Structural analysis of human nuclear membrane zinc metalloprotease mutant (E336A) in complex with synthetic CSIM peptide in progeria

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INTRODUCTION

Progeria also known as the Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare,fatal,congenital disease which involves a condition of premature aging that arises the chances of getting the abnormalities and prone to diseases just as in the case of oldage people.In context of epidemiological data one in 4-8 million births get affected by this syndrome in a ---manner with no gender differences.At present there are nearly 114 children across 39 countries got affected by this and in India there are about 66 children suffering with this syndrome.From this statistics its even considered as one of the rare genetic diseases.Children with this disease usually face the death at an age of 13 years.

Progeria condition is formed due to the mutations in the human nuclear membrane zinc metalloprotease ZMPSTE24 which leads to the improper functioning of the lamin processing causes the laminopathies.In wild type .ZMPSTE24 is the gene that codes for the protein metallopeptidase that cleaves the residues of the one of the three proteins of the Prelamin A to produce the mature lamin A as a part of the post transcriptional modifications.These mature lamin A polypeptides undergo dimerisation to form a higher order structure beneath the nuclear membrane and gives structure to the nucleus.ZMPSTE24 protease acts between the Cysteine and serine residues in the C-terminal of prelamin A followed by carboxymethylation of the farensyl cysteine in prelamin A.(Figure 1)After this the metalloprotease from the ZMPSTE24 cleaves between the tyrosine and leucine residues.Incase of Progeria there is a loss of aminoacid residues because of the mutants.So the ZMPSTE24 cannot act and do not produce the mature laminA that causes the chromatin associated with the lamin membrane to stress and leads to the upregulation of the P53 signalling activation and causes the cellular and tissue senescence. The diseased state is due to the mutation in the aminoacid sequences of the Prelamin A protein and because of that the symptoms like weakening of the muscles , poor eye sight which are the features of the old age people.The rare disease Progeria, accelerated aging, involve the proteins of Pre-lamin A of human nuclear membrane. One of the protein constituent parts is Human Nuclear Membrane Zinc Metalloprotease ZMPSTE 24.This protein is in complex with the CSIM peptide which is synthetically prepared peptide and this is similar to the insect nuclear membrane peptide

EXPERIMENTAL DESIGN

Regarding the atomic co-ordinates and other information about describing ZMPSTE24 metalloprotease protein is studied using the X-RAY Crystallography and the methods of the computational biology.A reference protein for the progeria disease is considered (PDBID:2YPT).Then the quality of the protein is checked out by using the standard values.The Rfree-Rwork values are calculated and compared to the <0.05 and the Rwork value is considered with respect to the one-tenth of the resolution.Coming to the clashscore,any crystal structure do not have the clear view of hydrogens.And from the Ramchandran outlliers defects and the possibilities in the stereochemistryof structure is done.Using the PyMol application the structure of the metalloprotease is examined.The structure has 4 protein chains, each with the same sequence alignment in the cartoon format.Structure has two macromolecules CAAX PRENYL PROTEASE 1 HOMOLOG and PRELAMIN -A/C and the ligand present in the molecule is Zinc ion.Using the tabs from the Graphics User Interface(GUI) and the sequence tabs of PyMol the structure is differentiated according to the colours gives the clear structure showing various colors for the secondary strtructures and the chains.

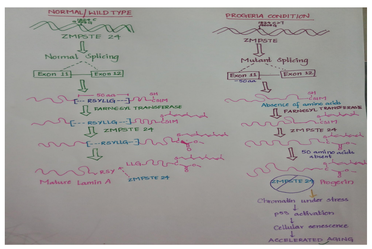

Figure 1:The difference between the wild type and the progeria condition

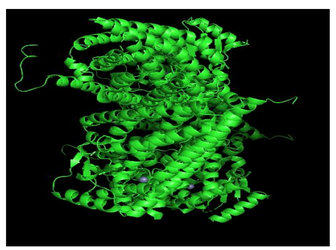

Figure 2:The structural analysis of the ZMPSTE 24 metalloprotein in association with the mutant.

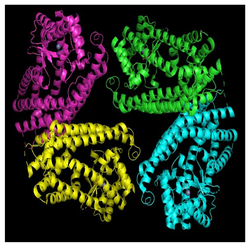

Figure3:Structure of the metalloprotease ZMPSTE24 as the 4 chains -PDBID:2YPT,PyMol

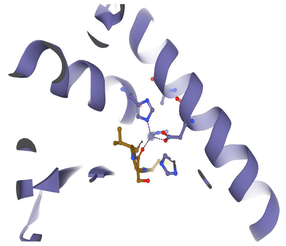

Figure 4:Structure of metalloprotease showing the hydrogen bonding of the ligand and the main chain protein. -PDBID:2YPT

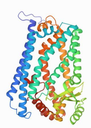

Figure 5:CAAX PRENYL PROTEASE 1 HOMOLOG

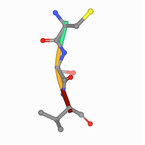

Figure6:PRELAMIN-A/C

RESULTS & DISCUSSION

The structure of theZMPSTE24 metalloprotease (PDBID:2YPT) has the Rfree-Rwork value as 0.037 which is less than the 0.05 and also the Rwork which is one-tenth of the resolution has the value of 0.18 which is approximately equal to the resolution(0.184).So the crystal structure value is good and it contains nearly 15 .alpha helices and 4 beta strands connected by the loops in the secondary structureAnd there are no water molecules present throughout the structure.But there are two hydrogen bonds present between the ligand and the protein with the help of amino acid residues.The ligand-Zinc ion is attached to the protein structure at the Glutamic acid-415 and Serine-662 residues by forming two hydrogen bonds.The bond length between the Glutamic acid residues and the ligand is of 21nm and the serine residue to that of the ligand is 2.0nm.

REFERENCES

1.Ahmed, M.S., Ikram, S., Bibi, N. *et al.* Hutchinson–Gilford Progeria Syndrome: A Premature Aging Disease. *Mol Neurobiol* 55, 4417–4427 (2018). https://doi.org/10.1007/s12035-017-0610-7

2. Sinha, Jitendra Kumar et al. "Progeria: a rare genetic premature ageing disorder." *The Indian journal of medical research* vol. 139,5 (2014): 667-74.

3. Quigley A, Dong YY, Pike AC, Dong L, Shrestha L, Berridge G, Stansfeld PJ, Sansom MS, Edwards AM, Bountra C, von Delft F, Bullock AN, Burgess-Brown NA, Carpenter EP. The structural basis of ZMPSTE24-dependent laminopathies. Science. 2013 Mar 29;339(6127):1604-7. doi: 10.1126/science.1231513. PMID: 23539603.

4.Lee, Seung-Jun & Park, Sung-Ha. (2013). Arterial Ageing. Korean circulation journal. 43. 73-79. 10.4070/kcj.2013.43.2.73

5.J. Barrowman,S. Michaelis, ZMPSTE24, an integral membrane zinc metalloprotease with a connection to progeroid disorders. *Biol. Chem.* 390, 761 (2009). doi:10.1515/BC.2009.080pmid:19453269

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