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APOPTOSIS REGULATOR BAX OF CHINESE TREE SHREW (*TUPAIA BELANGERI CHINENSIS*): MOLECULAR MODELING AND STRUCTURAL CHARACTERIZATION

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ABSTRACT

Apoptosis is a process of programmed cell death that is critical for removal of unnecessary, injured, or contaminated cells, and is linked with various biological processes, which includes cell growth, isolation, and propagation. Lack of apoptosis may guide to cancer and autoimmune diseases, while too much cell death may increase ischemic conditions and promote neurodegeneration. The first mammalian gene that was responsible for cell death to be acknowledged was B-cell leukemia/lymphoma 2 (Bcl2), which was cloned from hematopoietic cell lines obtained from a translocation hot spot in follicular lymphoma. The discovery of Bcl-2 led to the discovery of many other key cell death regulators, such as Bcl-2 like 1 (Bcl-x), Bcl-2 linked protein x (BAX). With this background the objective of this study was to determination of protein structure of BAX of Chinese tree shrew (*Tupaia belangeri chinensis*) with the assistance of various bioinformatical research methods. A structural model of the BAX protein was generated and further analysis was carried out to infer molecular characteristics.

KEYWORDS: Apoptosis regulator, BAX, Protein structure, Chinese tree shrew, *Tupaia belangeri chinensis,* Structural characterization.

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INTRODUCTION

During usual life process of human body the cell death has many means in tissue sculpting and functions modification such as in the immune system or central nervous system [1-2]. Apoptosis is a process of programmed cell death that is critical for removal of unnecessary, injured, or contaminated cells, and is linked with various biological processes, which includes cell growth, isolation, and propagation [3]. Lack of apoptosis may guide to cancer and autoimmune diseases, while too much cell death may increase ischemic conditions and promote neurodegeneration [4]. Cell death has been conceded as a biological process for past 150 years, while it was not prominent until the 1970s when the word apoptosis and necrosis were establish based on distinctive morphological features [5-7]. In the year 1983, cell death defining gene came with the identification of cell death 1 (CED-1) and cell death 2 (CED-2), such that genes are involved in cell death is *Caenorhabditis elegans (C. elegans)* [8]. The first mammalian gene that was responsible for cell death to be acknowledged was B-cell leukemia/lymphoma 2 (Bcl2), which was cloned from hematopoietic cell lines obtained from a translocation hot spot in follicular lymphoma [9]. Afterward it was shown that Bcl-2 was an antiapoptotic protein with a conserved ortholog in *C. elegans*, cell death 9 (CED-9) [10]. The appearance of mammalian Bcl-2 in *C. elegans* remarkably protected the cells against apoptosis. The discovery of Bcl-2 led to the discovery of many other key cell death regulators, such as Bcl-2 like 1 (Bcl-x), Bcl-2 linked protein x (BAX) and Bcl-2 homologues competitor (BAK), among numerous others [11-12]. In recent times computational biology research techniques make it possible to solve complex research questions in life science with *in-silico* research [13-16]. With this background the objective of this study was to determination of protein structure of BAX of Chinese tree shrew (*Tupaia belangeri chinensis*) with the assistance of various bioinformatical research methods. The extended objectives of this study was to critical dissection of BAX of Chinese tree shrew with reference to structural validation of the model, positioning of positive and negative charge over the structure and hydrophobicity molecular surface analysis.

MATERIALS AND METHODS

Amino acid sequence BAX of Chinese tree shrew was collected from National Centre for Biotechnology Information (http://ncbi/nlm/nih.gov) [17]. Signal P 4.1 server was used for detection of signal peptide within protein sequences (http://www.cbs.dtu.dk/services/SignalP/)[18]. Comparative structural model of BAX of Chinese tree shrew was created with the help of Swiss-model and iterative implementation of the threading assembly refinement algorithm [19-20]. Energy minimization critical step for structural refinements of molecular model of BAX of Chinese tree was performed by Swiss-PDB Viewer [21]. Confirmation of accuracy of molecular model obtained by structural modeling was analyzed by PROCHECK algorithm, ProSA and QMEANclust tool [22-24]. Positioning of positive and negative charge over the structure and hydrophobicity molecular surface analysis

was performed with the utilization of UCSF Chimera package [25].

RESULTS AND DISCUSSION

In recent years the expansion of cancer research confirmed that apoptosis is significantly involved in the guideline and treatment of tumor formation [26-27]. For a cell to stay alive mitochondria plays a central part by providing ATP via oxidation and phosphorylation. However the organelles inside the cell also has a dark side, at the surface are the family members of Bcl-2 protein which are lurking in cell death pathway as second mitochondria. Two pivotal members of the Bcl-2 family are the proapoptotic proteins BAX and BAK, which alter from risk-free monomers into toxic oligomers that form pores in the mitochondrial outer membrane (MOM).These pores are a means for proapoptotic factors for translation of cytochrome c to translocate to the cytoplasm.

The outcome is double:
- The loss of cytochrome c from mitochondria disable energy production and
- Cytosolic cytochrome c instigates a proteolytic cascade that destroys the cell [28-29]. A Bcl-2 family protein primarily regulates the signaling of mitochondria [30].

Based on their dissimilar structures and functions, the Bcl-2 family is grouped under two categories:
- Anti apoptotic proteins
- Proapoptotic proteins.

Proapoptotic proteins are further divided into two subclasses:
- Multidomain proteins (e.g., BAX and BAK)
- BH3-only proteins (e.g., Bim, Bid, Bad, Puma, Bik, Noxa, Hrk and Bmf).

Multidomain proapoptotic proteins such as BAX and BAK are crucial executive proteins which are liable for MOMP and a requisite gateway to mitochondrial dysfunction as well as cell death [31]. Cells which do not contain BAX and BAK proteins have proven to be entirely resistant to truncated Bid (t-Bid) induced cytochrome c discharge and cell death [31]. In 1993 a tumor suppressor gene known as Bax was first recognized as heterodimer with Bcl-2 family [32-33]. BAX is a 21 kDa protein consisting of 192 amino acids possessing nine α-helices. In the year 2000 its three-dimensional structure was resolved using nuclear magnetic resonance (NMR) [34]. The three dimensional structures of Bcl-2 family members consists of two central predominately hydrophobic alpha-helices bounded by six or seven amphipathic alpha-helices of varying length [35]. Molecular modeling approach was an advantageous alternative strategy when the three-dimansional structure not available. Previously modeled structure of biologically important molecules was generated and validated with the help of *in-silico* approach [36]. SignalP 4.1 server demonstrates that there is no signal signature is present within the sequence stretch of BAX of Chinese tree shrew. Molecular model structure of BAX of Chinese tree shrew was depicted in

Figure 1. PROCHECK analysis with Ramachandran plot examination is a gold standard for validation purpose of protein structure models. Ramachandran plot for BAX of Chinese tree shrew has been illustrated in Figure 4. In summary 100% of the residues of the BAX structure were observed in allowed and favored regions, which successfully substantiate the quality of generated protein structural model. PROCHECK tool also displayed 88.7 % of residues in the most favored regions, with 11.3 % residues in additionally allowed regions, respectively (Figure 4). This validates that the three dimensional modeled structure of BAX of Chinese tree shrew is satisfactory and accurate (Figure 1). The QMEANclust algorithm dictates a suitable quality of the structural atomic co-ordinates of BAX of Chinese tree shrew with a Z-Score of -0.683 and QMEANscore of 0.699 (Figure 5). As shown in Figure 6 the Z-score (ProSA tool) of BAX of Chinese tree shrew was -6.37. The calculation was correctly inside the range of scores normally regarded for proteins of equal size, demonstrating highly reliable structures. The manual analysis of BAX of Chinese tree shrew proposes that the total protein is composed by 173 numbers of amino acids. The presence of total number of positively charged amino acids is 19 (Figure 2). In contrast to that the total number of negatively charged amino acids is only 20 (Figure 3).
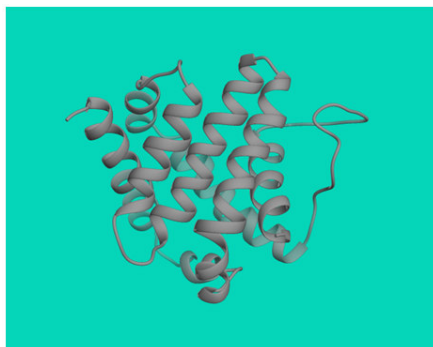

Figure 1
*Three-dimensional modeled structure of
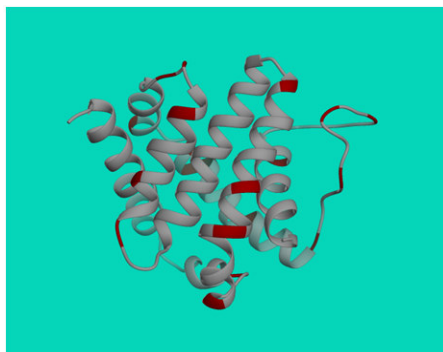BAX of Chinese tree shrew.*

Figure 2
*Negatively charged amino acid distribution on the
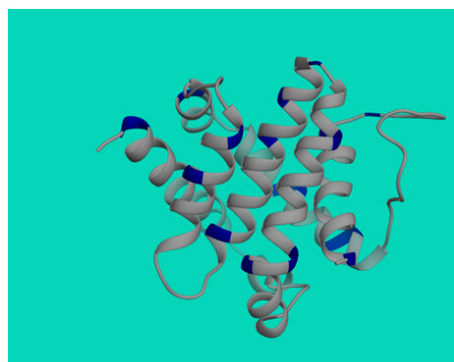modeled structure of BAX of Chinese tree shrew.*

Figure 3
*Positively charged amino acid distribution on the
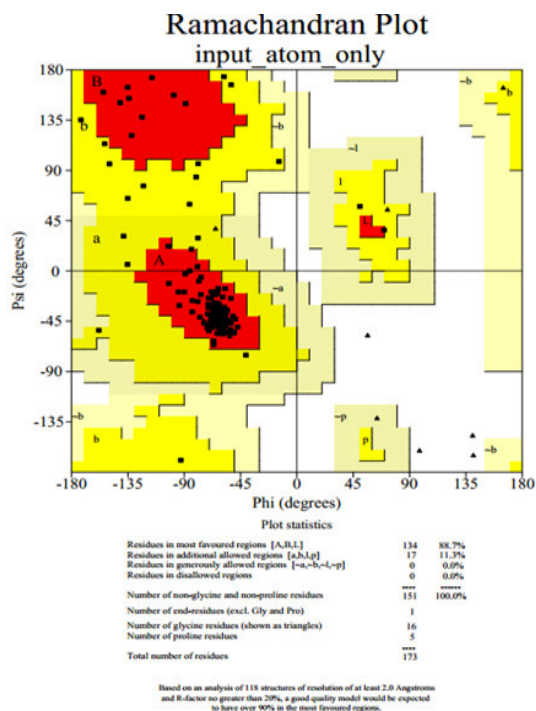Modeled structure of BAX of Chinese tree shrew.*

Figure 4
*Ramachandran plot analysis of molecular
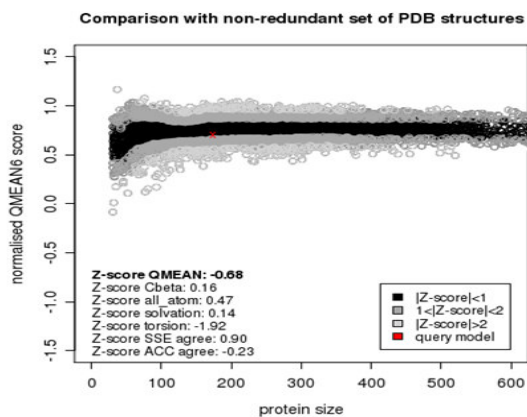model of BAX of Chinese tree shrew.*

Figure 5
*Stereo-chemical analysis (QMEANclust tool) of
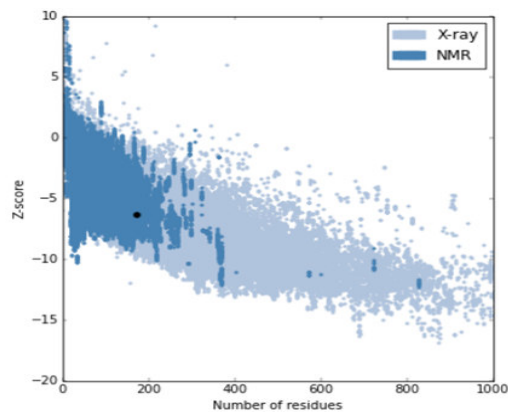Modeled structure of BAX of Chinese tree shrew*

Figure 6
*Stereo-chemical analysis (ProSA analysis) of
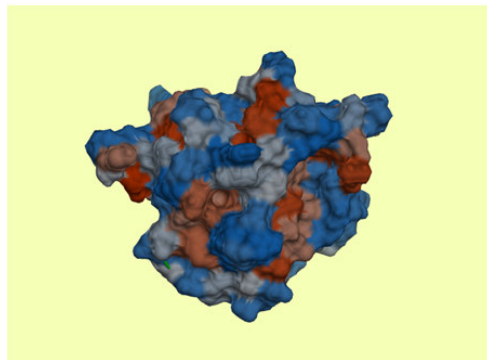Modeled structure of BAX of Chinese tree shrew.*

Figure 7
Hydrophobicity surface maps of BAX of Chinese tree shrew (dodger blue for the most hydrophilic, to white, to orange red for the most hydrophobic).

CONCLUSION

In the present study, we have effectively utilized comparative modeling approach to proffer the first molecular model structure of BAX of Chinese tree shrew. The apoptosis regulator BAX of Chinese tree shrew plays a significant role in physiological and cellular systems. Accordingly, it would be an interesting approach to deduce its molecular structure and structural characterization to propose mechanism of action. Therefore, a structural model of the BAX protein was generated and further analysis was carried out to infer molecular characteristics. The structural model data in supplementation to other pertinent post model examination data put forward molecular insight to apoptosis regulator BAX protein.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Golstein P. Cell death in us and others. Science. 1998; 281:1283.
2. Liu Z, Ding Y, Ye N, Wild C, Chen H, Zhou J. Direct Activation of Bax Protein for Cancer Therapy. Med Res Rev. 2016; 36:313-41.
3. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972; 26:239-57.
4. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol. 2014; 15:49-63.
5. Clarke PG, Clarke S. Nineteenth century research on naturally occurring cell death and related phenomena. Anat Embryol (Berl). 1996; 193:81-99.
6. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol. 1995; 146:3-15.
7. Karch J, Molkentin JD. Regulated necrotic cell death: the passive aggressive side of Bax and Bak. Circ Res. 2015; 116:1800-9.
8. Hedgecock EM, Sulston JE, Thomson JN. Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. Science. 1983; 220:1277-9.
9. Tsujimoto Y, Croce CM. Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. Proc Natl Acad Sci U S A. 1986; 83:5214-8.
10. Vaux DL, Weissman IL, Kim SK. Prevention of programmed cell death in *Caenorhabditis elegans* by human bcl-2. Science. 1992; 258:1955-7.
11. Farrow SN, White JH, Martinou I, Raven T, Pun KT, Grinham CJ, et al. Cloning of a bcl-2 homologue by interaction with adenovirus E1B 19K. Nature. 1995; 374:731-3.
12. Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, et al. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell. 1993; 74:597-608.
13. Panda S, Kumari L. Molecular modeling and structural analysis of arylesterase of *Ancylostoma duodenale*. International Journal of Pharma and Bio Sciences. 2016; 7:611-6.
14. Panda S, Chandra G. Sequence analysis and phylogenetic study of some toxin proteins of snakes and related non-toxin proteins of chordates. Bioinformation. 2013; 9:259-66.
15. Panda S, Chandra G. Physicochemical characterization and functional analysis of some snake venom toxin proteins and related non-toxin proteins of other chordates. Bioinformation. 2012; 8:891-6.
16. Panda S, Kumari L, Hui S, Panda S. Structural insight of homeobox DNA binding domain of Hox-

B7a protein of *Esox lucius*. Journal of PharmaSciTech. 2016; 6:1-4.

17. Coordinators NR. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2015; 43:D6-17.

18. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods. 2011; 8:785-6.

19. Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: An automated protein homology-modeling server. Nucleic Acids Res. 2003; 31:3381-5.

20. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics. 2008; 9:40.

21. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis. 1997; 18:2714-23.

22. Laskowski RA, Rullmannn JA, MacArthur MW, Kaptein R, Thornton JM. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J Biomol NMR. 1996; 8:477-86.

23. Sippl MJ. Recognition of errors in three-dimensional structures of proteins. Proteins. 1993; 17:355-62.

24. Benkert P, Schwede T, Tosatto SC. QMEANclust: estimation of protein model quality by combining a composite scoring function with structural density information. BMC Struct Biol. 2009; 9:35.

25. Goddard TD, Huang CC, Ferrin TE. Visualizing density maps with UCSF Chimera. J Struct Biol. 2007; 157:281-7.

26. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science. 1984; 226:1097-9.

27. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature. 1988; 335:440-2.

28. Westphal D, Kluck RM, Dewson G. Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis. Cell Death Differ. 2014; 21:196-205.

29. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell. 1996; 86:147-57.

30. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer. 2002; 2:647-56.

31. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science. 2001; 292:727-30.

32. Yin C, Knudson CM, Korsmeyer SJ, Van Dyke T. Bax suppresses tumorigenesis and stimulates apoptosis in vivo. Nature. 1997; 385:637-40.

33. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell. 1993; 74:609-19.

34. Suzuki M, Youle RJ, Tjandra N. Structure of Bax: coregulation of dimer formation and intracellular localization. Cell. 2000; 103:645-54.

35. Lalier L, Cartron PF, Juin P, Nedelkina S, Manon S, Bechinger B, et al. Bax activation and mitochondrial insertion during apoptosis. Apoptosis. 2007; 12:887-96.

36. Panda S, Kumari L, Panda S. Structural understanding of cytotoxin 1 of *Naja sputatrix*: a potential anticancer agent. Journal of Drug Delivery and Therapeutics. 2016; 6:59-63.