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Research Article

THE BIOPHYSICAL CHARACTERISTICS AND STRUCTURAL EXPLORATION OF PROGRAMMED CELL DEATH REGULATOR B-CELL LYMPHOMA 2-ASSOCIATED X PROTEIN OF CHINESE LIVER FLUKE (*CLONORCHIS SINENSIS*)

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ABSTRACT

Objective: The balance between deaths and cellular life is regulated by B-cell lymphoma 2 (BCL-2)-associated X protein (BAX) an important pro-apoptotic components of BCL-2 family. With this initial point, the aim of this study was to determine a comparative composite based structure of BAX of Chinese liver fluke and different structural analysis.

Methods: Protein amino acid of BAX of Chinese liver fluke mined from National Centre for Biotechnology Information (http://ncbi.nlm.nih.gov). Molecular model of BAX of Chinese liver fluke protein was generated by the comparative composite modeling tool Iterative Threading ASSEmbly Refinement suite. Afterward, I-TASSER generated molecular model was subjected to further structural improvements by energy minimization step. Distribution of negatively and positively charged amino acid over molecular modeled structure, distribution of secondary structural elements, and hydrophobicity molecular surface analysis was performed with the help of bioinformatical tools.

Results: Analysis of Ramachandran plot created by PROCHECK tool is a consensus standard for validation purpose of protein structural modeling. Altogether 97.8% of the residues were detected in allowed and favored regions, which in turn validate the quality of generated protein structural model. Total negatively and positively charged residues within the BAX of Chinese liver fluke were 23 and 20, respectively. Chimera package-guided hydrophobicity molecular surface analysis illustrates that molecule specific hydrophobicity surface is exclusive to BAX protein molecule.

Conclusion: Within the scope of this scientific investigation, we have successfully utilized molecular modeling approach to suggest the first molecular three-dimensional model structure of BAX of Chinese liver fluke. The synchronous balance between cellular deaths and cellular life is keeping up by BAX, an important pro-apoptotic family member of BCL-2 family. Consequently, it would be an exciting approach to resolve its structural characterization and molecular structure to propose mode of mechanism action.

Keywords: B-cell lymphoma 2-associated X protein, Chinese liver fluke, Molecular model, *Clonorchis sinensis*, Protein structure.

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INTRODUCTION

B-cell lymphoma 2 (BCL-2)-associated X protein (BAX) was identified as a regulator of cell death that controls apoptosis in normal and cancer cells. BAX is a vital key role player to mitochondrial dysfunctioning and a lead proapoptotic member of the BCL-2 family proteins. Apoptosis dysfunction leads to the cancerous cell becoming resistant to cure and thereby promoting tumorigenesis [1,2]. Activation of BAX induces permeabilization of mitochondrial membrane, thus leading to liberation of apoptotic factor cytochrome c and as a result death of cancer producing cells. In clinical use, numerous drugs are identified which in some way activates BAX. The proteins in BCL-2 family including BAX and BCL-2 homologous antagonist/killer normally act on the outer mitochondrial membrane to promote permeabilization and commit cells to apoptosis. The continuous retrotranslocation of healthy cells in cytosol controls BAX a pro-survival BCL-2 protein [1-3].

The balance between deaths and cellular life is regulated by BAX an important proapoptotic components of BCL-2 family. Since numerous medical circumstances can be grouped under diseases so some high priority scientific strategies needed to be developed this can pharmacologically target and break down the biochemistry of BCL-2 family [4]. In 1984, BCL-2 family founding member was discovered, as soon as a pediatric patient was diagnosed with acute lymphoblastic leukemia resultant cell line proved that it contain defective oncogenic translocation which merged with the heavy chain of immunoglobulin locus on chromosome 14 with BCL-2 gene on chromosome 18 with unidentified function [5]. In follicular lymphoma, the gene product t (14;18) breakpoint was identified, characterized, cloned [6-8], and discovered that it promotes cell survival [5,9,10].

In 1993, the initial BCL-2 interaction partner, BAX, was recognized as a immensely homologous protein that might self-associate or heterodimerize with BCL-2, but in prominent contrast to BCL-2, it promoted, to a certain extent than blocked, cell death after a stress stimulus [7]. With the invention of BAX, the rheostat cell death model was formulated, positioning the BCL-2, and BAX as the dark side and light side of whose relative levels dictated the equilibrium between death and cellular life [7]. With this initial point, the aim of this study was to determine a comparative composite based structure of BAX of Chinese liver fluke and different structural analysis.

METHODS

Starting material, i.e., protein amino acid of BAX of Chinese liver fluke mined from National Centre for Biotechnology Information (http://ncbi.nlm.nih.gov) [11]. SignalP 4.0 server was used for search of signal peptide signature within the protein sequence of BAX of Chinese liver fluke [12]. Molecular model of BAX of Chinese liver fluke protein was generated by the comparative composite modeling tool Iterative Threading ASSEmbly Refinement (I-TASSER) suite [13]. Afterward,

I-TASSER generated molecular model was subjected to further structural improvements by energy minimization step by Swiss-PDB Viewer (GROMOS 96 43B1 parameters set) [14]. The validation for three-dimensional structural model obtained by comparative composite modeling approach was evaluated by PROCHECK tool and ProSA-web tool [15,16]. Distribution of negatively and positively charged amino acid over molecular modeled structure, distribution of secondary structural elements, and hydrophobicity molecular surface analysis was performed with the help of UCSF Chimera package [17].

RESULTS AND DISCUSSION

BAX is a main proapoptotic constituent of the BCL-2 family of protein and a molecule essential for cell death that is broadly expressed equally in small cell (SCLC) and non-small cell lung cancer (NSCLC) cells signifying that BAX possibly will be a therapeutic target designed for lung cancer [18,19]. Activation of BAX proapoptotic function is expected to occur through a number of interdependent mechanisms to facilitate its translocation from the cytosol to mitochondria [20], insertion, and oligomerization into mitochondrial membranes after cellular stress [21]. Earlier reports point out that the post-translational modification of the proapoptotic action of BAX can be controlled by phosphorylation [22].

In patients with lung cancer survival, chances are low due to resistance to conventional health-giving interventions [23]. Apoptotic initiation in cancer cells is a well-known example in cancer therapy. BAX has been recognized as a promising prognostic marker in lung cancer patients, signifying that it could potentially offer a beneficial target in patients with lung cancer [24]. In recent times, it has been known that in the c-terminal tail the S184 phosphorylation site of BAX functionally controls the proapoptotic action of BAX [19,25].

BAX similar to any other three dimensional structure of its BCL-2 family proteins shows alike tertiary structures [26]. The center of the protein comprises of Helices α5 (Hα5) and Hα6 which are implanted within the further seven helices that are amphipathic and maintain their hydrophilic residues uncovered to the periphery [27]. Hα5 is known as mitochondria pore-forming domain and Hα6 known as transmembrane domain [28]. For the translocation of BAX to mitochondria, a mitochondrial addressing signal of N-terminal Hα1 is believed to be necessary [29]. Recent bioinformatical research methods were very helpful for the discovery process of new biologically and biomedically important protein molecules [30-39]. Therefore, varied structural forms of BAX molecules in the different organisms (e.g., Chinese liver fluke) must be identified for bio-medical interventions.

Analysis of Ramachandran plot created by PROCHECK tool is a consensus standard for validation purpose of protein structural modeling. Ramachandran plot for BAX has been depicted in Fig. 1. Altogether 97.8% of the residues were detected in allowed and favored regions, which in turn validate the quality of generated protein structural model (Fig. 2). ProSA-web was employed to investigate three-dimensional model of BAX of Chinese liver fluke for possible errors. As shown in Fig. 3, the Z-score of BAX of Chinese liver fluke was −5.57. The score was perfectly within the range of scores typically observed for proteins of corresponding size representing highly dependable three-dimensional structures.

Figs. 4 and 5 depict the distribution of positively and negatively charged amino acid over molecular modeled structure of BAX of Chinese liver fluke, respectively. Total negatively and positively charged residues within the BAX of Chinese liver fluke were 23 and 20, respectively.

Chimera package guided hydrophobicity molecular surface analysis (dodger blue for the most hydrophilic, to white, to orange-red for the most hydrophobic) which represents the molecular surface of protein, colored by amino acid hydrophobicity, illustrates that molecule

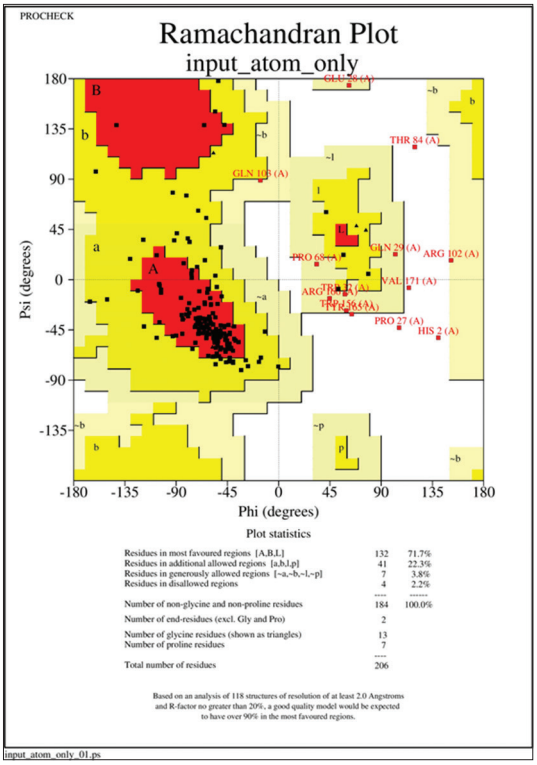

Fig. 1: Ramachandran plot (PROCHECK analysis) of molecular model of B-cell lymphoma 2-associated X protein of Chinese liver fluke

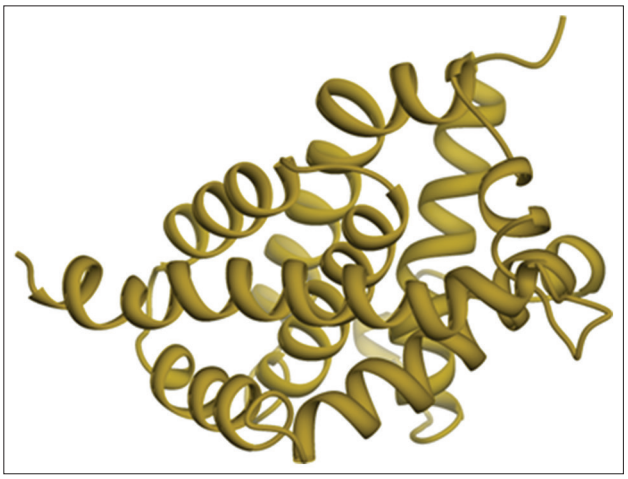

Fig. 2: Three-dimensional modeled structure of B-cell lymphoma 2-associated X protein of Chinese liver fluke

specific hydrophobicity surface is exclusive to BAX protein molecule in Fig. 6.

CONCLUSION

Within the scope of this scientific investigation, we have successfully utilized molecular modeling approach to suggest the first molecular three-dimensional model structure of BAX of Chinese liver fluke. The synchronous balance between cellular deaths and cellular life is keeping up by BAX, an important proapoptotic family member of BCL-2 family. Consequently, it would be an exciting approach to resolve its structural characterization and molecular structure to propose mode of mechanism action. Hence, a three-dimensional structural model of the BAX protein was generated. Additional investigation was performed to infer its molecular characteristics.

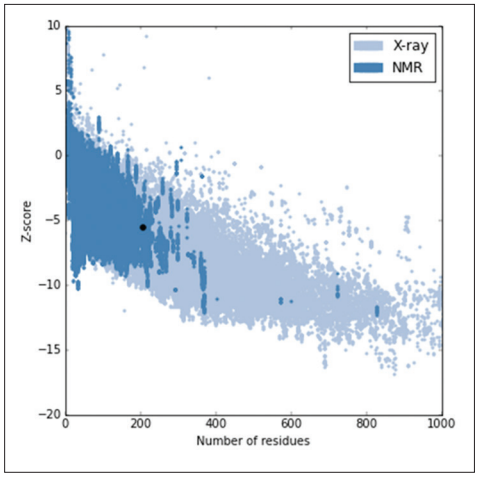

Fig. 3: ProSA-web analysis (Stereo-chemical validation) of modeled protein structure of B-cell lymphoma 2-associated X protein of Chinese liver fluke

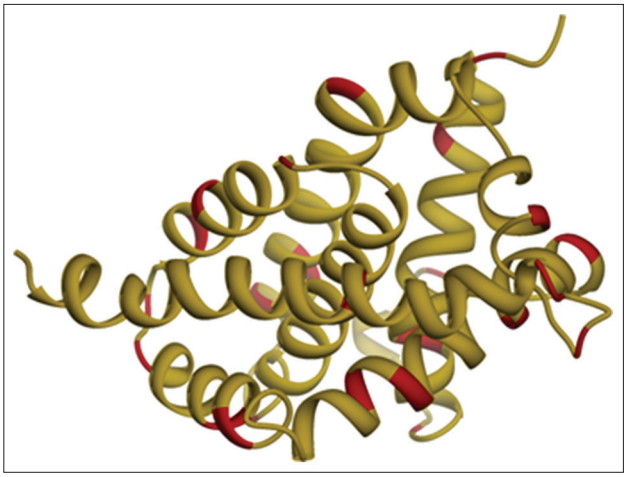

Fig. 5: Distribution of negatively charged (red) amino acid distribution within the B-cell lymphoma 2-associated X protein of Chinese liver fluke

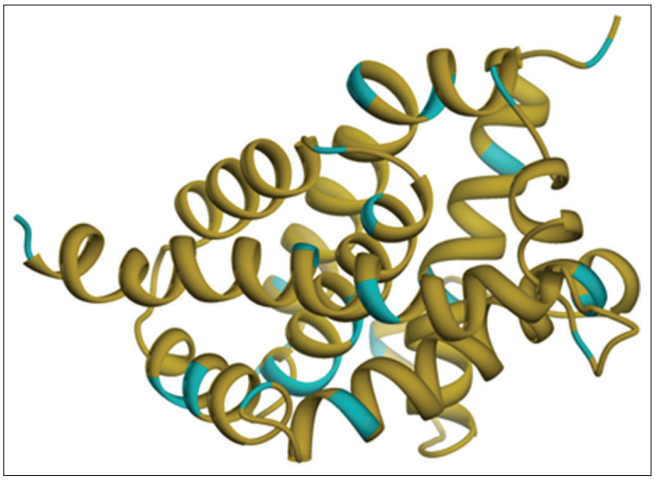

Fig. 4: Distribution of positively charged (blue) amino acid distribution within the B-cell lymphoma 2-associated X protein of Chinese liver fluke

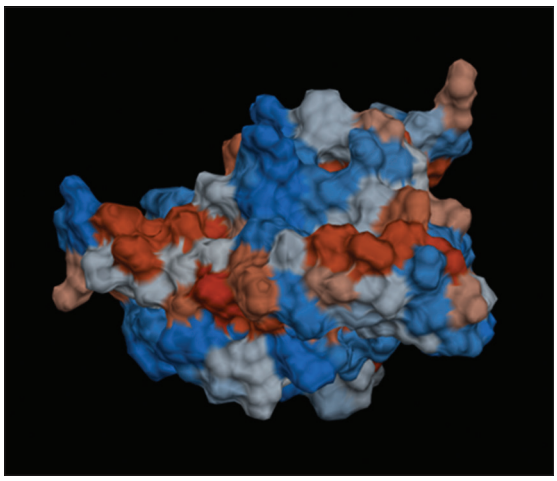

Fig. 6: Hydrophobicity surface maps of B-cell lymphoma 2-associated X protein of Chinese liver fluke (Dodger blue for the most hydrophilic, to white, to orange red for the most hydrophobic)

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