Computational analysis of DNMT1 mediated DNA methylation pathway regulating LIM-homeodomain 2 gene and PTGDS regulation for hair growth: A predictive approach for Androgenetic alopecia

[1]Arun R. Nair, [2]Dr. Mahavir Yadav, [3]Prof. Archana Tiwari

[1]M.tech Scholar, [2]Assistant Professor, [3]Professor and head of the department
[1]School of Biotechnology,
[1]Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, India

***Abstract*: Molecular docking is the study of interaction between protein ligand molecules, used as a powerful tool for drug designing. Androgenetic alopecia, where miniature of the hair takes place by converting testosterone to dihydrotestosterone via 5-alpha reductase. Hair follicle, a mini-organ developed in the epithelial layer is one of the complex structural and functional unit of the hair growth. DNMT1, DNMT3a, DNMT3b, DNMT3L and TETs group of proteins, which gives structural stability to the gene and expression by epigenetic modification of DNA, play an important role in hair growth. LHX2 is responsible for the initiation of anagen phase of hair growth. DNMT1 can be regulated by various ligands; in this paper we show the best affinity value of DNMT1 with 5- azacytidin. We also find luteolin, which has best affinity value with Prostaglandin D2 synthase, which converts PGH_2 to PGD_2, In future; these two molecules can be used as drugs to prevent hair loss in humans.**

***Keywords*: Molecular docking, Androgenetic alopecia, hair follicle**

Introduction:

The hair follicle is one of the most complex mini organs of the human body, productive protein fiber factory, which acts as a sensory organ and functions as of psychosocial communication, excretion, and protection, undergoes cyclic transformations between phases of growth (anagen), apoptosis-driven regression (catagen), and relative quiescence (telogen) [1]. Hair follicle demonstrates the unique ability to cyclically regenerate itself during our lifetime, based on epithelial–Mesenchymal interactions that drive waves of daughter cell populations, derived from resident epithelial, neural, and Mesenchymal stem cells, into defined strata of differentiation[2]. Hair loss, as well as unwanted hair growth (hirsutism, hypertrichosis), is a widespread problem. According to one calculation, androgenetic alopecia on its own eventually affects approximately 50% of the world's adult population [3][4].

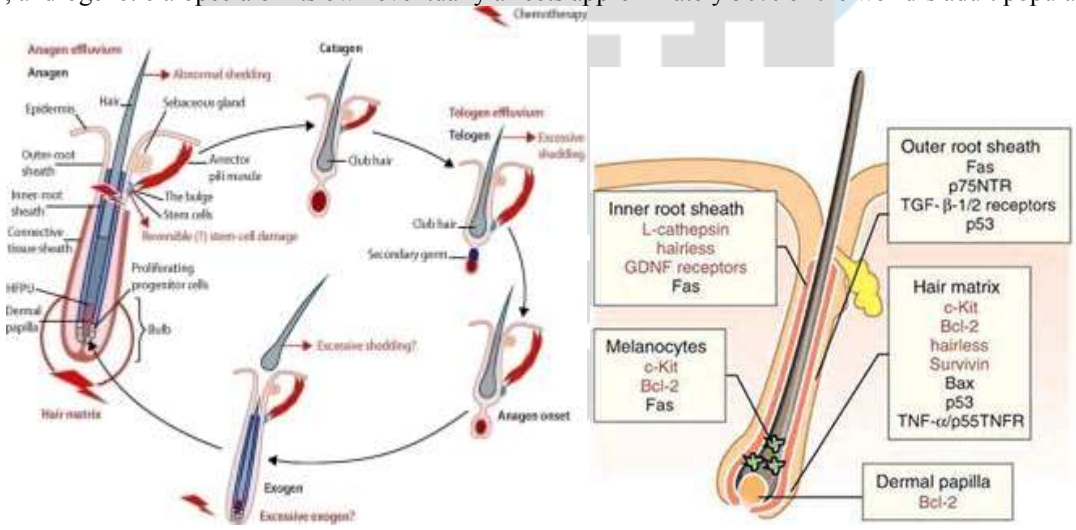


Figure1: Depicting the hair cycle and Hair structure (Stenn *et al.*, 2001).

Androgenetic Alopecia: Male Pattern Balding

Androgenetic alopecia, AGA, the most common cause of hair loss, affects 50% of men and 20% to 53% of women by age 50 years. It is a patterned form of hair loss in that the resultant baldness occurs in a highly predictable location [5]. For reasons we do not yet understand, AGA in females is more diffuse and less well patterned than in males [6][7]. Etiologically, AGA is caused by genetic and hormonal factors. While some genetic studies suggest an autosomal dominant inheritance with incomplete penetrance[8], other studies describe a unique polymorphism of the androgen receptor (X-chromosome) in these patients [9]. Early

studies demonstrated that AGA hair loss does not occur in the absence of androgens or the androgen receptor [10]; moreover, if androgens are administered to genetically predispose but androgen deficient males, the nonbald androgen recipients will now develop AGA.

LHX2

LIM-homeobox gene 2 (*LHX2*; previously called *LH-2* or *LH2A*) was first identified as a pre-B-cell-specific gene [11]. It is expressed during development and diverse functions are reported. Embryos lacking *LHX2* expression show a diminished forebrain due to hypoplasia of the cerebral. cortex and agenesis of the hippocampal anlagen. LHX2 appears to be crucial for patterning of the telencephalon. Furthermore, LHX2 is essential for eye formation. In the null embryos eye development arrests prior to the formation of an optic cup[12]. *LHX2* is also expressed in the progress zone of the growing limbs, however the limbs are developing normally in *LHX2-/-* mice since the LIM-homeobox genes *Lhx9* and *Lmx1b* compensate for LHX2 loss. *LHX2* is also expressed in the olfactory epithelium and required for differentiation of progenitors into mature olfactory sensory neurons [13][14].

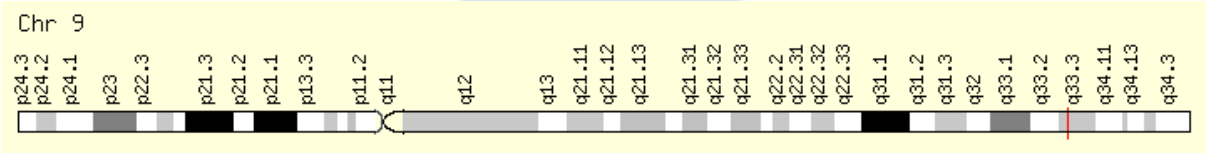


Figure 3: LHX2 gene on chromosome 9 in Human (https://www.genecards.org/cgi-bin/carddisp.pl?gene=LHX2).

Prostaglandin D2 Synthase

Prostaglandins (PG) are a family of structurally related eicosanoids that have regulatory roles in normal physiological as well as pathological contexts. Cyclooxygenase enzymes
catalyze the conversion of arachidonic acid to PGH2, which is converted to other prostanoid species including PGD2, PGE2, PGF2*α*, prostacyclin (PGI2), and thromboxane (TX)
A2 by the action of specific synthases [15].

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. Our results suggest that in mouse and human skin, a balance between PGE2 and PGD2 controls hair growth. Thus model predicts that efforts to reverse alopecia should optimally focus on both enhancing PGE2 and inhibiting PGD2 signaling. PGD2 inhibits hair growth, prostaglandins represent an underappreciated network for controlling the rate of hair lengthening.

Epigenetic

The term epigenetic refers to the study of heritable changes in phenotype or expression of genes that are not due to changes in the sequence of DNA [16].
Research into epigenetic has demonstrated that epigenetic regulation of gene expression has a critical role in normal development and cell functions, including imprinting, X-inactivation
and tissue-specific gene expression. In addition, disordered epigenetic gene regulation is a feature of a number of important human diseases, including cancer [17]. A number of processes have been implicated in epigenetic gene regulation including
DNA methylation; chromatin structure and modification; and untranslated RNAs. This review aims to introduce the reader to the concept of DNA methylation as a regulator of gene expression, with examples of its involvement in genomic imprinting and cancer.

DNA Methylation

DNA methylation refers to the addition of a methyl group (_CH3) covalently to the base cytosine (C) in the dinucleotide 5´-CpG-3´ (methylated cytosine residues are sometimes referred to as the fifth nucleotide). The term CpG refers to the base cytosine (C) linked by a phosphate bond to the base guanine (G) in the DNA nucleotide sequence. Most CpG dinucleotides in the human genome are methylated. However, unmethylated CpGs are not
randomly distributed, but are usually clustered together in 'CpG islands', which are in the promoter region of many genes (the region that facilitates transcription of a particular gene) [18]. The observation that the CpG islands in the promoters
of important genes that are expressed in most cells (often called 'housekeeping genes') are mainly unmethylated, and that methylation of CpG islands in cancer cells often leads to silencing of gene expression, led to the hypothesis that DNA methylation plays an important role in regulating gene expression[18]. One theory on the evolution of DNA methylation is that it evolved as a host defense mechanism to silence foreign DNA such as viral sequences, replicated transposable elements and other repetitive sequences [19].

Materials and Methods:

Protein Data Bank (PDB)

The Protein Data Bank (PDB) is a crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy, and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations.

CpG Island Finder
DataBase of CpG islands and Analytical Tool (DBCAT) is developed in order to recognize comprehensive mehtylation profiles of DNA alteration in human cancer. DBCAT is an online methylation analytical tool composed of three parts: a CpG Island Finder, a genome query browser and an analytical tool for methylation microarray data. The analytical tool can analyze raw data generated from scanners and search genes with methylated regions which could affect gene expression regulation (http://dbcat.cgm.ntu.edu.tw/).

Meth Primer 2.0
Meth Primer is an online platform which provides a number of tools and databases to facilitate the study of DNA methylation and epigenetics, including tools for designing primers and probes for various bisulfite conversion based PCRs, predicting CpG islands, and manipulating sequences. Meth Primer, based on Primer3, is a program for designing PCR primers for methylation mapping

Quma
Bisulfite sequencing, a standard method for DNA methylation profile analysis, is widely used in basic and clinical studies. This method is limited, however, by the time-consuming data analysis processes required to obtain accurate DNA methylation profiles from the raw sequence output of the DNA sequence, and by the fact that quality checking of the results can be influenced by a researcher's bias. We have developed an interactive and easy-to-use web-based tool, QUMA (Quantification tool for Methylation Analysis), for the bisulfite sequencing analysis of CpG methylation.

AutoDock Vina
AutoDock Vina is one of the apt and reliable software's available for drug discovery, molecular docking and virtual screening offering multi-core capability, high performance, enhanced accuracy and ease of use. This was designed and implemented by Dr. Oleg Trott. AutoDock Vina automatically calculates the grid maps and clusters the results in a way transparent to the user. AutoDock Vina significantly improves the accuracy of the binding mode predictions Additionally, AutoDock. It should be noted that all six of the other docking programs, to which it was compared, are distributed commercially. For its input and output, Vina uses the PDBQT molecular structure file format used by AutoDock.

PyMOL

PyMOL is an open-source molecular visualization system created by Warren Lyford DeLano. It is user-sponsored, open-source software, released under the Python License. PyMOL allows carrying out molecular docking, virtual screening and binding site analysis with PyMOL. The plug-in represents an interface between PyMOL and popular docking programs. Autodock Vina and makes extensive use of a Python script collection, Autodock Tools, for the setup of docking runs. Since visualization is crucial for structure-based drug design, the actual docking calculations can be launched from within PyMOL and the results be visualized.

UCSF Chimera
The molecular graphics program UCSF Chimera includes a suite of tools for interactive analyses of sequences and structures, structures automatically associate with sequences in imported alignments. Modeling and visualization of molecular structures are critical for gaining insight into biological function and molecular interactions, as well as for drug design and development. To address researcher needs, the extensible software platform UCSF Chimera has been developed by the resource for biocomputing, visualization, and informatics (RBVI). This software allows for 3- D visualization and structural analysis of molecular entities.

Rampage

Rampage is a bioinformatics tool used to design Ramachandran plot. A Ramachandran plot is a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure. A Ramachandran plot can be used in two somewhat different ways. One is to show in theory which values, or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein. G N Ramachandran used computer models of small polypeptides to systematically vary phi and psi with the objective of finding stable conformations (https://Ramachandran-plot.html).

Results and Discussion

5.6 Ramachandran plot:

DNMT1:

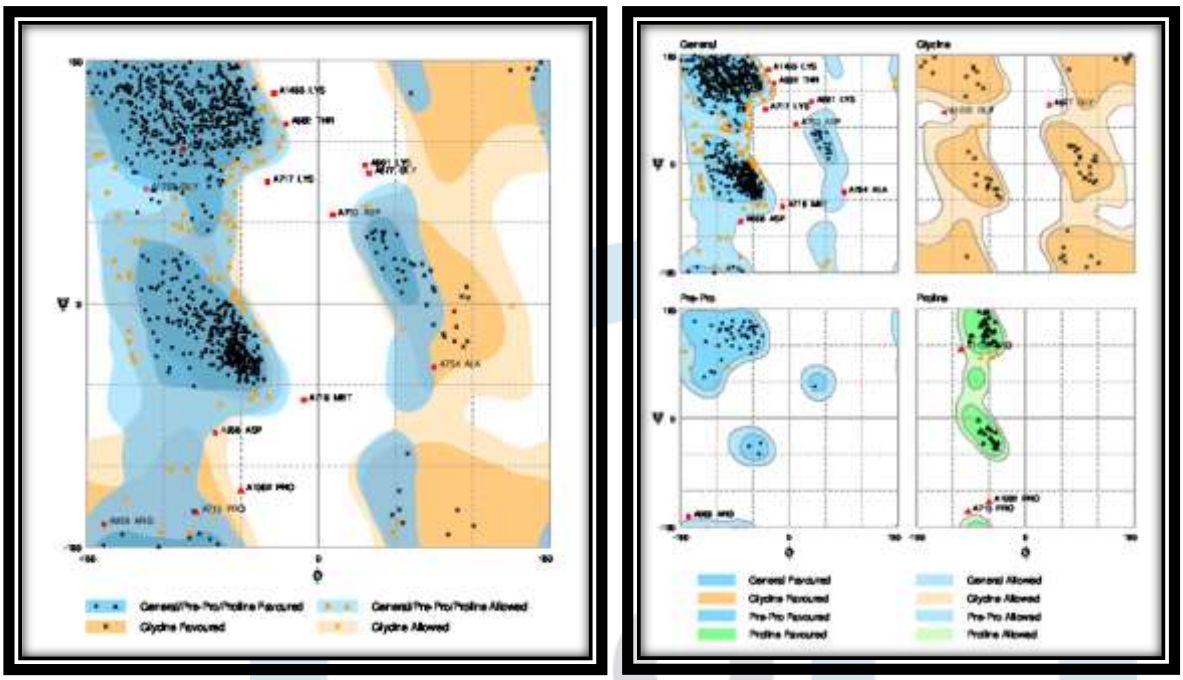


Figure 24: Ramachandran plot of DNMT1 (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

```
Residue [A1130 :PRO] (-105.83, 114.46) in Outlier region
Residue [A1223 :GLY] (-133.80,  84.53) in Outlier region
Residue [A1483 :LYS] ( -34.28, 155.66) in Outlier region
Number of residues in favoured region    (~98.0% expected)  :  869 ( 90.8%)
Number of residues in allowed region     ( ~2.0% expected)  :   74 (  7.7%)
Number of residues in outlier region                        :   14 (  1.5%)
```

PTGDS:

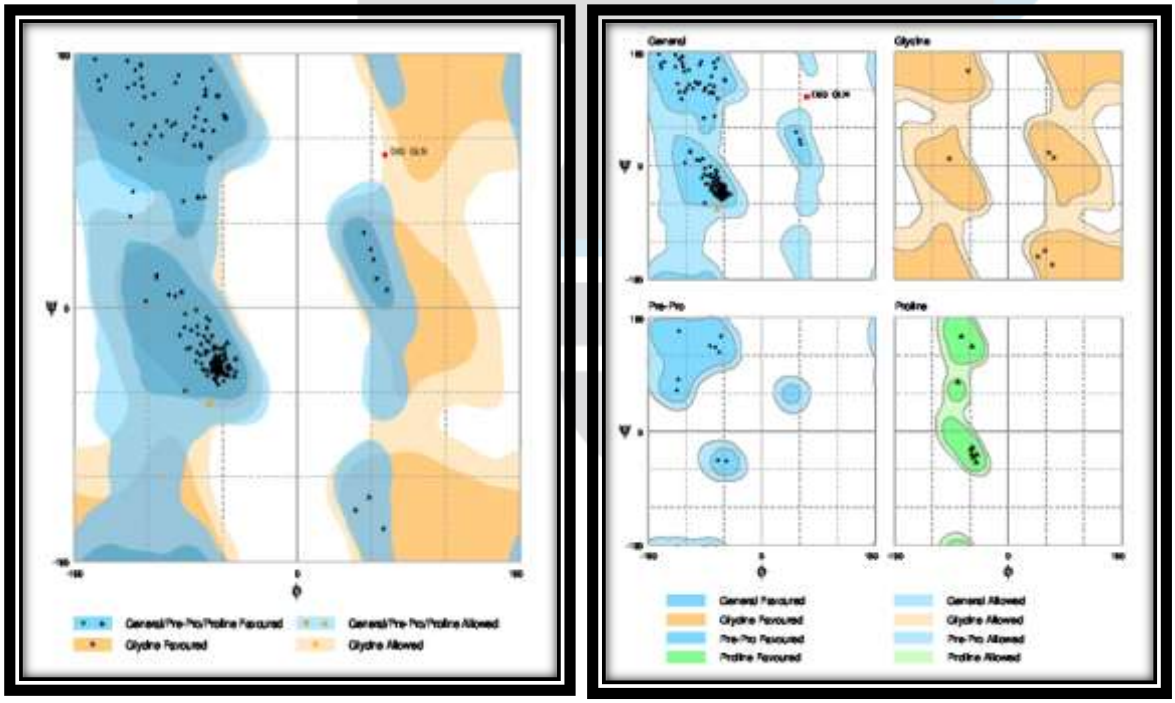
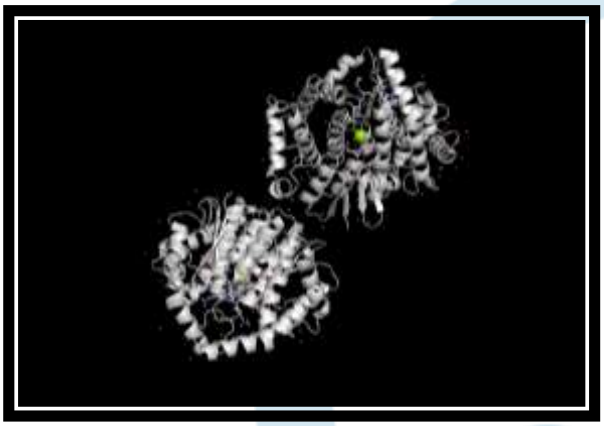
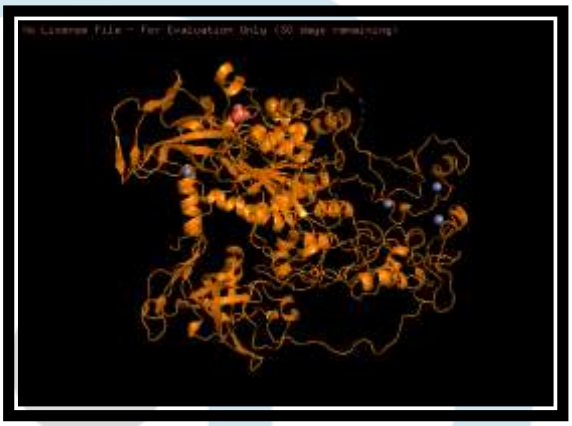


Figure 25: Ramachandran plot of PTGDS (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

```
Residue [D  12 :ARG] ( -70.57, -68.75) in Allowed region
Residue [D  63 :GLN] (  70.96, 108.35) in Outlier region
Number of residues in favoured region    (~98.0% expected)  :  194 ( 99.0%)
Number of residues in allowed region     ( ~2.0% expected)  :    1 (  0.5%)
Number of residues in outlier region                        :    1 (  0.5%)
```

Rampage used to design a Ramachandran plot through which protein structure was validate based on the φ (phi), ψ (psi) and ω (omega) angles. Ramachandran plot, Homology modeling, docking, was used for predicting the 3D model of the protein and (Protein Structure Analysis) to determine the stereo chemical quality. Molecular docking studies binding analysis, receptor protein was taken for the study as it is considered being a potential target for treatment of relies on RC plot, which determines the quality of the, compounds were selected for docking study. The validated model structures of Protein using "Ramachandran plot" this validation determines the quality of the protein structure, good quality in turn reflects in efficient and accurate docking results. On the basis of RC plot in docking minimum 88% of favorable region was required. Protein DNMT1 and PTGDS favored region was 98%, but it's not directly related to molecular docking.





PTGDS (5AIS) **DNMT1 (3SWR)**

Similarly PTGDS protein structure taken from the PDB data bank where all the structural information about the protein is available. With different PTGDS (PDB 5AIS) resolution 1.85 Å is taken and processed in the Chimera software tools.

DNMT1 protein structure taken from the PDB data bank where all the structural information about the protein is available. With different DNMT1 (PDB 3SWR) resolution 2.49 Å is taken and processed in the Chimera software tools.

Autodock Vina:

The first step was to prepare the ligand and receptor coordinate files which included the information needed by AutoGrid and AutoDock. These coordinate files are created in an AutoDock-specific coordinate file format, termed PDBQT, which includes: i) Polar hydrogen atoms; ii) Partial charges; iii) Atom types; iv) Information on the articulation of flexible molecules. The first two steps may be performed using the tools in the Edit menu of AutoDock Tools, or with other molecular modeling programs: i) Hydrogen atoms to the molecule was added. ii) Partial charges were added. Then, the molecule was read into the AutoDock Tools using the Ligand (for the ligand) or Grid (for the receptor) menus, and create the PDBQT file: iii) Deleted non-polar hydrogens and merge their charges with the carbon atoms. iv) Assigned atom types, defining hydrogen bond acceptors and donors and aromatic and aliphatic carbon atoms). AutoDock requires pre-calculated *grid maps*, one for each atom type present in the ligand being docked. This helps to make the docking calculations fast.

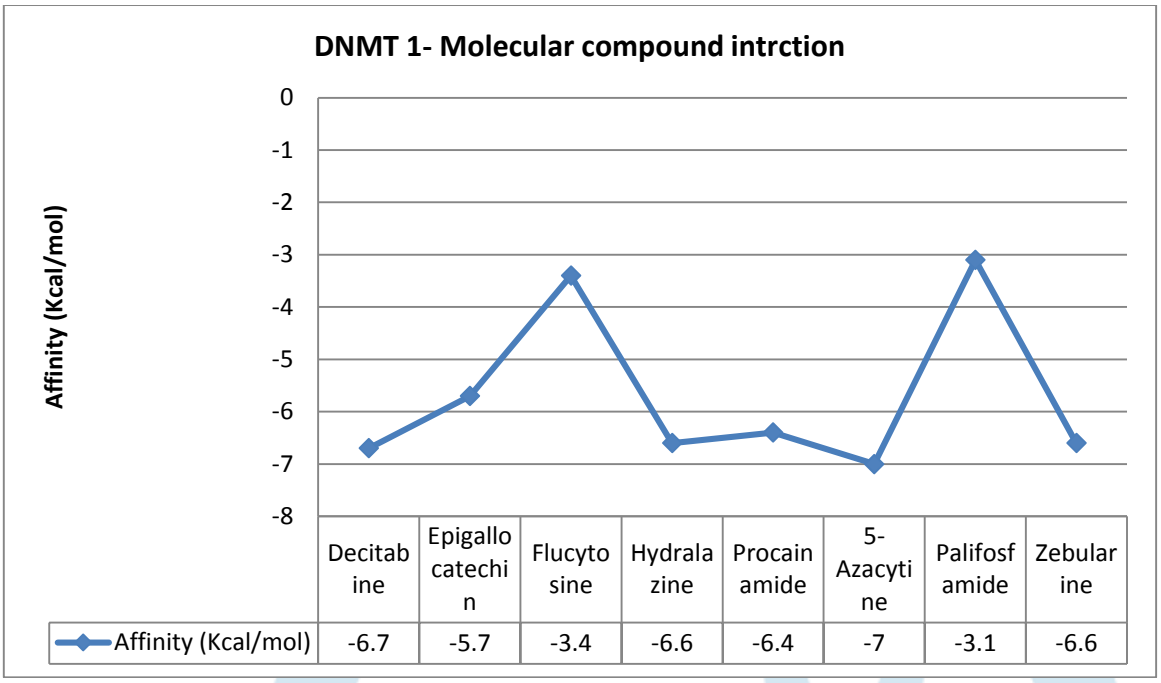


Chart1: Results of the different docking interaction of DNMT1with molecular compounds.

Autodock shows 9th conformant result 1st and 2nd values are considered best in case of standard result, if not found in 1st conformant then other conformant was selected, other 9th conformant just a references.

1st conformant of docking DNMT1-decitabine, DNMT1-Epigallocatechin, DNMT1-Flucytosine, DNMT1-Hydralazine, DNMT1-Procainamide, DNMT1-5-azacytine, DNMT1-Palifosfamide and DNMT1-Zebularine shows that different binding affinity and RMSD values binding affinity was -6.7, -5.7, -3.4, -6.6, -6.4, -7.0, -3.1 and -6.6 respectively. Docking complex DNMT1-Flucytosine and DNMT1-Palifosfamide have lowest binding affinity on the basis of this result interpret binding affinity of DNMT1 protein; DNMT1-Decitabine, DNMT1-5-azacytine and DNMT1-Procainamide highly regulate the DNMT1 protein in the process of DNA methylation/de-methylation.

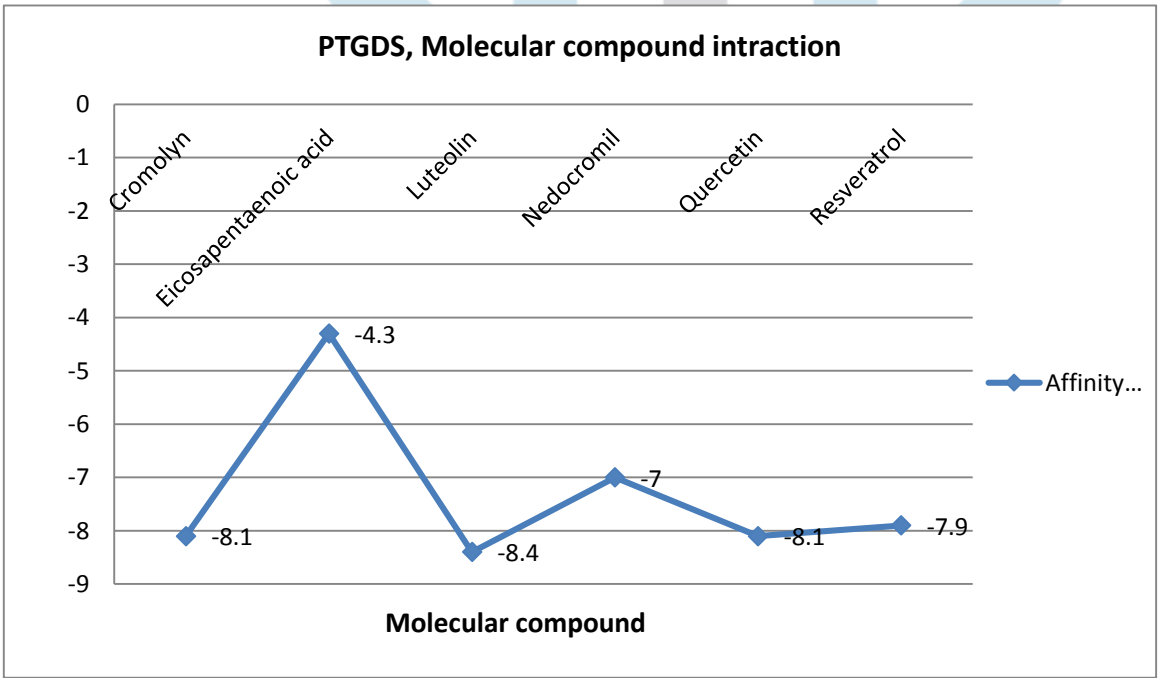


Chart2: Results of the different docking interaction of PTGDS with molecular compounds.

Autodock shows 9th conformant result 1st and 2nd values are considered best in case of standard result, if not found in 1st conformant then other conformant was selected, other 9th conformant just a references.

1st conformant of docking PTGDS-Cromolyn, PTGDS-Eicosapentaenoic acid, PTGDS-Luteolin, PTGDS-Nedocromil, PTGDS-

Quercetin and PTGDS-Resveratrol shows that different binding affinity and RMSD values binding affinity was -8.1, -4.3, -8.4, -7.0, -8.1 and -7.9 respectively. Docking complex PTGDS-Eicosapentaenoic acid have lowest binding affinity on the basis of this result interpret binding affinity of PTGDS protein, PTGDS-Cromolyn, PTGDS-Luteolin, PTGDS-Nedocromil, PTGDS-Quercetin and PTGDS-Resveratrol highly regulate the PTGDS protein.

5.9 Discussion:

Among eight molecular compounds, we performed interaction analysis with DNMT1, significant affinity values were -7.0, -6.7, -6.6 and -6.6. These values are associated with the molecular compounds 5-azacytine, Decitabine, Zebularine and Hydralazine respectively. In the previous study it was reported that Azacytidinecytidine and Decitabine in human acute myeloid leukemia (AML) cell lines responses to the cell viability, protein synthesis, DNMT1 depletion, hypomethylation of DNA, induction of DNA damage, cell cycle, induction of apoptosis, and gene expression. Both Azacytidinecytidine and Decitabine regulated molecular end points related to drug incorporation into DNA, including DNMT1 depletion, DNA hypomethylation, and induction of the DNA damage marker phospho-H2AX [20]. In this study we show the interaction of both Azacytidinecytidine and Decitabine with DNMT1.

The most extensively studied nucleosides with known DNA methylation inhibitory activity, 5-Azacytidinecytidine and 5-Azacytidine-2'-deoxycytidine; exhibit myelo suppression even when used at low doses has spurred the search for less toxic alternatives. Among non-nucleoside demethylating agents, hydralazine is a good candidate for clinical development mainly due to the fact that its extensive use for decades for cardiovascular conditions has demonstrated its tolerability and safety. Existing data show that this agent is able to demethylate and re-activate tumor suppressor gene expression in patients with cancer. In this study, we observed that zebularine decreased the level of DNMT1, DNMT3a, and DNMT3b in HepG2 cells. These results were similar to the reports that DNMT inhibitor induces the depletion of DNMT1, 3a, or 3b protein in human bladder, breast, and cervical cancer cells. Because tight covalent complexes of zebularine and DNMT could lead to compositional change in DNMT protein, it is plausible that DNMTs can be degraded via an ubiquitination system, consequently being observed in the reduction of its expression [21].

Since the molecular compounds regulates the LHX2 gene by regulating DNMT1, DNA methyltransferase enzymes which will not add the methyl group to the promoter region and our status was hypermethylation. Thus our methyl pool will be reduced and up regulation of the expression of the LHX2 gene will take place which is necessary for the development of the anagen phase of the hair growth.

As per the study conducted among the six molecular compounds, docking interaction with PTGDS protein significant values were -8.1, -8.4, -7.0, -8.1 and -7.9. These values are associated with the molecular compounds Cromolyn, Luteolin, Nedocromil, Quercetin and Resveratrol respectively. Que and cromolyn (100 mM) can effectively inhibit secretion of histamine and PGD2. Cord derived Human Mast cells activated by IgE-FcRe1 cross linking, released histamine and prostaglandin (PG) D2, which were inhibited (30–65%) by 5 min pre-treatment with cromoglycate (10 nM) or nedocromil (10 nM), as well as dexamethasone (2 nM) and human recombinant Anx-A1 Null mice(1–10 nM) (Yazid *et al.*,2013). Resveratrol at low concentrations (≤ 10 μM) inhibited PGD2 biosynthesis but not degranulation [22].

PGD2 inhibits hair growth in explanted human hair follicles and when applied topically to mice. Hair growth inhibition requires the PGD2 receptor G [23]. Prostaglandin D2 synthase (PTGDS) is elevated at the mRNA and protein levels in bald scalp compared to haired scalp of men with AGA. The product of PTGDS enzyme activity, prostaglandin D2 (PGD2), is similarly elevated in bald scalp. During normal follicle cycling in mice, *PTGDS* and PGD2 levels increase immediately preceding the regression phase, suggesting an inhibitory effect on hair growth (Garza *et al.*,2012). Since the molecular compounds Cromolyn, Luteolin, Nedocromil, Quercetin and Resveratrol regulates PTGDS protein that inhibits the PTGDS to convert from PGH2 to PGD2 which will not result in the hair loss. As per the study conducted 5-azacytine-DNMT1interaction observed with best binding affinity value -7.0 and Luteolin-PTGDS interaction also observed with best binding affinity value -8.4. Both these ligands needs to be further validated for the *in-vitro* work.

Conclusions

The hair follicle is one of the most complex mini organs of the human body, productive protein fiber factory, which acts as a sensory organ and functions as of psychosocial communication, excretion, and protection, undergoes cyclic transformations between phases of growth (anagen), apoptosis-driven regression (catagen), and relative quiescence (telogen) [1]. Hair loss, as well as unwanted hair growth (hirsutism, hypertrichosis), is a widespread problem. According to one calculation, androgenetic alopecia on its own eventually affects approximately 50% of the world's adult population [3].

DNA methylation refers to the addition of a methyl group (_CH3) covalently to the base cytosine (C) in the dinucleotide 5´-CpG-3´ (methylated cytosine residues are sometimes referred to as the fifth nucleotide). The term CpG refers to the base cytosine (C) linked by a phosphate bond to the base guanine (G) in the DNA nucleotide sequence. Most CpG dinucleotides in the human genome are methylated. 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. Through computational approach we can identify the molecular compounds that regulate the gene which could be utilized for the future drug therapy.

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.
As per the study conducted LHX2 gene is expressed low in the hair follicle, epigenetic modification is done to DNMT1 that unregulated LHX2 gene expression promoting hair growth. In the study where the Prostaglandins D2 synthase helps to convert PGH_2 to PGD_2 that leads to the hair loss. By molecular modification to the Prostaglandin D2 synthase we could inhibit the conversion and reduce the hair fall.
In accordance with previous experimental reports, computational results suggest that DNMT1 can bind to different substrates with comparable binding affinities and the hydrogen abstraction step in the catalytic cycle acts as the rate-limiting step, this bioinformatics analyses the interaction pattern of molecular compound with DNMT1 and PTGDS protein as a predictive approach for Androgentic alopecia.

DNMT1 and PTGDS molecule are available in PDB. Crystal structure of both human proteins was generated with the help of PDB databases. It is a novel work in the field of methylation study. Downloaded crystal structure of Proteins was docked with different molecular compounds using Auto Dock Vina bioinformatics tool. Among eight molecular compounds, we performed interaction analysis with DNMT1, significant affinity values were -7.0, -6.7, -6.6 and -6.6. These values are associated with the molecular compounds 5-azacytine, Decitabine, Zebularine and Hydralazine respectively. In the previous study it was reported that AZACYTIDINE and DECITABINE in human acute myeloid leukemia (AML) cell lines responses to the cell viability, protein synthesis, DNMT1 depletion, hypomethylation of DNA, induction of DNA damage, cell cycle, induction of apoptosis, and gene expression. Both AZACYTIDINE and DECITABINE regulated molecular end points related to drug incorporation into DNA, including DNMT1 depletion, DNA hypomethylation, and induction of the DNA damage marker phospho-H2AX [20]. In this study we show the interaction of both AZACYTIDINE and DECITABINE with DNMT1.

The most extensively studied nucleosides with known DNA methylation inhibitory activity, 5-Azacytidinecytidine and 5-Azacytidine-2'-deoxycytidine,exhibit myelo suppression even when used at low doses has spurred the search for less toxic alternatives. Among non-nucleoside demethylating agents, hydralazine is a good candidate for clinical development mainly due to the fact that its extensive use for decades for cardiovascular conditions has demonstrated its tolerability and safety. Existing data show that this agent is able to demethylate and re-activate tumor suppressor gene expression in patients with cancer. In this study, we observed that zebularine decreased the level of DNMT1, DNMT3a, and DNMT3b in HepG2 cells. These results were similar to the reports that DNMT inhibitor induces the depletion of DNMT1, 3a, or 3b protein in human bladder, breast, and cervical cancer cells. Because tight covalent complexes of zebularine and DNMT could lead to compositional change in DNMT protein, it is plausible that DNMTs can be degraded via an ubiquitination system, consequently being observed in the reduction of its expression [21].
Since the molecular compounds regulates the LHX2 gene by regulating DNMT1, DNA methyltransferase enzymes which will not add the methyl group to the promoter region and our status was hypermethylation. Thus our methyl pool will be reduced and up regulation of the expression of the LHX2 gene will take place which is necessary for the development of the anagen phase of the hair growth.

As per the study conducted among the six molecular compounds, docking interaction with PTGDS protein significant values were -8.1, -8.4, -7.0, -8.1 and -7.9. These values are associated with the molecular compounds Cromolyn, Luteolin, Nedocromil, Quercetin and Resveratrol respectively. Que and cromolyn (100 mM) can effectively inhibit secretion of histamine and PGD2. Cord derived Human Mast cells activated by IgE-FcRe1 cross linking, released histamine and prostaglandin (PG) D2, which were inhibited (30–65%) by 5 min pre-treatment with cromoglycate (10 nM) or nedocromil (10 nM), as well as dexamethasone (2 nM) and human recombinant Anx-A1 Null mice(1–10 nM) (Yazid *et al.*,2013). Resveratrol at low concentrations (≤ 10 μM) inhibited PGD2 biosynthesis but not degranulation [22].
PGD2 inhibits hair growth in explanted human hair follicles and when applied topically to mice. Hair growth inhibition requires the PGD2 receptor G[23]. Prostaglandin D2 synthase (PTGDS) is elevated at the mRNA and protein levels in bald scalp compared to haired scalp of men with AGA. The product of PTGDS enzyme activity, prostaglandin D2 (PGD2), is similarly elevated in bald scalp. During normal follicle cycling in mice, *PTGDS* and PGD2 levels increase immediately preceding the regression phase, suggesting an inhibitory effect on hair growth [23]. Since the molecular compounds Cromolyn, Luteolin, Nedocromil, Quercetin and Resveratrol regulates PTGDS protein that inhibits the PTGDS to convert from PGH2 to PGD2 which will not result in the hair loss.

Future Prospects

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 [10]. Interaction between genetic and environmental factors in disease development most likely involves epigenetic modifications. The study so far suggested that epigenetics mechanism (DNA methylation/de-methylation) also play a key role in the regulation of the LHX2 gene. DNA methylation protein DNMT1 and PTGDS protein both are regulated so as to effect the hair growth. This study investigate the substrate binding and catalytic mechanisms of DNMT1 and PTGDS on different substrates through computational approaches based on the result, permitting to dry lab results, it was concluded that significant affinity values were -7.0, -6.7, -6.6 and -6.6. These values are associated with the molecular compounds 5-azacytine, Decitabine, Zebularine and Hydralazine respectively is a best molecular compound. Docking interaction with PTGDS protein significant values were -8.1, -

8.4, -7.0, -8.1 and -7.9. These values are associated with the molecular compounds Cromolyn, Luteolin, Nedocromil, Quercetin and Resveratrol respectively.

- Computational results show that DNMT1-5-Azacytidineacytine is the best interaction which might be control LHX2 level.

- PTGDS-Luteolin is the best interaction which may down regulate the PTGDS protein and helps not to convert PGH2 to PGD2 that leads to hair loss.

- Therefore, this molecular compound should be validated in *in vitro/ex vivo* and *in vivo*

this might help in study further mechanism of hair growth.

References

[1] Dry FW (1926). The coat of the mouse (mus musculus). J Genet 16:187-340
[2] Paus R, Foitzik K (2004). In search of the "hair cycle clock": A guided tour. Differentiation 72:489-511
[3] Paus R, Cotsarelis G (1999). The biology of hair follicles. N Engl J Med 341:491-497
[4] Whiting DA (2001). Possible mechanisms of miniaturization during androgenetic alopecia or pattern hair loss. J Am Acad Dermatol; 45:S81-6.
[5] Hamilton JB (1951). Patterned hair loss in man: Types and incidence. Ann NY Acad Sci; 53:708-28.
[6] Norwood OT (1975). Male pattern baldness: Classification and incidence. South Med HJ; 68:1359-65.
[7] Norwood OT, Lehr B (2000). Female androgenetic alopecia: A separate entity. Dermatol Surg; 26:679-82.
[8] Bergfeld WF(1995). Androgenetic alopecia: An autosomal dominant disorder. Amer J Med; 98:95S-8S.
[9] 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.
[10] Imperato-McGinley J (2002). 5alpha-reductase-2 deficiency and complete androgen insensitivity: Lessons from nature. Adv Exp Med Biol; 511:121-31.
[11] Xu X, Mannik J, Kudryavtseva E, Lin KK, Flanagan LA, Spencer J, Soto A, Wang N, Lu Z, Yu Z, Monuki ES, Andersen B (2007) Co-factors of LIM domains (Clims/Ldb/Nli) regulate corneal homeostasis and maintenance of hair follicle stem cells. Dev Biol. 312:484-500.
[12] 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.
[13] Kolterud Å, Alenius M, Carlsson L, Bohm S (2004a) The Lim homeobox gene Lhx2 is required for olfactory sensory neuron identity. Development. 131:5319-26.
[14] Curtiss J, Heilig JS (1998) DeLIMiting development. Bioessays. 20:58-69.
[15] D. L. Simmons, R. M. Botting, and T. Hla (2004). "Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition," *Pharmacological Reviews*, vol. 56, no. 3, pp. 387–437.
[16] Egger, G., Liang, G., Aparicio, A., & Jones, P. A. (2004). Epigenetic in human disease and prospects for epigenetic therapy. *Nature*, *429*(6990), 457-463.
[17] Jones PA, Baylin SB (2002). The fundamental role of epigenetic events in cancer. *Nat Rev Genet* ;3:415–28.
[18] Bird, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature*, *321* (6067), 209-213.
[19] Hedges DJ, BatzerMA (2005). From the margins of the genome: mobile elements shape primate evolution. *Bioessays* ;27:785–94. doi:10.1002/bies.20268
[20] 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.
[21] Nakamura, K., Aizawa, K., Nakabayashi, K., Kato, N., Yamauchi, J., Hata, K., & Tanoue, A. (2013). DNA Methyltransferase Inhibitor Zebularine Inhibits Human Hepatic Carcinoma Cells Proliferation and Induces Apoptosis. *PLoS ONE*, *8*(1), e54036.
[22] Shirley, D., McHale, C., & Gomez, G. (2016). Resveratrol preferentially inhibits IgE-dependent PGD_2 biosynthesis but enhances TNF production from human skin mast cells. *Biochimica et Biophysica Acta*, *1860*(4), 678–685.
[23] 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.