COMPARATIVE PROTEIN MODELLING OF METALLOTHIONEIN 1B PROTEIN ACTING AS A POTENTIAL CHEMOPROTECTOR AGAINST HEPATIC NEOPLASIA

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ABSTRACT: Background and objectives: Primary liver cancer also called hepatocellular carcinoma or hepatoma is the fifth most common cancer in the world with a poor prognosis. An Indian medicinal plant *Acanthus ilicifolius* shows encouraging results in preventing liver cancer cells from progressing. The aqueous leaf extract (ALE) of the plant was substantially effective in preventing hepatic DNA alterations and sister-chromatid exchanges (a type of chromosomal damage) in tumor-bearing mice. The ALE treatment was able to limit liver metallothionein 1B expression, a potential marker for cell proliferation, and lengthen the mean survival of animals to a significant extent. The findings suggest that *Acanthus ilicifolius* may be used as a potential chemoprotector against hepatic neoplasia. **Methods:** The protein ID from Swissprot data base was selected for obtaining the description and function of the protein, its domains structure, post-translational modifications etc. BLAST analysis was performed to identify template protein sequence for metallothionein protein. The comparative modelling of the Metallothionein 1Bby different algorithms like Hidden Markov Model of homology modelling and multiple threading alignments and iterative alignments methods for *Ab initio* was performed by using the programs like Swiss Model, CPH Model, Wurst and I.TASSES respectively.

Results: The obtained models were verified with structure validation programs like, PROCHEK & SAVS. **Interpretation and Conclusions:** Schrodinger was used for energy refinement of the model. Active site determination through CASTp and surface visualization by Molegro Virtual Docker suggests that this protein can act as a potential drug target.

Keywords: *Acanthus ilicifolius,* Metallothionein, *Ab initio,* Active site determination.

INTRODUCTION

Liver cancer, also called primary liver cancer, is a form of cancer that develops within the liver tissue. Normally, the liver's cells grow and divide in a regulated manner (only a specific number of cells are produced in order to keep the liver healthy and functioning properly). When this process is impaired, the liver's cells grow and divide uncontrollably and in an exaggerated manner -causing tumors to form. There are two types of tumors: benign (the term refers to a non-cancerous mass or growth which is not life threatening) and malignant (the term refers to a cancerous mass or growth which can invade and destroy the adjacent tissues and organs inside the body causing death).Hepatocellular carcinoma (HCC, also called malignant hepatoma) is a primary malignancy (cancer) of the liver. Most cases of HCC are secondary to either a viral hepatitide infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of hepatic cirrhosis) (Kumar *et al*., 2003).

An Indian medicinal plant *Acanthus ilicifolius* shows encouraging results in preventing liver cancer cells from progressing, dubbed chemoprevention. The leaves of *Acanthus ilicifolius* are used to treat rheumatism, neuralgia and poison arrow wounds. It is widely believed among mangrove dwellers that chewing the leaves will protect against snake bite. The aqueous leaf extract (ALE) of the plant was substantially effective in preventing hepatic DNA alterations and sister-chromatid exchanges (a type of chromosomal damage) in tumor-bearing mice.ALE treatment was able to limit liver metallothionein expression, a potential marker for cell proliferation, and lengthen the mean survival of animals to a significant extent. The findings suggest that *Acanthus ilicifolius* may be used as a potential chemoprotector against hepatic neoplasia. (Chakraborty *et al.,* 2007)

Metallothionein (MTs) were discovered in 1957 by Margoshes and Vallee. Due to their high metal content and unusual bioinorganic structure, they are distinguished as metalloproteinase.(Thirumoorthy et al., 2007). MT molecules contain 20 residues of cysteine, which approximately amounts to 30% of amino acid content. Large amounts of cysteine with sulphydril groups determine protein activity. Four major isoforms (MT-1 through MT-4) have been identified in mammals. Mt-1 and MT-2 have ubiquitous tissue distribution particularly in liver, pancrease, intestine, and kidneys, whereas MT-3 is found in brain. MT-4, is found in epithelial cells (Krizkova *et al.,* 2009). The human metallothionein (MT) IB gene (hMT-IB) is located in a region of human DNA containing at least four tandemly arranged MT genes. As deduced from its sequence, hMT-IB is likely to encode a functional protein. A high level of expression of the endogenous hMT-IB gene could be detected only in human hepatoma and renal carcinoma cell lines. The 5' flanking region of the hMT-IB gene was highly methylated in HeLa cells, a nonexpressing cell type, but it was not methylated in a hepatoma (expressing) cell line (Heguy *et al.,* 1986). Cheminformatics approach requires three dimensional proteins for it's *in silico* studies, as hMT-IB crystal structure is not present in protein data bank so protein modelling is performed by homology and *Ab initio* both to compare the algorithms applied in each server and software.

MATERIALS AND METHODS

In Silico: 3D structure modelling and analysis

The sequence of the Metallothionein1B protein was taken from SWISSPROT databases (ID code P07438).Structure prediction of Metallothionein 1B protein has been based on the availability of the template sequence of Metallothionein 1B protein (PDB code 4MT2). The search for sequence similarity within databases has been performed with the BLAST program. *Ab intio* protein modelling was done using wurst and I-Tasser tool. The stereo-chemical quality of the models was verified with the program PROCHECK (Laskowski *et al.,* 1993) in order to select the best model. The fasta sequence of MT 1B was submitted to SWISS MODEL SERVER (Schwede *et al.,* 2003) and the structure was obtained. The energy minimization of all the structures modeled by different homology modelling methods was done with the help of SCHRÖDINGER Impact module (Schrödinger, 2005). The structures modeled by SWISS MODEL SERVER , CPH Model (explain about cph), Wurst and I-TASSER were having the energies –2.14866, -2.14533, -1.560673, -1.22620 KJ/mole respectively. So the minimum energy was found for MT 1B modeled by SWISS MODEL and CPH.

The evaluation of the four generated models of MT 1Bwas performed by two tools - PROCHECK (command line) and SAVS (Structural Analysis and Verification Server) (Kiefer *et al.,* 2009). The secondary structure evaluation was done with the help of SPDV.

CASTp: Active site determination was done for the modeled protein to further work on its docking studies. Active site determination gives us an idea to make a grid before docking (Dundas et al, 2006).

RESULTS:

The modeled structure of MT 1B obtained by homology modelling contains alpha helices, beta sheets and loops. The homology modelling was done by SWISS MODEL (fig.1 (A)), CPH MODEL (fig.1 (B)), I-Tasser (fig.1(C)), and finally Wurst (fig.1 (D)).

The structures modeled by SWISS MODEL(fig.2 (a)), CPH MODEL(fig.2 (b)), Wurst (fig.2 (c)),I=Tasser (figure.2 (d)) shows the amino acids percentage in the favorable region as 80.4%, 78.4%, 93%and 88.2% respectively and amino acid percentage in disallowed region as 3.9%, 0.0%, 2.3%, 0.0% respectively. This allocates that model builded by CPH MODEL and I-Tasser builds almost similar structure, whereas out of these models Wurst shows the highest percentage of allowed region amino acids. The next comparison was done by secondary structure evaluation with the help of SPDV and the alpha helices and beta sheets were analysed as follows (Table 1).

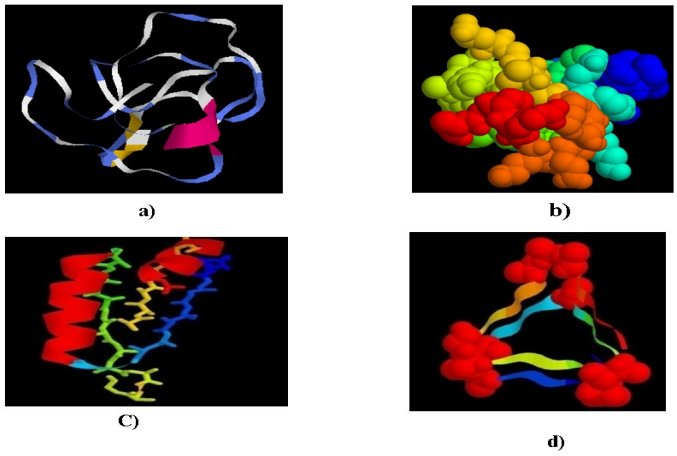

Fig. 1: Model Protein of Metallothionein 1B by comparative modelling.

CASTp: Computed Atlas of Surface Topography of Proteins gives a prediction of active site and the number of amino acid envolved in it.

The green color (figure.3)shows the active site position of the builded ptrotein starting from sixth amino acid to some interaction of 61 amino acid .The major coverage of amino acid to be in binding cavities lies in between 6-9, 20-23, 25-26, 31-36and 44-46 amino acids which are mainly four turn alpha helixes. Surface of protein was designed by Molegro Virtual Docker and cavity can be visualized in the green color (figure.4).

Table 1 Secondary structure

Secondary Structure	Swiss Model	CPH Model	Wurst	I-Tasser
HELIX	-	-	19-32 54-60	-
SHEETS	-	-	9-13 37-39 48-52	6-8 14-19 36-38 41-44 48-52 57-59
COILS	1-61	1-61	7-8 14-18 33-36 40-47 53,61	1-5 9-13 39,40 45-47 53-56 60,61

The PROCHECK results obtained for MT 1B by four different methods are as follows:

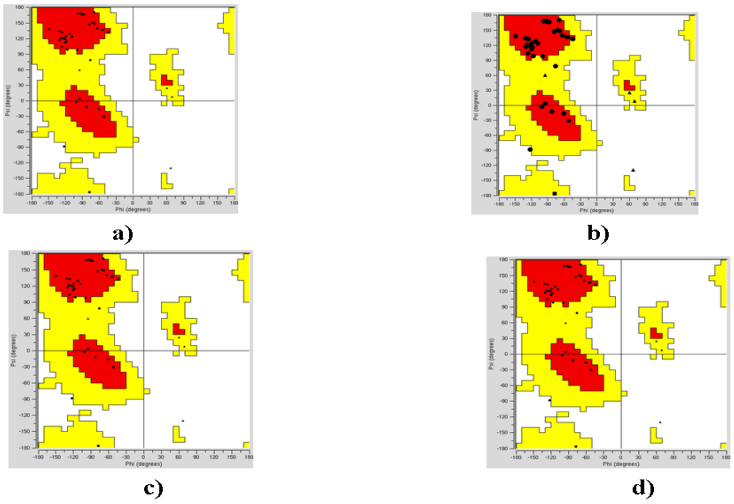

Fig.2: Ramachandran plot of the structure of Metallothionein protein obtained through various comparative modelling tools.

Fig.3: Active side information by CASTp.

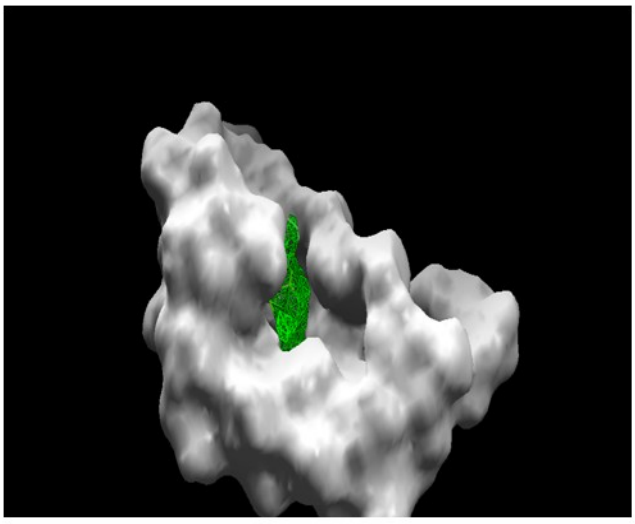

Fig.4: Surface of protein with cavity.

CONCLUSION

The alpha helices were obtained from position 24 to 449 with beta sheets in between them. A little variation was obtained between Modeler and Schrodinger structure prediction. The energy value for the both structure and Ramachandran plot was also almost similar so the best structures are modeled by Modeler and Schrödinger. Then the active site determination by CASTp and surface visualization with active site information gives us an opportunity to further work on its docking studies and its binding activities.

Acknowledgement

We take this opportunity to acknowledge the help we had received from several individuals for completing this work. We express our heartfelt gratitude to the management of Karunya University, Coimbatore for all their support.

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