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Bcl-2 Targeted Structural Based Computer Aided Drug Design (CAAD) For Therapeutic Assessment of Ricin in Prostate Cancer

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ABSTRACT

Cancer is referred as uncontrolled growth of abnormal cell mass. Out of the several types of cancer, prostate cancer (PC) has become a major public health problem in men worldwide. Bcl-2 and p27 proteins are important regulatory molecules of cell cycle. Failure of cell cycle regulation leads to uncontrolled cell proliferation and causes cancer. For designing an effective structural based targeted drug, the assessment of protein-protein and protein-ligand interaction is indispensable. Therefore for treatment of PC, we selected a ribosome inactivating protein, Ricin, for assessment of its therapeutic nature. In the present work through CLUSTAL-W phylogenetic analysis, we found that Bcl-2 protein was found more conserved than p27. Further Bcl-2 was selected as target molecule for docking study with Ricin protein and other chemically synthetic inhibitor molecules i.e. 2-difluoromethylornithine (DFMO) and Sarcosine, as lead molecule. Through HEX5.1 docking software docking was performed between targeted receptor and lead molecules. Energy maximum (Emax= -93.12) and energy minimum (Emin= -163.07) was observed for docking complex of optimised and energy minimised structure of Bcl-2 receptor with Ricin, which in turn shows that it is highly stable interaction. On the other hand, for synthetic inhibitors, we found energy maximum (DFMO; Emax= -77.17, Emin= -117.83 and Sarcosine; Emax= -72.23, Emin= -103.00) and energy minimum, which are significant more as compared to Ricin docking complex. Due to ricin docking complex having less energies shows stable interaction with Bcl-2. We also observed that Ricin is less toxic (lesser log P value) as compared to other molecules by toxicity analysis by ADME/TOX server. These evidences show this Ricin could be better drug for PC. Further results are needed to validate by *in vitro* and *in vivo* study to make proper elucidation of drug for better PC treatment.

Keywords: Ricin, Prostate cancer, Molecular Docking, Bcl-2, Anti-cancer.

INTRODUCTION

Cancer is referred as uncontrolled growth of abnormal cell mass, also known malignancy. Prostate cancer (PC) has become a major public health problem in men. [1] Bcl-2 and p27 proteins are most important cell cycle regulatory molecules. Bcl-2 is an anti-apoptotic protein; it plays the role in the regulation of the cell cycle. [2]

Bcl-2 is member of the Bcl-2 family that regulates the apoptosis. Bcl-2 is overexpressed in several tumours including prostate cancer (PC) and supresses the apoptosis stimuli. [3] p27 is an inhibitor of cyclin dependent kinase complex and it promotes the cell progression. This is also oncogenic protein which is downregulated during PC progression. [4] Selection of a potential therapeutic target for the treatment is a daunting task. Ricin is a stored toxic protein found in castor beans (*Ricinus communis*). The Ricin protein is comprised of two glycoprotein chains "A" and "B" that

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are joined by disulphide bond. [5] The "B" chain helps in internalization of Ricin through endocytosis process. The chain "A" reaches cytosol and exhibits N-glycosidase activity, which hydrolyses glycosidic bond of a specific adenine residue in conserved region of 28S rRNA leading to ribosome inactivation. [6] This ribosome inactivation results inhibition of protein synthesis and subsequently cell death. Ricin is having divers effect, its chain "A" has been used to develop immunotoxin which shows anticancer and anti-AIDS activity through ribosome inactivation, release of cytokines, and generation of oxidative stress and activation of apoptotic pathways. [7] Targeted drugs are evolved for better treatment of disease. The protein-protein and protein-ligand interaction plays a significant role in structural based drug designing. Molecular docking analysis of drug and target molecule shows interaction force and proper orientation of drug. [8-9] The prediction of putative protein-ligand interaction studied by computational docking methods are increasing importance in the field of structure based drug designing. [10]

Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed toward improving the methods in drug discovery. Hex is interactive molecular graphics software for calculating and displaying feasible docking between protein-ligand, assuming the ligand is rigid, and it can docks with receptor target molecule based on their 3D structure. It is one of the few docking programs which have built-in graphics to view the results. [11]

This study is aimed to select the target molecule and molecular docking analysis of lead molecules Ricin, DFMO and Sarcosine structural based drug designing against target receptor for the treatment of PC.

MATERIALS AND METHODS

Sequence retrieval

The protein sequence of Bcl-2 and p27 (Kip1 protein) were retrieved in FASTA format from NCBI database (http://www.ncbi.nlm.nih.gov/) and used for our studies.

Phylogenetic analysis

CLUSTAL-W produces biologically meaningful sequence alignments of divergent sequences and uses to calculate the best match for the selected sequences and lines them up so that the identities, similarities and differences can be seen. Phylogenetic analysis was performed using CLUSTAL-W tool while taking Bcl-2 and p27 protein sequences and found the results in the form of dendrogram that shows the sequence similarities and BOXSHADE result that visualizes conserved region.

Geometric optimization and energy minimization

For geometric optimization and energy minimization of Drug and receptor molecules was performed using Argus lab. Argus lab is the introductory molecular modelling software package. [12] It does different type of calculation cleaning geometry, optimizing geometry and energy minimization to make the molecule stable and in proper geometry.

Molecular Docking

Docking was performed between receptor (Bcl-2) and lead, natural molecule Ricin and other synthetic drugs i.e. DFMO and Sarcosine. Docking represents the binding of drug molecule with the receptor binding sites. Stable interaction shows the minimum energy level in Docked complex. Energy minimum and maximum was observed in the result of individual Docked complex.

Toxicity analysis

Toxicity analysis was done by ADME/TOX server. According to Lipinski 'rule of five' log P value should less than 5 (<5) that shows the less toxic drug. [13] We analysed the toxicity (logP) of all three lead molecules i.e. Ricin, DFMO and Sarcosine.

Prostate cancer pathway

Kyoto Encyclopaedia of Genes and Genomes (KEGG) is the knowledge database for the systemic execution of the gene product and metabolites. Database covers metabolic pathways, disease, signalling and other chemical ligands pathways. [14]

We explored the prostate cancer pathway by using KEGG pathway tool, which shows the involvement of Bcl-2 protein.

RESULTS AND DISCUSSION

Retrieval of sequence

Protein sequences of Bcl-2 and p27 have 459 and 191 amino acid residues, respectively. These both are known protein which belongs to human, the sequence are given below

Bcl-2

>gi|4505959|ref|NP_002689.1| POU domain, class 2, transcription factor 2 [Homo sapiens]
MVHSSMGAPEIRMSKPLEAEKQGLDSPSEHTDTERN
GPDTNHQNPQNKTSPFSVSPTGPSTKIKAEDPSGDSA
PAAPLPPQPAQPHLPQAQLMLTGSQLAGDIQQLLQ
LQQLVLVPGHHLQPPAQFLLPQAQQSQPGLLPTPNL
FQLPQQTQGALLTSQPRAGLPTQPPKCLEPPSHPEEP
SDLEELEQFARTFKQRRIKLGFTQGDVGLAMGKLYG
NDFSQTTISRFEALNLSFKNMCKLKPLLEKWLNDAE
TMSVDSSLPSPNQLSSPSLGFDGLPGRRRKKRTSIETN
VRFALEKSFLANQKPTSEEILLIAEQLHMEKEVIRVW
FCNRRQKEKRINPCSAAPMLPSPGKPASYSPHMVTP
QGGAGTLPLSQASSSLSTTVTTLSSAVGTLHPSRTAG
GGGGGGGAAPPLNSIPSVTPPPPATTNSTNPSPQGS
HSAIGLSGLNPSTGPGLWWNPAPYQP

p27

>gi|2982673|dbj|BAA25263.1| p27 [Homo sapiens]
MALNGAEVDDFSWEPPTEAETKVLQARRERQDRISR
LMGDYLLRGYRMLGETCADCGTILLQDKQRKIYC
VACQELDSDVDKDNPALNAQAALSQAREHQLASA
SELPLGSRPAPQPPVPRPEHCEGAAAGLKAAQGPPA
PAVPPNTDVMACTQTALLQKLTWASAELGSSTSLET
SIQLCGLIRACAEALRSLQQLQH

Phylogenetic analysis

Through CLUSTAL-W analysis, we observed dendrogram of Bcl-2 and p27 proteins shows that both protein are evolutionary close to each other based on their sequence similarity. The BOXSHADE result in yellow colour shade is showing conserved region. In the given result, this is clear that that Bcl-2 is having more conserved region than p27, so we selected Bcl-2 as target receptor for further study.

Dendrogram

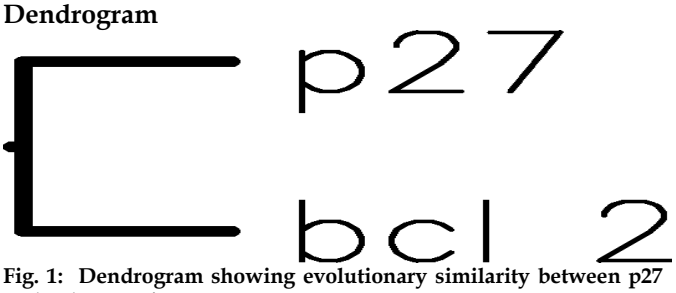

Fig. 1: Dendrogram showing evolutionary similarity between p27 and Bcl-2 protein

BOXSHADE

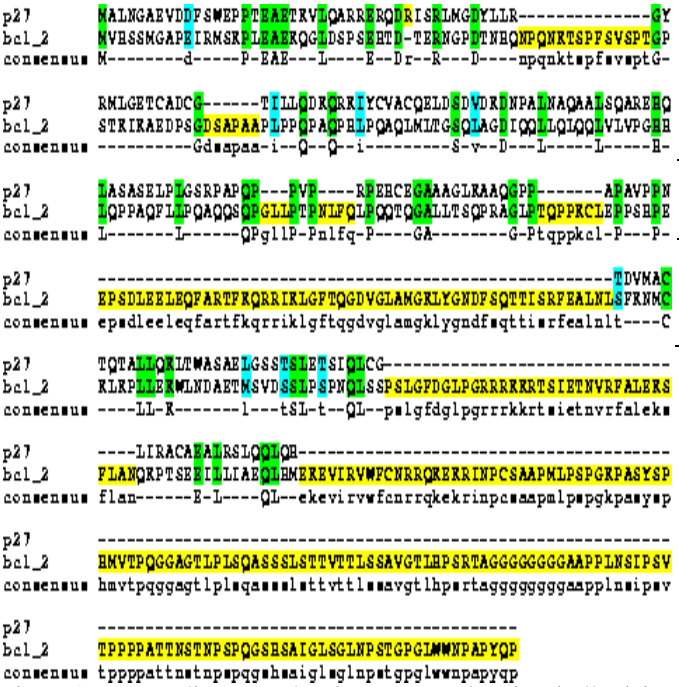

Fig. 2: Sequence alignment showing conserved region (yellow) in p27 and Bcl-2 protein

Argus lab

Optimized and Energy Minimized structure of Ricin, Sarcosin and DFMO

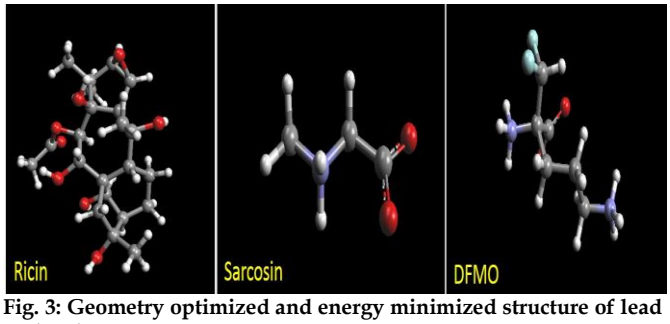

Fig. 3: Geometry optimized and energy minimized structure of lead molecules

Through Argus lab software we optimized and energy minimized the structure of lead molecules (Fig. 1).

Molecular Docking

Docking of receptor molecule Bcl-2 with Ricin and other synthetic lead molecule was performed individually and found the minimum binding energy of Ricin docked with receptor (**Emin** = -163.07, **Emax** = -93.12) than other molecules Sarcosin (**Emin** = -103.00, **Emax** = - 72.23) and DFMO (**Emin** = -117.83, **Emax** = -77.17). Less binding energy denotes the high interaction and binding stability with the receptor molecule that is the essential property of a good drug. [15]

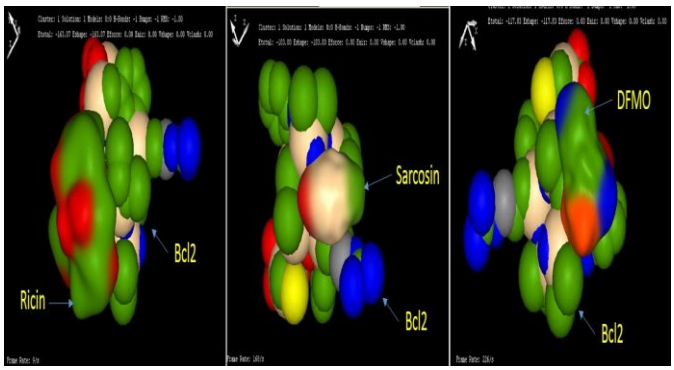

Fig. 4: Docking complex of lead (Ricin, Sarcosine and DFMO) with receptor molecule (Bcl-2)

Table 1: Toxicity analysis

S. No	Receptor name	Ligand name	Log p of ligand	Energy level	
				E-min.	E-max.
1.	Bcl 2	Ricin	-0.21	-163.07	-93.12
2.	Bcl 2	Sarcosine	< -2	103.00	-72.23
3.	Bcl 2	2-difluoromethylornithine (DFMO)	< -2	-117.83	-77.17

Toxicity analysis

We also found that Ricin is less toxic than other lead molecules. Through ADME/Tox server found less log P values (-0.21) representing less toxicity than Sarcosin (< -2) and DFMO (< -2) (Table 1).

These lead molecules fulfill the Lipinski ' rule of five' and Ricin shows the better results as compared to other lead molecules.

KEGG pathway analysis

Through KEGG pathway analysis found Bcl-2 is involved in prostate cancer pathway. Bcl-2 is triggered by NF-kappa B signalling and it prevents the apoptotic pathway. Bcl-2 is responsible for cell survival.

In the present work through phylogenetic analysis we found Bcl-2 protein is having more conserved region than p27 so that Bcl-2 selected as target receptor molecule. In course of cancer, Bcl-2 is promoted to over expression that prevents the cell apoptosis and uncontrolled cell proliferation leading to cancer. This conserved region shows the less chance to change in the structure and Structural based drug design has become powerful for long lasting effect. Docking results show the stable docking of Ricin and its less toxicity are evidences toward Ricin to make as a better drug for the treatment of PC. *In silico* results are need to be validated by *in vitro* experiments in laboratory.

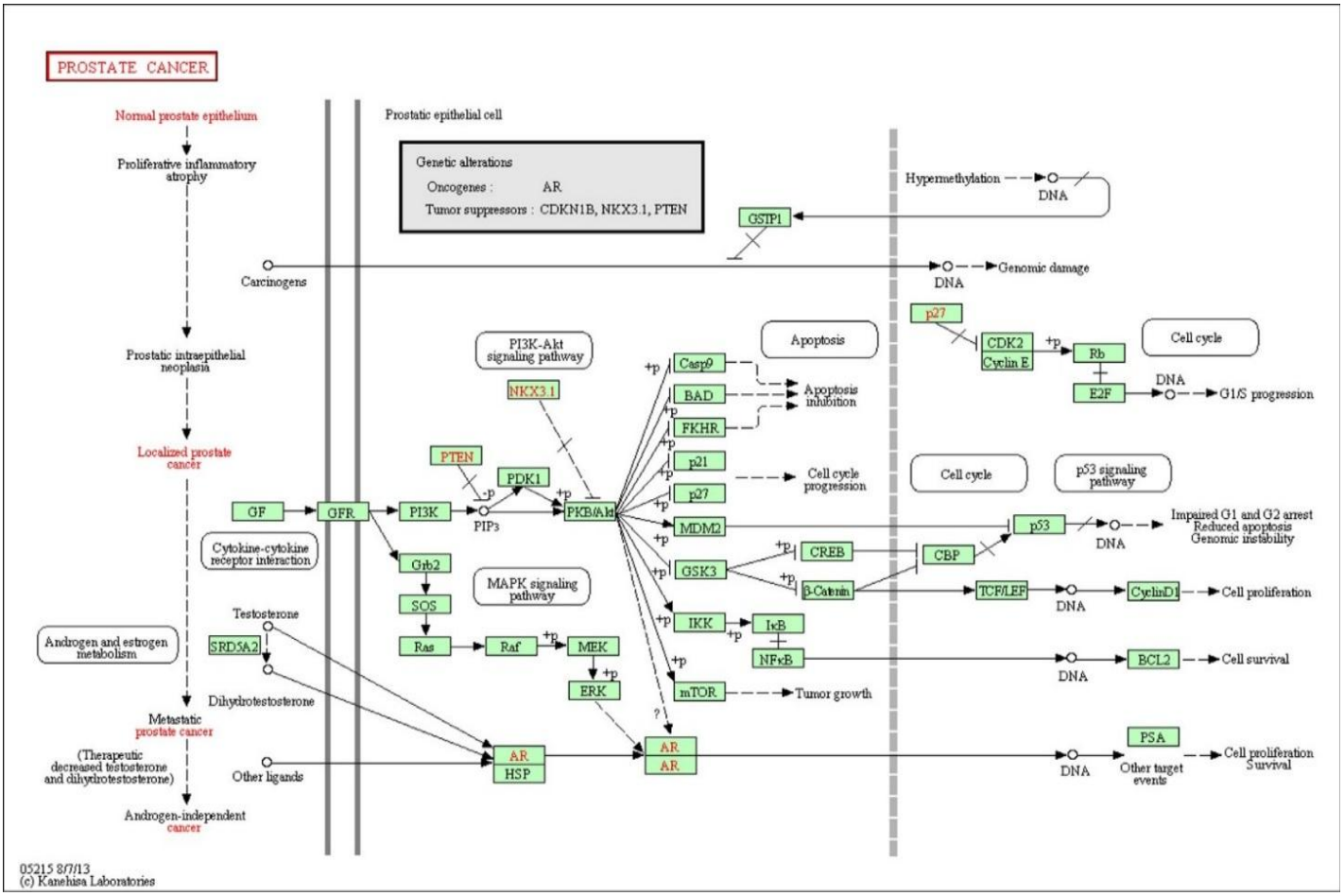

Fig. 5: Prostate cancer KEGG pathway showing involvement of Bcl-2

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