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COMPARATIVE HOMOLOGY MODELING AND DOCKING OF LAMIN A MOLECULE AND ITS INCIDENCE IN PROGERIA

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ABSTRACT

Progeria is a rare and fatal autosomal recessive disorder affecting children, usually newborn babies causing accelerated ageing. It is caused due to mutation in a gene called LMNA which leads to instability of nucleus thereby causing rapid ageing. This means that an individual carrying a mutation in a single gene does not show any sort of symptoms as it is recessive in nature. Moreover 90% of the children have mutation on the gene that encodes protein Lamin A. The gene involved is LMNA which codes this protein and due to one point mutation (mostly) defective fibrous protein is formed leading to unstable nucleus thereby causing premature ageing. Researches are being done as no cure is available till date but some drugs are used in order to subsidies the effects caused. This work deals with establishing a drug or to manipulate analogues of approved drug and to check its effectiveness and binding activity over the receptor protein through docking. For this purpose number of ligands and their analogues were prepared and docked on the receptor lamin A whose structure was obtained through a request from EBI-Swiss model with PDB id as q369. Out of all the drug molecules docked one specific analogue Indinavir showed great results with energy of -94.2 Kcal/mol and good binding capacity, So this drug was manipulated and modified and again docked where better results were obtained with a binding energy of -105.5 Kcal/mol. Using this modified analogue might further aid in the treatment of this disease and can be subjected to clinical validations.

KEYWORDS : ***Progeria, Lamin A, homology modeling and docking***

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INTRODUCTION

Progeria is very rare yet fatal autosomal recessive disorder affecting children, usually newborn babies causing accelerated ageing. It is often quoted as, progressively terminal condition in which the normal ageing characteristics are copied at a much faster rate, of upto eight times approximately. Its various symptoms are similar to normal ageing[1]. Progeria was for the first time described simultaneously but independently by Dr. Jonathan Hutchinson in 1886 and by Dr. Hastings Gilford in 1897. Progeria has different forms, depending upon the type and place of mutation on the gene, but the most known one is the classic type also named after the scientists who discovered it, as Hutchinson Gilford Progeria syndrome [HGPS][2]. It is an autosomal recessive disease and 90% of the children have mutation on the gene that encodes protein Lamin A. The gene involved is LMNA which codes this protein and due to point mutation (mostly) defective fibrous protein is formed leading to unstable nucleus there by causing premature ageing. Under normal conditions, gene LMNA encodes protein Prelamin A which has a Farnesyl group attached to it, once this group is cleaved Lamin A protein is formed and it does not remain attached to the nucleus rim thereby maintaining the normal stable condition of the nucleus. The function of farnesyl transferase is to allow anchoring of Prelamin A to the nucleus and later its subsequent removal. Normal Lamin A is a fibrous protein, a scaffolding component[2] and a major structural protein of nuclear lamina (along with Lamin-C). This Nuclear Lamina participates in organizing the chromatin material and also supports the nuclear envelope[3]. However, in Progeria the LMNA gene encodes the prelamin A protein and at later stage the farnesyl group does not get detach as it was supposed to leading to an abnormal form of Prelamin A being synthesized called as 'Progerin'[4,5,6] which remain anchored to the nucleus leading to its abnormal unstable shape. This happens in most of the cases as a result of the point mutation at 1824 position of the LMNA Gene where by replacing a cytosine by a thymine creating a truncated form Progerin whose further processing is disrupted leading to its accumulation[7,8] into the nucleus thereby causing HGPS.

Homology modeling of protein, is about constructing an atomic-resolution model of the "target" protein using its respective amino acid sequence and an experimental three-dimensional structure of a related homologous protein often referred as the "template". In other words it involves taking a known sequence with an unknown structure and mapping it against a known structure of one or several similar proteins[9,10]. Homology modeling is an important part in *in-silico* drug designing. *In-silico* methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding/active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities and further optimize the molecules to improve binding characteristics. [11,12]. The present study was oriented to develop a homologous structure of the target protein (Lamin A) and to try developing a better drug for the same through docking studies as till date no cure is available for this disease.

MATERIALS AND METHODS

The sequence of Homo sapiens lamin A/C (LMNA), transcript variant 4, mRNA with accession ID NM_001257374.1 submitted (6-May-2012) by Jimenez-Escrig A, Gobernado I, Garcia-Villanueva M and Sanchez-Herranz A. was selected for the *in-silico* analysis and it was retrieved from NCBI.

(i) Structure Analysis

The primary analysis of the sequence was done using ProtParam and Sosui followed by the secondary analysis by GOR-IV. Since the tertiary structure was not available in PDB, it was generated by using Swiss Model (Automated mode) and a PDB-ID was assigned in EBI (personal workspace). The generated structure (Entity-1) is validated using ProCheck by generating a Ramachandran Plot; a tool of PDBsum.

(ii) Structure Visualisation and Energy Minimisation

For structure visualization and energy minimization Prime was used. The structure was imported in Maestro which is Schrödinger's powerful, unified, multi-platform graphical user interface (GUI). Based on this structure, a structure was built with three template structures using Maestro and was saved as PDB structure (Entity-2) which had comparatively lower energy and required less energy minimization iterations.

(iii) Active Site Prediction

Active site prediction of the generated PDB structure (Entity-2) was carried out by Q-Site Finder. Three drugs related to the treatment of Progeria; Farnesyltransferase Inhibitor, Pravastatin and Zoledronic acid were selected and their analogous structures were searched from the Drug Bank. From a list of drugs generated, drugs with approved structure were selected.

(iv)Identification of Particular Receptor and Ligand

Many analogues of drugs were taken and there structures were drawn in 2D-Sketcher tool of Schrodinger and were prepared as ligands by the LigPrep application.

Docking of the PDB structure (Receptor) and the ligand (drug) was done using the GLIDE tool of Schrodinger. The drug Indinavir with maximum negative energy showed good binding energy. The structure of this drug was further modified and all the analogues were also docked against the receptor [Lamin A] and the binding energies obtained showed significant improvement. Their Interaction image was also obtained for better understanding.

RESULTS AND DISCUSSION

Progeria a very fatal and rare segmental autosomal recessive disorder causing restrictive dermopathy and rapid ageing due to De novo mutation in a gene LMNA, hence is also called as laminopathies[12,13]. So in order to develop effective drug, as no proper cure is present till date, we started from scratch with the gene. Literature survey indicated that mutation in Gene LMNA is responsible for this disease so nucleotide sequence with Accession Id - NM_001257374.1 was retrieved from NCBI and its Fasta sequence was used for the *in-silico* analysis[14].

For primary analysis ProtParam was used through which it was deduced that the protein is hydrophilic and interactive in nature as GRAVY score came to be -0.792 and the instability index computed was to be 54.80 classifying the protein as unstable, reason for which was later deduced as absence of end terminal caps and a missing gap. For knowing the nature of protein sequence that whether it is transmembrane or soluble SOSUI was used indicating soluble nature of peptide [12]. As a part of structural analysis GOR IV was used for secondary prediction of alpha helix and beta sheets, resulting into alpha helix holding 44.26% and random coil 41.09% of total amino acids. As it was observed that the peptide string is interactive – visualization tools were used but for this PDB structure was needed which was not available so a request was sent to EBI- Swiss Model for its generation. Structure was generated with PDB ID as 'q369' using Template as 1IVT A with sequence identity of 98.36%.

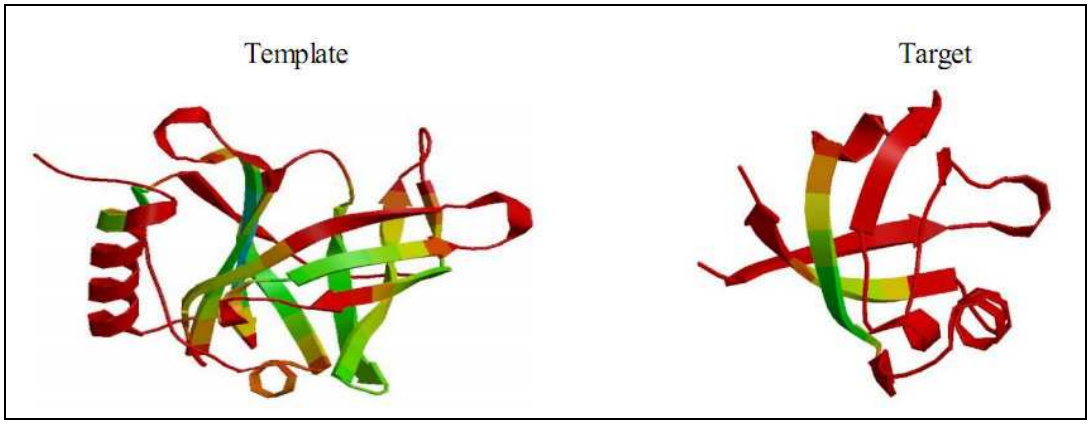

Figure1
Comparisons of the template structure (1IVT A) and the target protein with PDB ID generated as q369

The generated structure had a Q Mean Score of .66 stating that the structure is 66% reliable and Z score of -1.51 explaining the absolute quality to be a little lower but accepted after validating from PDBsum - ProCheck. Ramachandran Plot was created and G factor was calculated as 0.39 showing a minute unusualness in sequence and outliers were identified; one in third quadrant [Valine 432] and three in fourth quadrant [Glysine 368,333, Threonine 413] , later using Maestro for Schrodinger these were tried to be removed via Prime refinement.

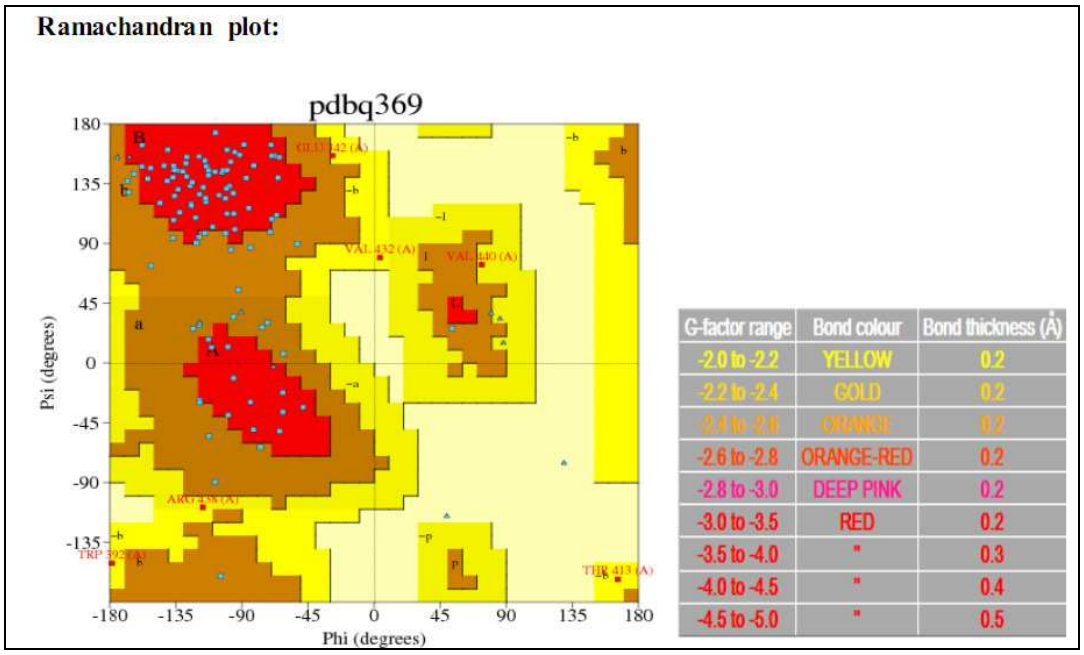

Figure2
Structure validation by generating Ramachandran Plot using ProCheck

Further for homology modeling and docking - Maestro, a GUI for Schrodinger was used where the receptor [Lamin A] was imported, energy analysis was done followed by energy minimization of upto - 1654.42 Kcal/mol which was a big value hence structure prediction was also computed as the PDB structure was Auto generated and showed great amount of energy minimization possible. Hence for this purpose comparative modeling was done and model was rebuilt in Maestro based on the results after running BLAST through which structures showing maximum similarity were identified with PDB ID as 1IVT, 1UFG ,IIFR and based on these the structure of receptor was rebuild. The receptor now had both terminus capping and the gap was filled too therefore not much of energy minimization was required to be done (-1.640kcal/mol).

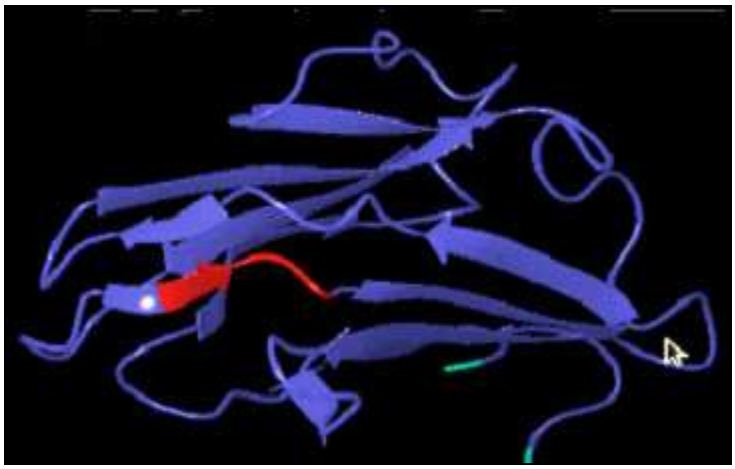

Figure3
Homology model rebuilt in Maestro using 1IVT, 1UFG and IIFR as templates

The structures of Drugs commonly used in the treatment of Progeria were searched namely Farnesyl transferase, Zolendronic acid and Pravastatin and their structures taken from DrugBank along with structures of approved analogous drugs. These drugs were drawn in Maestro using 2D-sketcher and all possible conformations were built to dock on the Receptor [Lamin A] using Glide.

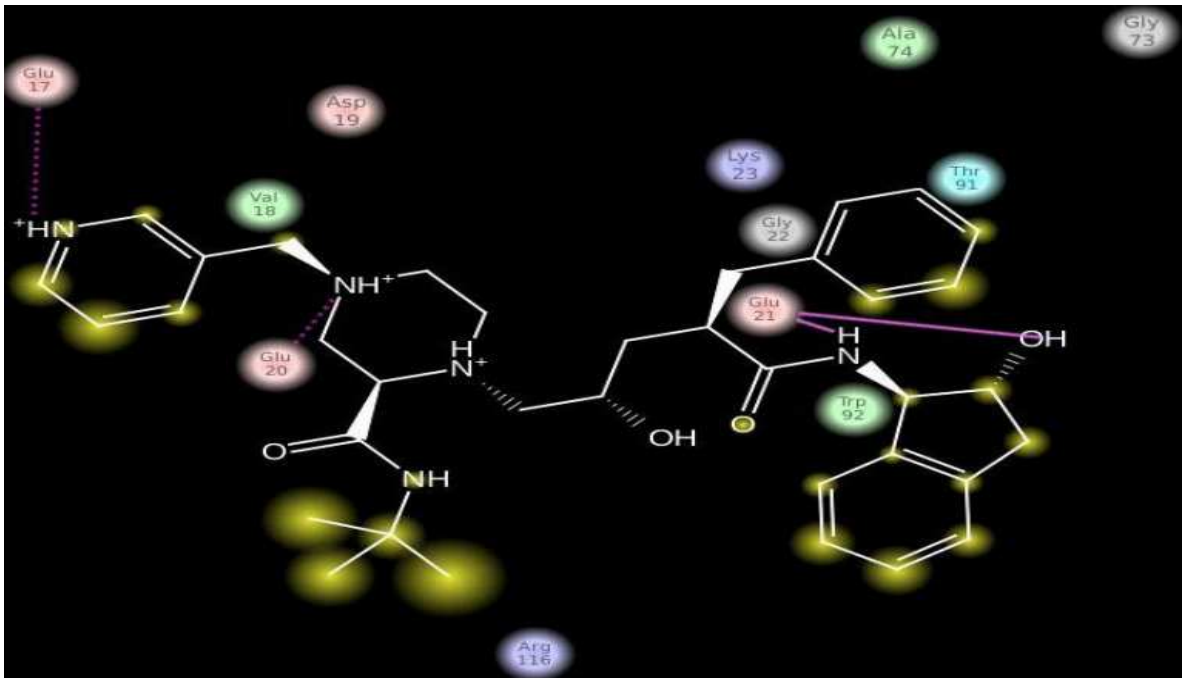

Figure 4
Binding energy of drug Indinavir with receptor (Lamin A) [-94.7Kcal/mol]

Interesting results were obtained out of all, the drug Indinavir showed better binding capacity with the receptor with 94.70 Kcal/mol. Hence, this drug was further modified and manipulated to develop better structures showcasing more binding capacity as compared to Indinavir.

Table 1
Interaction of Indinavir-Analogues with the Receptor

Structure Name	Binding Energy (in Kcal/mol)
Ind_Ph_NH-CH3	-86.32
Ind_ana_F	-88.8
Ind_ana_Cl	-88.8
Ind_NH-CH3	-94.2
Ind_ana_NH^+	-87.2
Ind_ana_Ph_OH	-101.49
Ind_Ph_NH_OH_$NH2^+$	-105.5

The structure showed binding capacity of 105.5 Kcal/mol which was a significant value in all the results obtained.

CONCLUSION

Progeria a very fatal and rare segmental autosomal recessive disorder causing restrictive dermopathy and rapid ageing and is caused due to mutation in the LMNA gene. LMNA gene encodes various proteins of which Lamin A is of remarkable importance as it is responsible for the nucleus stability of a cell. Therefore this project was aimed at designing the structure of Lamin A protein and studying the binding of various drugs with this protein through docking techniques. The drug

Indinavir was shown to have best binding energy and hence this drug was further modified to improve the binding capacity. Research work can further be initiated towards validating and standardizing this result through clinical and preclinical studies.

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