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COMPUTATIONAL PREDICTION OF PROTEIN INTERACTIONS IN BCL-2 PROTEIN AND THEIR ROLE IN APOPTOSIS

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ABSTRACT

Protein-protein interactions (PPIs) participate in a diverse range of biological processes inside a cell such as cell to cell communication, developmental process and most of the major metabolic processes. These interactions play a vital role in predicting the functions of a target protein and drug ability of molecules. Apoptosis is considered as a major component of biological processes, it involves number of proteins that are actively participating and regulating the survival of cells. In this work protein functional region among mammals along with their interactions were studied in mitochondrial mediated apoptosis. There are many key proteins which actively participate in mitochondrial apoptosis process. Among these proteins, Bcl-2 family of proteins has great significance in apoptosis process. It possesses different classes of proteins which promote cell survival and cell death named as Anti-apoptotic proteins and Pro-apoptotic proteins respectively Along with these BH3 proteins acts as a mediator if it interacts with pro-apoptotic proteins. Using Bioinformatics tools, Bcl-2 family proteins were investigated to know homology with other mammals. Further observation was made on evolutionary relationship among closely related mammalian species. PPIs was studied in detail and found that they have great potential to be used in therapeutic purposes and research.

KEYWORDS: Protein-protein interactions (PPIs), Apoptosis, Anti-apoptotic proteins, Pro-apoptotic proteins, BH3 proteins.

INTRODUCTION

Proteome refers to the complete set of proteins produced by an organism. Proteome varies over time and changes from one cell to another cell. Proteomic data reveals important information on many biological problems. Protein interaction databases can be searched to understand any specific protein interaction and infer the result of these interactions. It is necessary to first interpret proteomics data in order to decode the pathways of protein interaction and to understand the role of cellular systems in human disease.

In the present study, prediction of protein functional regions and visualization of mitochondrial apoptosis process has been performed. Apoptosis has been accepted as a distinctive and specific mode of "programmed cell death," which allows elimination of genetically determined cells. Apoptosis starts normally throughout the course of development, aging and as a balance to maintain cell pool in tissues. Apoptosis is responsible for many neurodegenerative diseases, autoimmune disorders and cancer.

Mechanism of Apoptosis

The mechanism of apoptosis is highly complex and sophisticated among species. It is conserved and directly controlled by genes.[1] It involves many molecular events. Apoptotic cells exhibit several biochemical modifications such as protein cleavage, protein cross linking and DNA break down.[2] There are two main pathways of apoptosis - extrinsic pathway (death receptor pathway) and intrinsic pathway (mitochondrial pathway). One more pathway is also known that involves T-cell mediated cytotoxicity and perforin-Granzyme dependent killing of the cell (Figure 1).

The extrinsic pathway initiates apoptosis with the involvement of trans-membrane receptor-mediated interactions. These interactions are complex with death receptors that belong to the superfamily of tumor necrosis factor (TNF) receptor gene.[3]

The intrinsic pathway of apoptosis allows non-receptor-mediated stimuli that generate intracellular signals which act directly on targets within the cell and are strictly mitochondrial-initiated events. The control and regulation of mitochondrial apoptotic event is governed by Bcl-2 family of protein.[4] Till now, around 25 Bcl-2 family members have been identified.[5] These proteins can be either pro-apoptotic (Bax, Bak, Bcl-10, Bid, Bad, Bim, Bik and Blk) or anti-apoptotic (Bcl-2, Bcl-XL, Bcl-x, Bcl-W, Bcl-XS, BAG) (Figure 2). These proteins determine the fate of the cell in the apoptotic process. Bax and Bak are some

pro-apoptotic proteins that permeabilize and oligomerize the mitochondrial outer membrane while Bak or Bax.[6] are responsible to promote apoptosis either activating or by inhibiting the anti-apoptotic proteins such as Noxa, Bad, Bim and Bid.[7] Certain pro-apoptotic proteins like Bax, Bak and Bid as well as all anti-apoptotic proteins exhibit a multi-region BH domain which is evolutionary related.[8]

Bcl-2 family of proteins regulates apoptosis mainly by mitochondria. However, other organelles such as endoplasmic reticulum are also involved in this process. It has been known that the main function of Bcl-2 proteins is to regulate the release of cytochrome C from the mitochondria by altering the permeability of outer membrane.[9] The membrane permeability is directly controlled by Bax or Bak.[10] Bax and Bak are multi domain protein. Bax is localized in cytosol and transported in mitochondria only when it receives stimulus. where as Bak reside in mitochondria.[11] Several models such as direct activation model,[12] displacement model,[13] embedded together model,[14] and unified model[15] have been proposed to understand the role of these proteins in apoptosis (Figure 3). In the direct model, the BH3-only molecules can directly activate the multi-domain molecules Bax and Bak to initiate the mitochondrial events.[16]

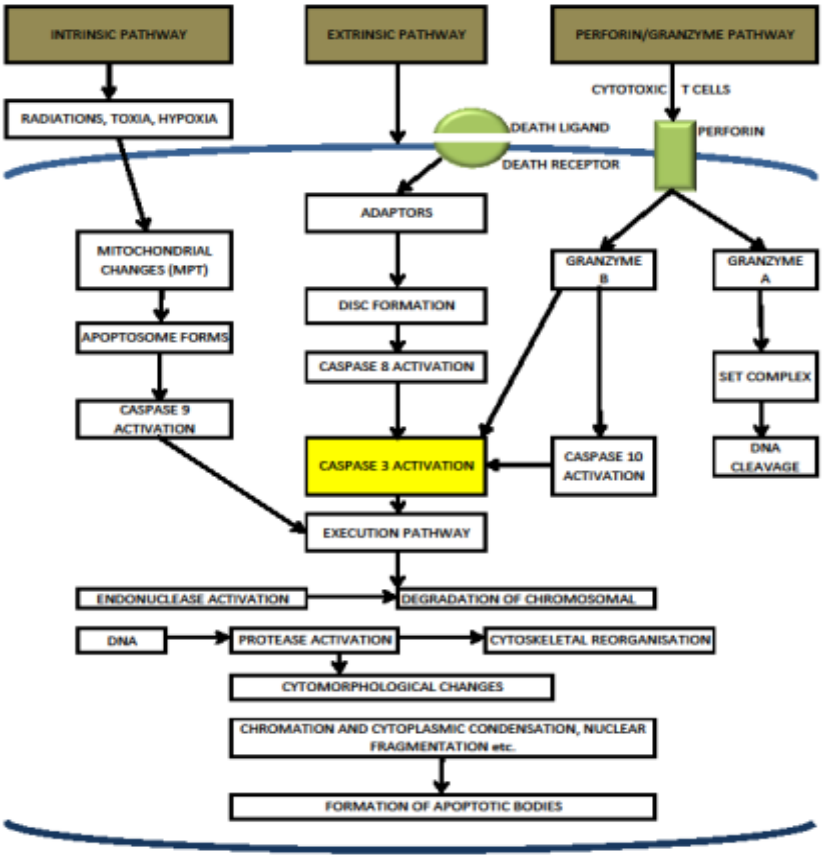

Figure 1: Mechanism of Apoptosis.

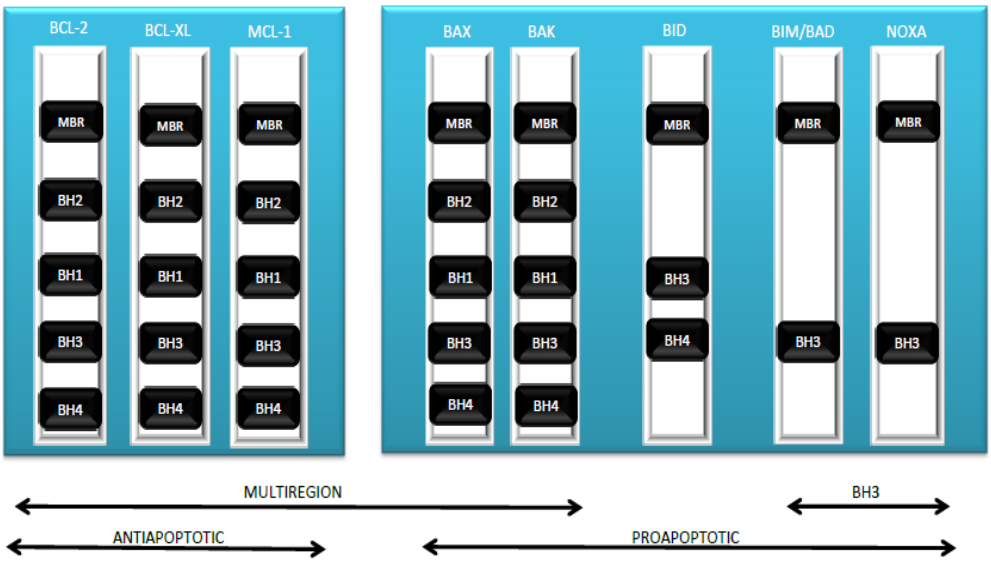

Figure 2: All BH domains in Anti-apoptotic and pro-apoptotic protein and interactions between the domains of two pro-apoptotic proteins such as BID and BAX.

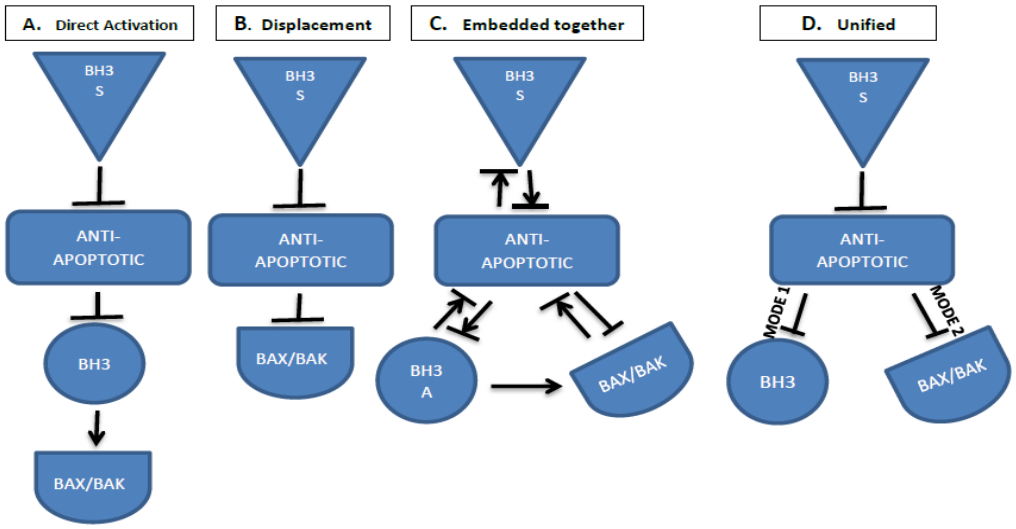

Figure 3: Schematics of the basic mechanism proposed by various models for mitochondrial outer membrane regulation by Bcl2 proteins Symbols shows activation (↑), inhibition (⊥), mutual recruitment/sequestration (↑⊥).

METHODOLOGY

Mitochondrial Bcl-2 protein (Homo sapiens) was taken from Swissprot database. During apoptosis, role of Bcl-2 family members, their mode of action and overview of each class of proteins was studied. Eleven mammalian species with identity more than 90% were selected based on BlastP algorithm. Multiple sequence alignment among these species was performed to see the dissimilar regions (Figure 5) and a phylogenetic tree was constructed. Motifs and secondary structure in Bcl-2 were predicted using PDB protein ID 5UUL. Domains that were

conserved domain amongst different species were detected using NCBI CDD. Protein interaction of Bcl-2 was studied using UCSE genome browser (Figure 4). It was revealed that Bcl-2 was the key interacting molecule forming a network with other protein molecule. Individual functions of each protein in context to proteins interactions need to be find out experimentally for future benefits in research and therapeutics.

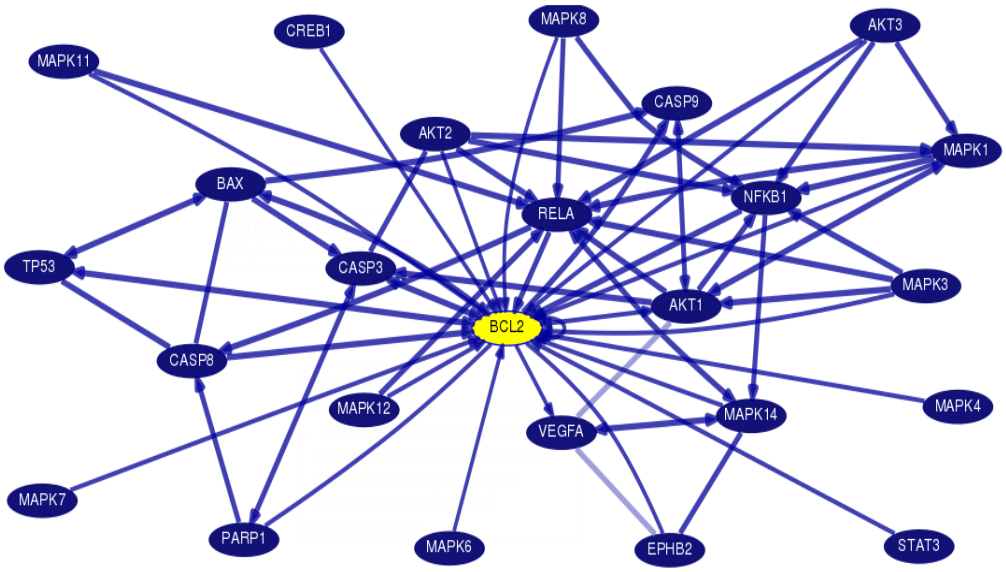

Figure 4: Visualization of protein interaction network of Bcl-2 using UCSC genome browser

RESULTS

It is clear from the recent studies that the Bcl-2 family of proteins is classified by certain regions of similarity known as BH-1, BH-2, BH-3, BH-4 or more particularly as B cell homology domains. These proteins possess functions like regulating fate of the cells. Bcl-2 family of proteins consists of anti-apoptotic proteins that exhibit all four BH domains, pro-apoptotic proteins possess three BH domains. These homology regions were viewed through Expasy Prosite by taking Bcl-2 sequence from Uniprot. Results obtained from Prosite shows that Bcl-2 family consists of high homology with BH-3 region.

Sequence of Bcl-2 in FASTA format

>sp|P10415|BCL2_HUMAN Apoptosis regulator BCL-2OS=Homo sapiens GN=BCL2 PE=1 SV=2

MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGH

TPHPAASRDPVARTSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDF

AEMSSQLHLTPFTARGRFATVVEELFRDGVNWGRIVAFFEFGGVMCVESVNREMSP
LVDNIALWMTEYLNRHLHTWIQDNGGWDAFVELYGPSMRPLFDFSWLSLKTLLSLA
LVGACITLGAYLGHK

Accession	Description
AAA35591.1	bcl-2 protein [Homo sapiens]
PNJ64341.1	BCL2 isoform 1 [Pongo abelii]
XP_003914501.1	apoptosis regulator Bcl-2 isoform X3 [Papio anubis]
XP_012301848.1	apoptosis regulator Bcl-2 [Aotus nancymaae]
NP_033871.2	apoptosis regulator Bcl-2 isoform 1 [Mus musculus]
XP_021028902.1	apoptosis regulator Bcl-2 [Mus caroli]
XP_021053579.1	apoptosis regulator Bcl-2 [Mus pahari]
XP_003788464.1	apoptosis regulator Bcl-2 [Otolemur garnettii]
XP_005081511.2	apoptosis regulator Bcl-2 [Mesocricetus auratus]
NP_058689.1	apoptosis regulator Bcl-2 [Rattus norvegicus]
XP_022440032.1	apoptosis regulator Bcl-2 [Delphinapterus leucas]

Figure 5: Multiple Sequence Alignment based on sequence similarity.

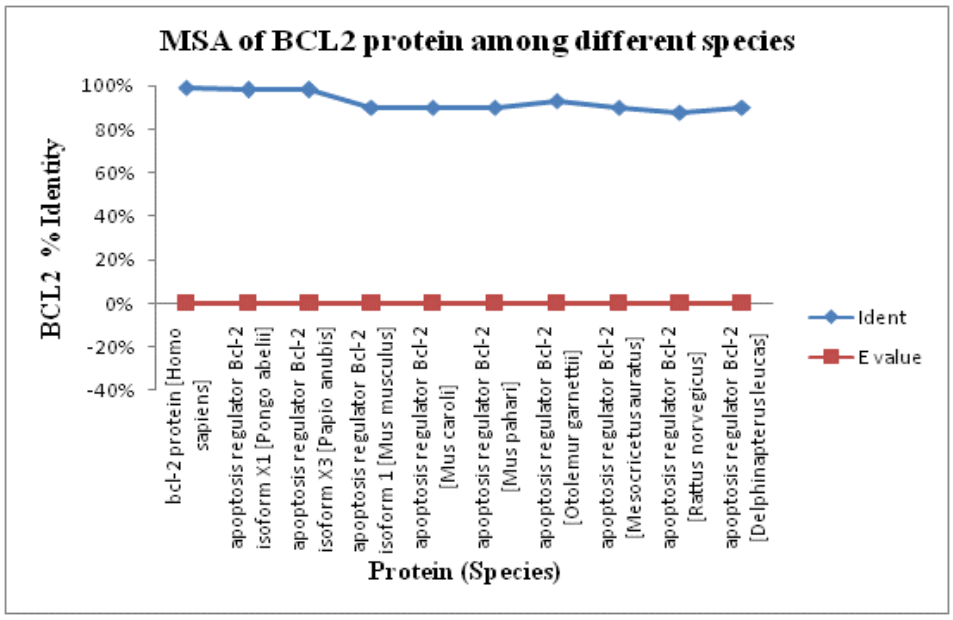

Figure 6: Graphical view of Multiple Sequence Alignment of Pro-apoptotic Proteins.

Blastp was performed first for human apoptosis regulator protein. On the basis of e-value and sequence identity, multiple sequence alignment was performed (Figure 5) by taking eleven homologous species. A graphical output was generated and visualized (Figure 6).The purpose was to see the conserved region as well as phylogenetic relationship among these proteins. To show phylogenetic relationship between the proteins, a phylogenetic tree was generated (Figure 7).

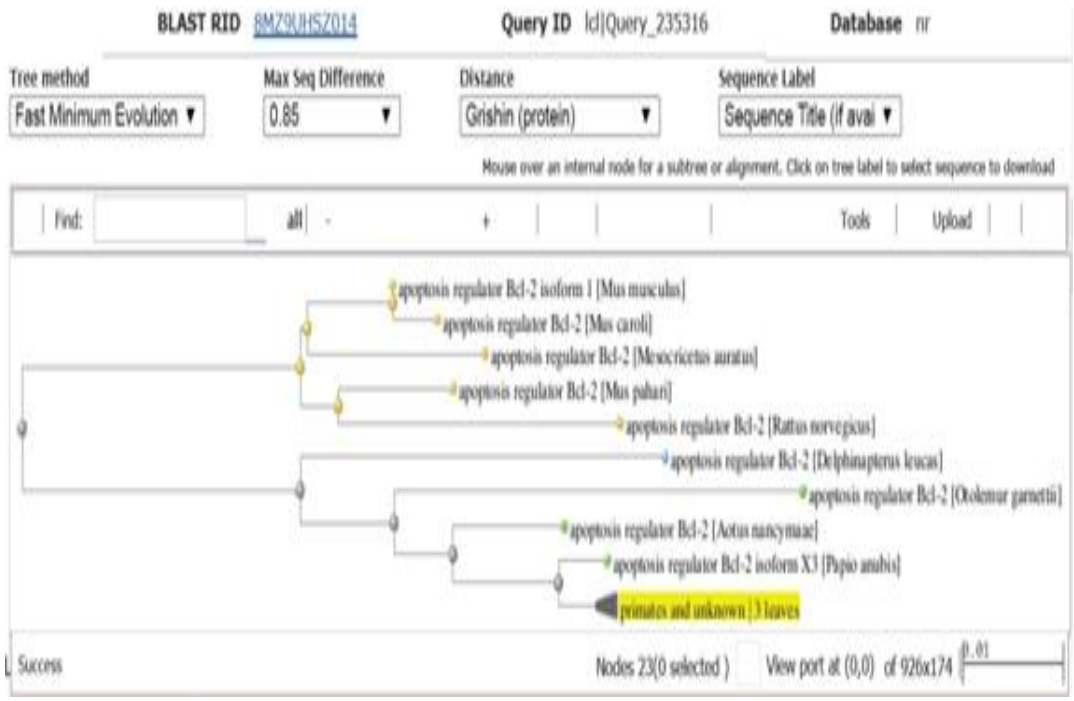

Figure 7: Phylogenetic tree showing relationship among mammals.

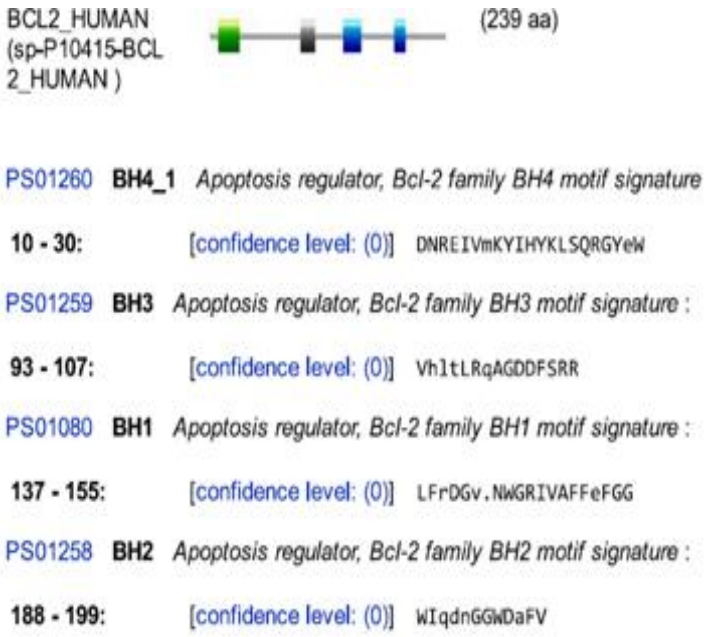

Figure 8: Different domains prediction of Bcl-2 using Prosite.

Various domains in Bcl-2 protein were predicted using Prosite tool provided by Expasy server (Figure 8). Different domains were visualized, which were present in pro-apoptotic as well as in anti-apoptotic protein, as protein interacts through domain. Domain is functional part of protein. Motif and secondary structure was obtained from PDB for protein ID 5UUL. This conserved region was checked across different homologous species (Figure 10).

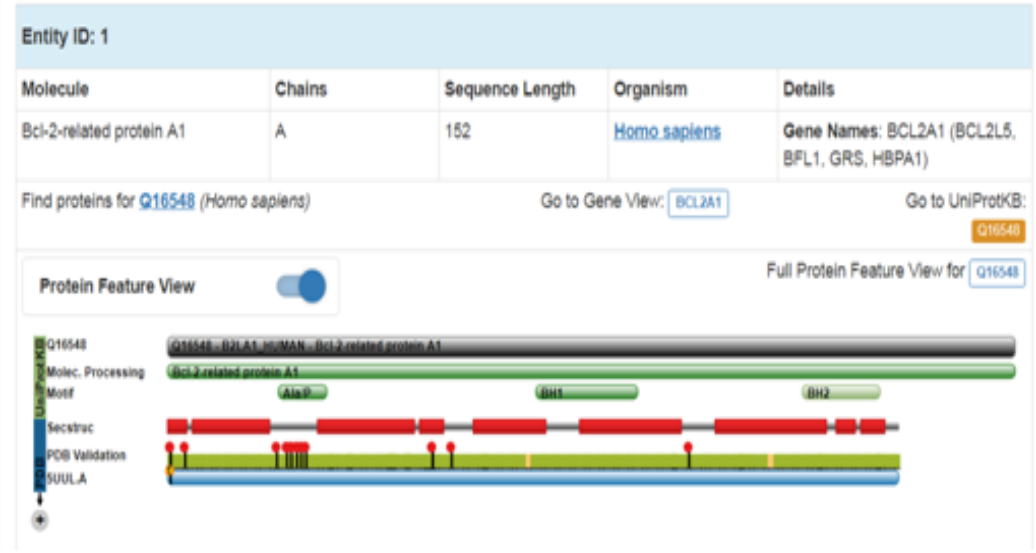

Figure 9: Prediction of secondary structure and motif in Bcl-2 using PDB ID 5UUL.

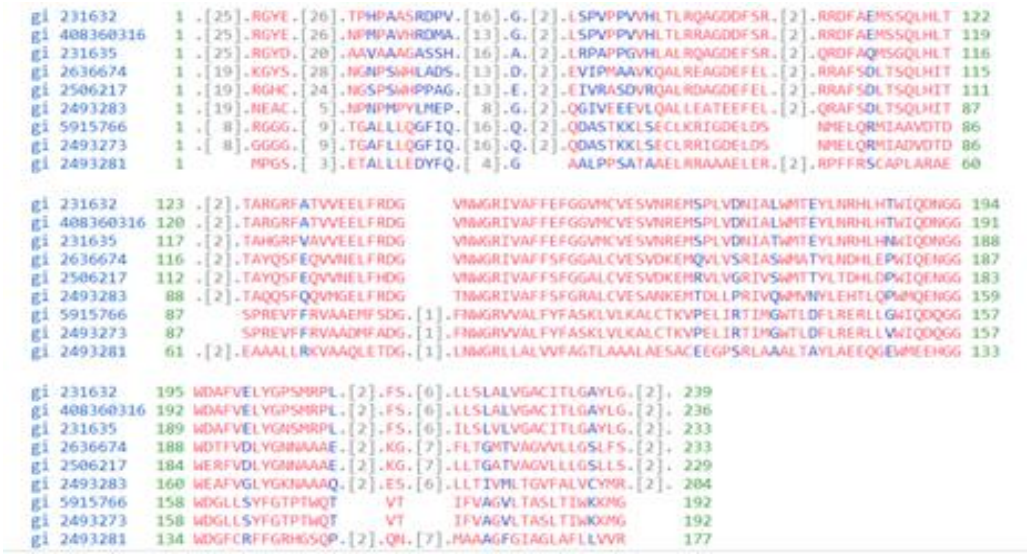

Figure 10: Predicting conserved domain in Bcl-2 family using NCBI CDD.

DISCUSSION

Bcl-2 family has a number of roles in different physiological process. Bcl-2 and the closely related Bcl-X(L) are anti-apoptotic key regulators of programmed cell death. Bcl-2 family protein can interact with each other. They also interact with anti-death and pro-death members. Bax is only such protein which interacts with all of them. They are assumed to function via heterodimeric protein-protein interactions, binding pro-apoptotic proteins such as Bad (Bcl-2-antagonist of cell death), Bid and Bim by specifically interacting with their BH3 regions. BH domains are perilously involved among family members in protein interaction. Domains involved in the interactions of two death proteins such as Bid and Bax are the BH3 domain of the BH3 only molecule. The BH3 molecule is made up of BH1, BH2 and BH3

domains. Mutations at any one of the domain can disrupt such interaction. Apoptosis is a conserved process. The strong sequence homology between Bcl-2 family members suggests that they all have been descended from common gene.

Interfering with this heterodimeric interaction via small-molecule inhibitors may prove effective in targeting various cancers as this protein is conserved among species. Common domain was also visualized in pro- and anti- apoptotic proteins. Mutation in these domains needs to be studied. For therapeutic purpose, this domain needs to be studied in detail as domain interaction plays key role in apoptosis.

CONCLUSION

Functional region of protein is domain. A protein can have more than one domain with different features. There are several underlying facts which can be decoded by studying features of domain. Domains can be conserved among species. From earlier studies, it was found that this Bcl-2 family consists of high homology with BH3 domain. Studies show that BH-3 acts as a potent mediator of cell death. Moreover mutations in any one of these domain can disrupt protein interaction. This BH3 region has high structural similarity with pro-apoptotic proteins. The consensus region of these two might be responsible for apoptosis by promoting cell death. Although the exact mechanism of action of Bcl-2 family of proteins is not clear, the role of these protein in the anti and pro-apoptotic processes need to be investigated further. This could provide alternatives to treat various diseases including neurodegenerative diseases, cancers, myocardial diseases, atherosclerosis and many more.

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