Journal of Pharmacology Research, ISSN: 0976–7134 & E-ISSN: 0976–7142 , Vol. 1, Issue 1, 2010, PP-01-08

Computational approach towards the B-Cell Lymphoma-2 Protein: A noticeable target for Cancer proteomics

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Abstract- In the present time, the emergence of Cancer is one of the crucial challenge for Bioinformaticians. The Enormous growth of cancer patient is now increasing worldwide due to its multidimensional complication. It is a group of diseases in which cells are aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues), and sometimes metastatic (spread to other locations in the body). Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication, or are inherited, and thus present in all cells from birth. The tumor is formed from uncontrolled growth of cell. The apoptosis is a cell death mechanism which control cell growth. Current research showing the BCL2 protein family playing an important role in cancer. It is present on outer membrane of mitochondria, consisting 239 amino acids. It suppress apoptosis, regulates cell death by controlling the mitochondrial membrane permeability. It also inhibits caspase activity either by preventing the release of *cytochrome c* from the mitochondria and/or by binding to the apoptosis activating factor (APAF-1). Hypermethylation is one of the effective concept in the origin of Cancer, due to extra methylation at the 5^{th} carbon of the cytosine residues. In our approach, we have to show the binding interaction in between BCL2 and a small ligand,

3-NITRO-N-{4-[2-(2-PHENYLETHYL)-1,3-BENZOTHIAZOL-5-YL]BENZOYL}-4-{[2-(PHENYLSULFANYL)ETHYL]AMINO}BENZENESULFONAMIDE and using this ligand (C36H30N4O5S3)it is supposed to become inactivated. They are closely attached to active site of BCL2 protein and then deactivate the protein. Thus, according to the concept of protein-ligand interaction in near future, these molecules can be used as drug in the treatment of cancer.

Introduction

As per the current research and outputs, Cancer is a group of diseases in which cells are grow and divide without respect to normal limits, invasive that is invade and destroy adjacent tissues, and sometimes spread to other locations in the body(metastatic). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited in their growth and don't invade or metastasize (although some benign tumor types are capable of becoming malignant).Cancer may affect people at all ages, even fetuses, but risk for the more common varieties tends to increase with age. Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication, and thus present in all cells from birth. Complex interactions between carcinogens and the host genome may explain why only some develop cancer after exposure to a known carcinogen. New aspects of the genetics of cancer pathogenesis, such as DNA methylation, and microRNAs are increasingly being recognized as important. Genetic abnormalities found in cancer typically affect two general classes of genes[1]. Cancer-promoting oncogenes are often activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the ability to become established in diverse tissue environments. Cancer is usually classified according to the tissue from which the cancerous cells originate, as well as the normal cell type they most resemble. These are location and histology, respectively. A definitive diagnosis usually requires the histologic examination of a tissue biopsy specimen by a pathologist, although the initial indication of malignancy can be symptoms or radiographic imaging abnormalities [2]. Most cancers can be treated and some cured, depending on the specific type, location, and stage. Once diagnosed, cancer is usually treated with a combination of surgery, chemotherapy and radiotherapy. As research develops, treatments are becoming more specific for different varieties of cancer. There has been significant progress in the development of targeted

therapy drugs that act specifically on detectable molecular abnormalities in certain tumors, and which minimize damage to normal cells. The prognosis of cancer patients is most influenced by the type of cancer, as well as the stage, or extent of the disease. In addition, histologic grading and the presence of specific molecular markers can also be useful in establishing prognosis, as well as in determining individual treatments [3].

Apoptosis

Apoptosis is a form of programmed cell death in multicellular organisms. It is one of the housekeeping mechanism for the human beings and their cell regulation. It is one of the main types of programmed cell death (PCD) and involves a series of biochemical events leading to a characteristic cell morphology and death, in more specific terms, a series of biochemical events that lead to a variety of morphological changes, including blebbing, changes to the cell membrane such as loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Processes of disposal of cellular debris whose results do not damage the organism differentiates apoptosis from necrosis. In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis, in general, confers advantages during an organism's life cycle. For example, the differentiation of fingers and toes in a developing human embryo occurs because cells between the fingers apoptose; the result is that the digits are separate. Between 50 billion and 70 billion cells die each day due to apoptosis in the average human adult.[4,5] For an average child between the ages of 8 and 14, approximately 20 billion to 30 billion cells die a day. In a year, this amounts to the proliferation and subsequent destruction of a mass of cells equal to an individual's body weight. Research on apoptosis has increased substantially since the early 1990s. In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in an extensive variety of diseases. Excessive apoptosis causes hypotrophy, such as in ischemic damage, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer.

BCL2 Protein

With practical assumptions it is found that, the BCL2 protein playing an important role in cancer specific in tumors. It is present on outer membrane of mitochondria. It contains 239 amino acids. It suppress apoptosis, regulates cell death by controlling the mitochondrial membrane permeability. It inhibits caspase activity either by preventing the release of cytochrome c from the mitochondria and/or by binding to the apoptosis activating factor (APAF-1). With some observations and Bioinformatics approach, the BH4 motif is required for anti-apoptotic activity and for interaction with RAF-1. [6]The phosphorylation/dephosphorylation on ser-70 regulates anti-apoptosis activity. If BCL2 lacking the BH4 motif then it work as pro-apoptotic activity due to release of cytochrome c into cytosol promoting further caspase activity.

Theme

As mentioned above, BCL2 protein is responsible for the controlling the mitochondrial membrane permeability and inhibit release of cytochrome C. Due to this caspase activity is blocked. All proteins are having specific active sites for their functions. The proteins are deactivated by binding small molecule to active site. How this molecule is bind to protein study by using molecular docking technique. This technique is a most important technique in drug discovery. The molecule which gives best results is used for drug development.[6,7] If BCL2 protein is docked, then it will be inactive and due to this cytochrome c is released from mitochondria and these cytochrome c is cause caspase activity and cell undergoes apoptosis and tumor cells are death that is remove tumors. This may alternative treatment for tumor.

Role of Hypermethylation in Cancer Genomics

Before Transcription the DNA and cytosine base pair is in un-methylated form→After transcription, 70% DNA and cytosine base pair is turn into methylated form→Sometimes Hypermethylation takes place at the 5^{th} Carbon position of Cytosine due to which cancer begins and initiated→ Hence, with the help of gene silencing approach we can silence the effective part of hypermethylated gene with their expression→ For this silencing, RNAi (Interfering RNA) playing a crucial role.

MATERIALS AND METHODS

We are going to work on this topic with all this advance in-silico technologies. We have been selected some important tools like NCBI, PDB,Geno3d,Cn3d,PYMOL,patchdock respectively. We are using some noticeable steps which are given bellow.

Retrieval of the Protein sequence

Go to NCBI tool.
Select theBCL2 protein from protein database .
We find out the BCL2 Apoptosis regulator protein from homo sapiens species.
Taking the BCL2 protein sequence of cancer in FASTA format.

BCL2_HUMAN RecName: Full=Apoptosis regulator Bcl-2
MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPHPAASRDPVAR
TSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMSSQLHLTPFTARGRFATVVEEL
FRDGVNWGRIVAFFEFGGVMCVESVNREMSPLVDNIALWMTEYLNRHLHTWIQDNGGWDAFVELYGPS
MRPLFDFSWLSLKTLLSLALVGACITLGAYLGHK

Prediction of PHYSICOCHJEMICAL PROPERTIES OF PROTIEN

Open expasy protparam tool.
Paste the peptide sequence in protparam toolbox.
Get amino acid composition, atomic composition, Extinction coefficients,
Estimated Half-Life & instability index.
Protein sequence contains total 239 amino acids.

Identification of regulatory motifs in protein sequence

Open 3Dinsight motif finder database.
Paste the peptide sequence in 3Dinsight.
Determination of four motifs BH1,BH2, BH3 & BH4.

Secondary structure prediction via HNN server.

PDB Blast

Go to the Blast tool.
Select protein blast.
Paste the Peptide sequence in query box.
We predict three parameters.
sequence producing significant alignment.
E-value.
Score.

Retrieve similar 3d structure.

select the third structural link.
selected link is BCL2 protien isoform1 & it's PDB ID is 1GJH
click on the score.
View alignment in Cn3d.
View 3d structure in cn3d software.

Analysis of Ramachandran Plot & model Energy

Open Geno3d.
Paste the Peptide sequence in Geno3d.
We find list of the templates.
Select the top three Templates.
We retrieve Ramachandran plots of three templates.
We find the minimum energy of Model1 from three models
Model1,model2 & model3.

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Identification of Active site of 1GJH protein

Selet the 3D structure of protein.
Use the Pymol visualization software
Active site is predict by using Pymol.
This Active site location based on amino acids residues in between(3 to 207)

HAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPHPAASRDPVARTS
PLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMSSQLHLTPFTARGRFATVVEELFR
DGVNWGRIVAFFEFGGVMCVESVNREMSPLVDNIALWMTEYLNRHLHTWIQDNGGWDAFVELYGPSM
R

Prediction of ligand for related protein & its properties

Open the homepage of PDB
Put the ID of Related protein in PDB.
Predict the ligand 3-NITRO-N-{4-[2-(2-PHENYLETHYL)-1,3-BENZOTHIAZOL-5-YL]BENZOYL}-4-{[2-(PHENYLSULFANYL)ETHYL]AMINO}BENZENESULFONAMIDE

Docking of receptor protein 1GJH & ligand 3-NITRO-N-{4-[2-(2-PHENYLETHYL)-1,3-BENZOTHIAZOL-5-YL]BENZOYL}-4-{[2-(PHENYLSULFANYL)ETHYL]AMINO}BENZENESULFONAMIDE

Open the patchdock software.
Put the ligand & receptor's PDB file in patchdock.
Get the desired result of docking.
ACE(Atomic contact energy) of top 3 solutions is -468.11, -366.33 & - 478.88 respectively.
Validation of best model on the basis of minimum energy concept.

Flow chart of our work: In our mentioned work, we are targeting the BCL 2 protein against the initiation of cancer, and our methodology is given below:-

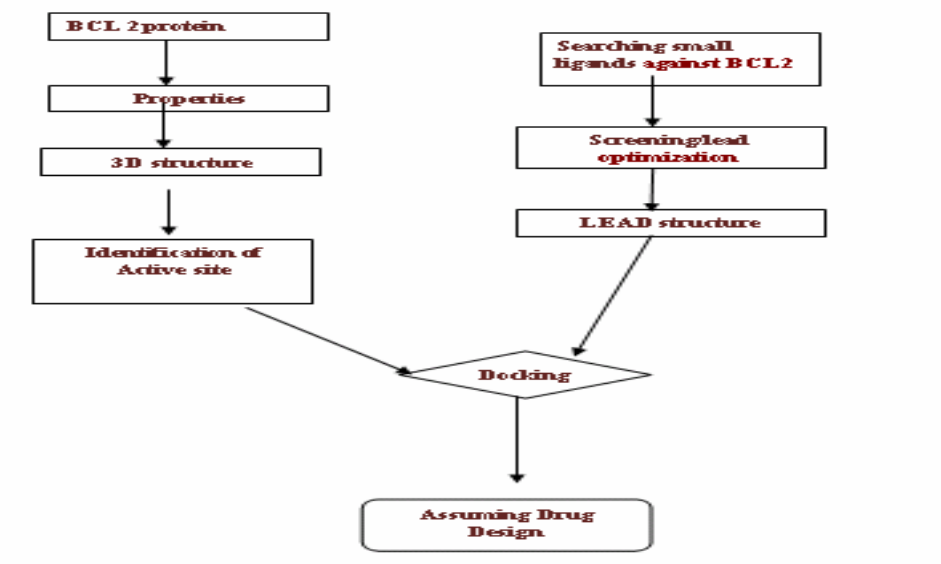


Fig. 1- Flow chart of computational docking

Result and discussion

Prediction of responsible motifs for the protein regulation.

motifs BH1,BH2,BH3 & BH4 are identified.

BH1: (137-155) LFRDGVNWGRIVAFFEFGG

BH2: (188-199) WIQDNGGWDAFV

BH3: (93-107) VHLTLRQAGDDFSRR

BH4: (10-30) DNREIVMKYIHYKLSQRGYEW

Secondary structure prediction via HNN server.

Hierarchical Neural Network result for : BCL2xx0 protein
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 121 is 50.63%
Ambigous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

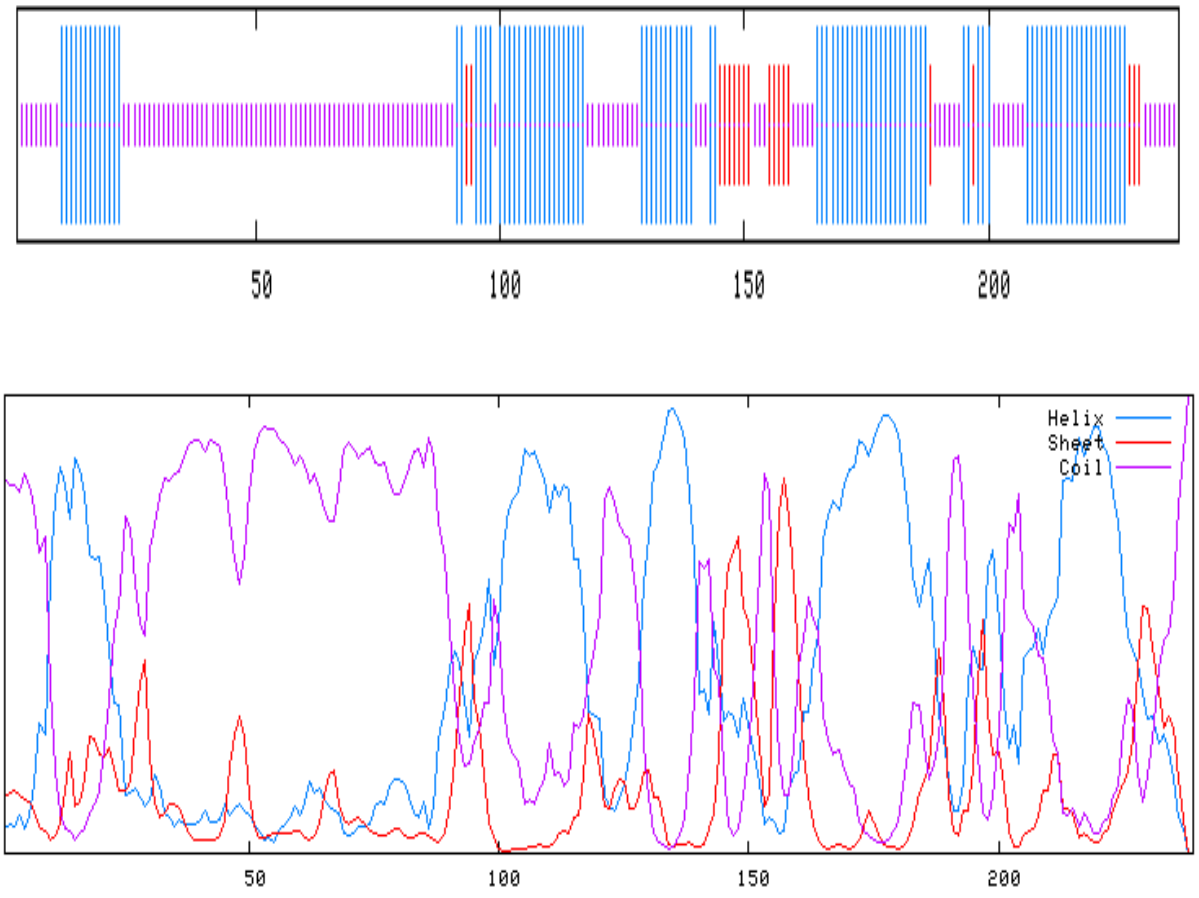


Fig. 2a,b- Predicted 2d structure of BCL2 protein.

Journal of Pharmacology Research, ISSN: 0976–7134 & E-ISSN: 0976–7142 , Vol. 1, Issue 1, 2010

Fig. 3- Predicted 3d structure of BCL-2 protein.

Homology modeling results, including Model energy & Ramachandran Plot of Model 1, Model 2 & Model 3

- Models energy (kcal/mol) :
. model 1 : -4650.91
. model 2 : 29049.40
. model 3 : -4644.91

Stereochemical quality of models with PROCHECk :

	core	allowed	generously	disallowed
Model 1	61.0%	24.7%	8.8%	5.5%

. ramachandran plot : jpeg postscript
. ramachandran plots for all residue types : jpeg

Model 2	57.7%	27.5%	9.3%	5.5%

. ramachandran plot : jpeg postscript
. ramachandran plots for all residue types : jpeg

Model 3	61.0%	26.4%	5.5%	7.1%

. ramachandran plot : jpeg postscript
. ramachandran plots for all residue types : jpeg

Prediction of Active site using Cast P & Pymol visualization software :-

Used the sequence of amino acid of protein BCL2.pdb

PDB format file view in cast p and Pymol

Predict active site of BCL2 in cast p and Pymol

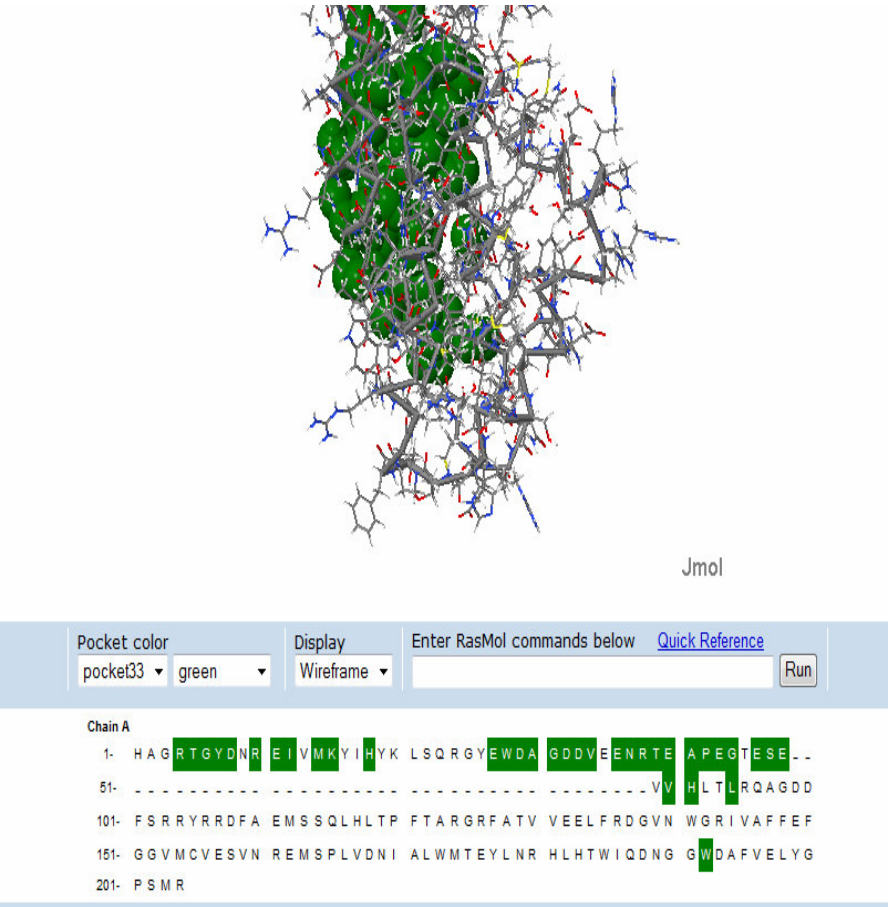
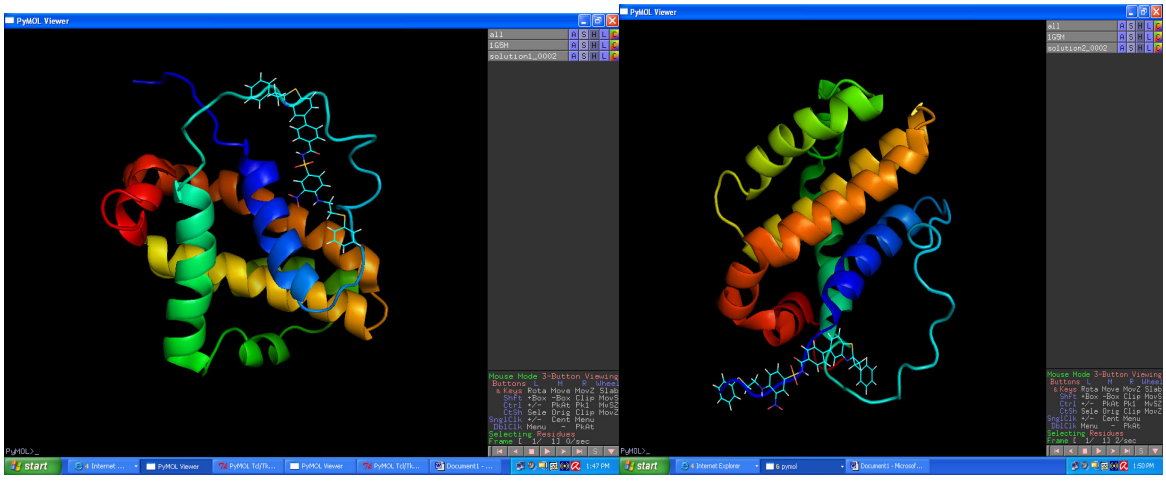
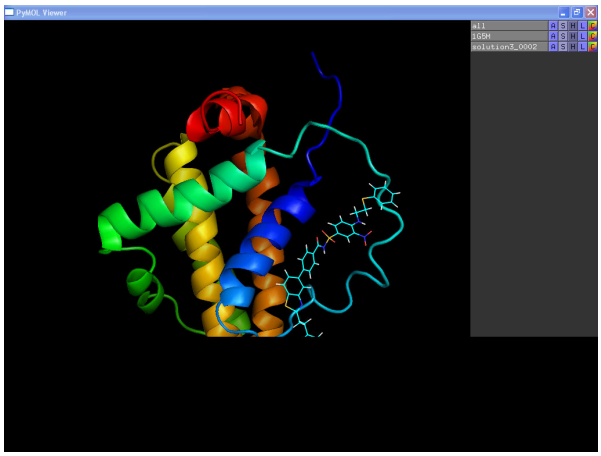


Fig. 4- Prediction of (binding pocket) active site of BCL-2 protein with their respective sequence

Docking Interpretations and comparison



Docking 1 Docking 2



Docking 3

Sr. No.	Binding Interactions	Docked energy in kcal/mol Or Atomic contact energy.
1	Receptor model 1 and Ligand 1	- 468.11.
2	Receptor model 2and Ligand 1	- 366.33
3	Receptor model 3and Ligand 1	- 478.88

Hence, the 3rd model and its docking energy is best for the future proceedings.

References

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