rich and CD34-positive hair follicle progenitor cells. J Clin Invest; 121:613– 622.

[19] Waddington, C. H. (1952). Selection of the genetic basis for an acquired character. *Nature*, *169*(4302), 625-626.

[20] Aiello, L. P., Avery, R. L., Arrigg, P. G., Keyt, B. A., Jampel, H. D., Shah, S. T., & Nguyen, H.V. (1994).Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *New England Journal of Medicine*, *331*(22), 1480-1487.

[21] Bird, A. (2007). Perceptions of epigenetics. *Nature*, *447*(7143), 396-398.

[22] Egger, G., Liang, G., Aparicio, A., & Jones, P. A. (2004). Epigenetics in human disease and prospects for epigenetic therapy. *Nature*, *429*(6990), 457.

[23] Sen GL, Reuter JA, Webster DE (2010). DNMT1 maintains progenitor function in self-renewing somatic tissue. Nature. 2010; 463:563–567.
[24] Robertson KD (2005). DNA methylation and human disease. Nat Rev Genet. Aug; 6(8):597–610.
[25] Solovyev V.V., Salamov A.A. (1997). The Gene-Finder computer tools for analysis of human and model organisms genome sequences. In Proceedings of the Fifth International Conference on Intelligent Systems for Molecular Biology (eds.Rawling C.,Clark D., Altman R.,Hunter L.,Lengauer T.,Wodak S.), Halkidiki, Greece, AAAI Press,294-302.
[26] Abdulhameed, S., Pradeep, N.S., &Sugathan, S. (Eds.). (2017). Bioresources and Bioprocess in Biotechnology: Volume1: Status and Strategies for Exploration. Springer, 262-272.
[27] Hollenbach, P. W., Nguyen, A. N., Brady, H., Williams, M., Ning, Y., Richard, N., … MacBeth, K. J. (2010). A Comparison of Azacitidine and Decitabine Activities in Acute Myeloid Leukemia Cell Lines. *PLoS ONE*, *5*(2), e9001.