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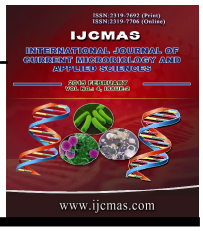

Original Research Article

Insilico docking evaluation of bacterial derived anticancer (Lectin) Protein with different oncogene protein

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Keywords

Oncogene proteins, Lectin, *Pseudomonas stanieri*, Molecular docking

A B S T R A C T

The present study focused on molecular docking analysis to identify the potential compound derived from bacterial organisms *Pseudomonas stanieri* against cancer genes. The 3D structure of the cancer protein was retrieved from protein data bank (PDB) and protein binding sites of the test compound were identified. The result revealed, among the 10 different cancer causing gene (BRCA-1, BRCA-2, V-src, Vir C1, Tra M, Atao1, CYCD3, NIP4, Nod C, Nod D1) lectin protein binds very effectively with 4 cancer genes (BRCA-2, V-Src, N1P4, Nod C).

Introduction

Cell is a structural and functional unit of all living organism. Our body is made up of billion to trillions of cells which carry out different functions like digestion, breathing, thinking, sensation and so on. All these functions can be carried out only by healthy cells, any abnormalities in the cells leads to alteration in the functioning. These abnormal cells are known as cancer cells. Breast cancer is a cancer that starts in the cells of the breast in women and men. Worldwide, breast cancer is the second most common type of cancer after lung cancer.

In 1994, Researcher's isolated Breast cancer –1 (BRCA-1) gene in chromosome 17, one of the 23 pairs of chromosomes from base pair 38,449,843 to base pair 38,530,933. It is a human gene, some mutations of which are associated with a significant increase in the risk of breast cancer. Breast cancer Type 2 Susceptibility protein (BRCA –2) is a human gene, located on the chromosome 13, from base pair 31,787,616 to base pair 31,871,804. BRCA –2 appears to be a cancer – causing gene when altered. In 1911, Peyton Rous discovered tumor inducing retrovirus called Rous sarcoma virus. It contain a gene called V-src (viral – sarcoma) which involved in the formation of cancer in mesodermal connective tissues (Rosalyn *et al*., 2000).

Reason for breast cancer is still unknown but factors like late pregnancy, early menstruation, late menopause [Hulka and Stark ,1995], race, older age, lack of breast feeding , less children [Yager and Dvidson, 2009] hormone therapies , mutation in genes influence the growth of tumour.

Agrobacterium tumefaciens causes crown gall, a neoplastic disease of dicotyledonous plants, by transferring a specific segment of DNA from its large Ti plasmid to plant cells. The virulence genes of *Agrobacteriu*m are required for this organism to genetically transform plant cells (scott *et al*., 1986). VirC1gene is essential for the transfer and integration of T-DNA into the chromosomal DNA of plant cells. Tra genes are regulated by a novel regulated protein, designated as TraM. Null mutations in traM causes strongly increased conjugation, tra gene transcription and IAA production.

The *Arabidopsis thaliana* amine oxidase 1 (Atao1) gene of *Arabidopsis thaliana* encodes an extracellular copper amine oxidase expressed during early stages of vascular tissue development, which induces root gall formation. The CYCD3 gene expression in *Arabidopis thaliana* displays an increased rate of leaf initiation, which is shown to be associated with distinct changes in the structural organization of their shoot apical meristem. *Medicagotruncatula* consist of more than 300- nodule specific genes. NIP4 coding for small secreted polypeptides involved in the formation of indeterminate nodule (Peter *et al*., 2004).

Rhizobium sp releases some growth hormones to cortical cells; as a result the chromosome number may get doubled. By continuous growth, the mass of cells protrudes from the surface of the root, in the form of nodule (Yelton *et al*., 1987). Nod C and Nod D is nodule forming genes in *Rhizobium*. Nod C codes for 426 amino acids that causes swelling and curling of root hairs.

A nod D gene code for a protein that binds with flavonoids produced by legumes and induces the transcription of nod genes, which encodes 311 amino acids. Almost all microorganism express surface exposed carbohydrates. The carbohydrates may be covalent bound as in glycosylated teichoic acids to peptidoglycan, or non-covalently bound as in capsulated polysaccharides. Every surface exposed carbohydrate is a potential lectin reactive site. The ability of lectins to complex with microbial glycoconjugates has made it possible to employ the proteins as probes and sorbents for whole cells.

Carbohydrate binding proteins, called lectins, are well known to bind to both plasma and inter membranes in a variety of cell types. The result of such an interaction is the initiation of series of cellular events that include oxyradical production, phagocytosis, and modulation of the action of lymphokinases. The interaction between a lectin and the cell membrane depends on the type of carbohydrate moiety present in the membrane. Since locations are specific for a particular sugar, lection binding has been used as a tool to provide insight into the biochemical composition of membranes and to identify cell types in cytochemical studies of both issue slices and cultured cells.

In the present study, the isolated marine bacterium *Pseudomonas stanieri was used* for the production of lectin compound. 10 different cancer genes (HUMAN-BRCA-1, BRCA-2, V-src, ***Agrobacterium tumefaciens***- Vir C1, Tra M, ***Medicagotruncatula***- Atao1, CYCD3, NIP4, ***Rhizobium***-Nod C, Nod D1) were retrieved from gene bank.

The 3 dimensional structure and binding efficiency of lectin in complex with these cancer genes proteins were predicted using computer assisted molecular docking. These findings will provide a target based drug discovery for tumor.

Materials and Methods

Isolation of microorganism

The marine water sample was collected from Rameswaram, Tamil nadu. It was serially diluted and plated on marine agar medium. After incubation, the isolated bacteria was tentatively identified by biochemical test and confirmed by 16s r RNA sequence analysis.

Production of lectin

Bacteria was grown at 37°C with vigorous shaking for 3 days in nutrient broth and supplied with 0.4% trehalose. The harvested cells were washed 3 times and disintegrated by homogenization. After incubation, cell free extracts are collected and subjected to heating at 65°C for 15 minutes then ammonium sulfate precipitation (70% saturation) was performed.

Haemagglutination assay

1% Human erythrocytes was prepared and washed with phosphate buffered (0.025 M, pH 7.2), isotonic NaCl solution (PBS) for 3 times. A 50µl of prepared lectin was serially diluted with 50µl of PBS to produce twofold dilutions. To this 1% erythrocyte suspension (50µl) was added to each well and kept for 2 hours at room temperature. The haemagglutinating activity was examined as described (Gilboa-Garber, 1982).The activity was represented by the number of wells in which there was haemagglutinaton, along the twofold dilutions (e.g. 7 positive wells = positive reaction up to dilution of 1:128 or original activity of 128 haemagglutination units).The haemagglutination inhibition intensity was represented by the highest number of dilutions at which no significant haemagglutination occurred.

16s rRNA amplication for identification of isolate

The forward and reverse primer sequence 5’ GATTAGATACCCTGGTAGTCCAC3’and 5’CCCGGGAAACGTATTCCACGG 3’ were used for the amplified the 16s rRNA of isolated bacteria. (Bioserve Biotechnologies (India) pvt.Ltd. Hyderabad).

Lectin Gene Amplification

A Lectin gene primer sequence was designed using available Lectin Gene sequence from the gene bank. The forward and reverse primer was designed, so as to carry out the specific gene amplification. The forward primer sequence 5’GCCTGCCGTCGCAGATCAACC 3’ and the reverse primer sequence 5’GCGGGCGGGCAGACCGTCC 3’ were used for amplification of the Lectin Gene.

Retrieval of cancer genes protein

The 10 different cancer gene (BRCA-1, BRCA-2, V-src, Atao1, Cycd3, Nip4 ,Vir C1, Tra M, Nod C, Nod D1) and drug (lectin) were used in this study. The sequence data for cancer gene proteins was obtained from the Swiss-Prot. Swiss-Prot strives to provide reliable protein sequences associated with a high level of annotation, a minimal level of redundancy and high level of integration with other databases.

Homology modeling

Among all current theoretical approaches, comparative modeling is the only method that can reliably generate a 3D model of a protein from its amino acid sequence. Modeling of protein structures usually requires extensive expertise in structural biology and the use of highly specialized computer programs for each of the

individual steps of the modeling process [Tramontano et al., 2001]. The method of homology modeling is based on the observation that protein tertiary structure is better conserved than amino acid sequence [Marti-Renom et al.,2000]

Molecular docking

Hex 5.1 is an interactive molecular graphics program. It is used for docking of the ligand (lectin) with the target (cancer gene) protein. Currently, Hex understands protein structures in PDB format.

Prediction of active site

VIDA is an interactive molecular graphics active site prediction program. It is used for docking of the ligand (lectin) with the target (cancer gene) protein. VIDA Understands protein structures in PDB format. Binding sites of amino acids in cancer genes with lectin were identified using VIDA (offline software).

Result and Discussion

Lectin producing microbes was isolated from the marine water. It was confirmed as *Pseudomonas stanieri* by 16s rRNA analysis. The bacterial gene sequences were deposited in the gene bank (Accession number EU 732701) and the lectin gene sequences was deposited in gene bank (Accession number EU 732704). The cell free extract lectin protein and supernatant protein are used for haemagglutination assay. The haemagglutinating activity was occurred at dilution of 1:12 of *Pseudomonas stanieri.* Mannose binding Lectin Protein was confirmed by the analysis of BLAST search tool. Sequence Analysis and database searches were performed with National Centre for Biotechnology Information BLAST server (http:// www. ncbi.html. nih.gov). In our study , we retrieval 10 different tumour causing genes from NCBI gene bank for similarity study and inhibition of that gene protein by isolated *Pseudomonas stanieri* lectin protein was performed using molecular docking bio-informatics tools.

The docking energy level of each cancer gene with lectin was calculated by using Hex version 5.1 and the results were show in table 1. In Hex version 5.1, the docking energy level of cancer genes was compared and noted that the lectin with the cancer gene N1P4 gives the lowest energy level of about -640.58. This shows that lectin may be high effective against cancer (tumorigenesis) protein. In VIDA software, the active sites of between the cancer gene and lectin can be detected. The lectin docked with the BRCA-2 at the active site LEU:1522(plate.1). The lectin docked with the V-src at the active sites GLU:356, GLY:355, ASP:351, MET:484, TYR:482.(plate 2). The lectin docked with the N1P4 at the active sites PRO:153, ALA:287, THR:217, PHE:152, LEU:272.(plate 3). The lectin docked with the Nod C at the active sites GLN:277, GLN:278, LEU:230,GLN:229.(plate 4). Among the 10 cancer gene, lectin protein binds very effectively with 4 cancer genes (BRCA-2, V-Src, N1P4, Nod C). All the 10 genes sequences were aligned using clustal – W. There is no similarity between these sequences. The 3D structure of cancer gene protein and lectin protein were visualized by swiss – model Rasmol software, and also the structure can be down loaded in the PDB format (Joachimia k *et al*., 2001). From this, we find out amino acid positions, α –helix and β –sheet of proteins.

The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking

methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization. The setting up of the input structures for the docking is just as important as the docking itself, and analyzing the results of stochastic search methods can sometimes be unclear.

Docking was performed using Hex (Navia and Murko, 1992), it reveals that energy value ranging from -301.81 to -663.82 Kcal/ mol (Table 1). When the energy value is high, the binding capacity of ligand (lectin) is higher with receptor molecules. VIDA is one of software used for detecting molecular docking and active site of the molecules. In this tool, we got four cancer genes (BRCA2, V-src, Nod C,NIP4) were effectively bound with the lectin compound. The docking model and active sites were displayed (plate 1 –4).

Plate 1 illustrates the representative interacting surface between BRCA 2 and lectin. In this, amino acids of lectin make direct contact with BRCA 2 protein by ionic interaction. The docked energy scores were higher in the V-Src protein bound with lectin (-517.74 Kcal/mol) the interaction regions are GLU:356, GLY:355, ASP:351 MET:484, TYR:482. Jayakanthan *et al*., (2009) reported that the cancer causing H – Ras p 21 mutant protein docked with lead. All the leads were docked into effector region forming interaction with ILE 36, GLU 37, ASP 38 and SER 39.

Ras means protooncogenes codes for small proteins of 189 aminoacids with molecular weight of 21 KDa protein. Activation of point mutations in the Ras is one of the most frequent genetic alterations associated with human cancer. Approximately 90% of these activating mutations occur in codons 12 and 59, identifying these codons as hot spot targets. Specifically, the mutation replaces the aminoacid glycine with the aminoacid valine at position 12 (Jayakanthan *et al*., 2009). The lectin protein inhibit the activation of Src. Normally, the activated Src in cancer indicates that this protein may play a significant role in the progression of many cancers. The recent Molecular docking method was used to inhibit the cancer causing protein specifically and increase the apoptosis tumors cells.

RAI gene is responsible for cancer or leukemia, RAI protein inhibited by the p53 gene protein (acts as tumor suppressor, cell cycle arrest and induction of apoptosis), but progression of carcinogenesis by p65. Tomoda *et al*., (2008) found that RAI interacts with p 53 and p65 with distinct amino acid residues, and that substitution of aminoacid residues on RAI that ruined the interaction with p53 did not substantially damage the interaction with p65. Nematode gene (NIP4) protein docked with lectin protein, the binding energy values are higher (-640.58 Kcal/mol). The root knot nematode highly damages the commercial crops. Molecular docking findings reveal that, the nematodes are killed by lectin compounds.

Nod C and Nod D are nodule forming genes in *Rhizobium sp*. Nod C codes for 426 aminoacids that causes swelling and curling of root hairs. Our finding shown that Nod C proteins are effectively binding with lectin (-663.82 Kcal/mol) and inhibit the nodule formation. But *Rhizobium Sp* produces nodules with legume plant. So that, most of the legume rhizosphere soil bacteria may not produce lectin. The findings clearly indicate feasibility and accuracy of such computational methods in interpreting the

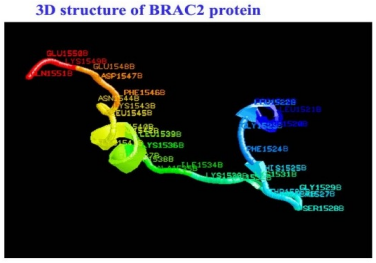
biological significance of interacting aminoacids. Lectin would be an effective drug to inhibit function of cancer gene protein. Moreover, in future invitro analyses will carry out for nematicidal activity and confirmation of whether lectin producing bacteria present or not in the rhizosphere soil of legume plant.

Table.1 Energy binding value of isolated *Pseudomonas stanieri* Lectin gene with various Cancer genes

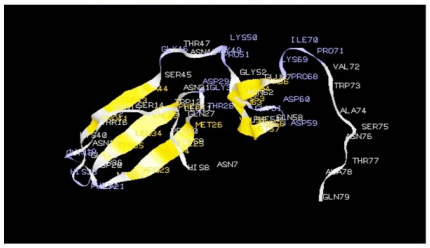
S.No	Cancer genes	Binding energy values
1	BRCA 1	-345.33
2	BRCA 2	-550.20
3	V-SRC	-517.74
4	VIR C1	-301.81
5	TRA M	-468.87
6	ATAO1	-467.36
7	CYCD3	-319.45
8	NIP4	-640.58
9	NOD C	-663.82
10	NOD D	-465.55

PLATE 1

3D structure of BRAC2 protein



3D structure of lectin protein



Molecular Docking between lectin and BRAC2 proteins

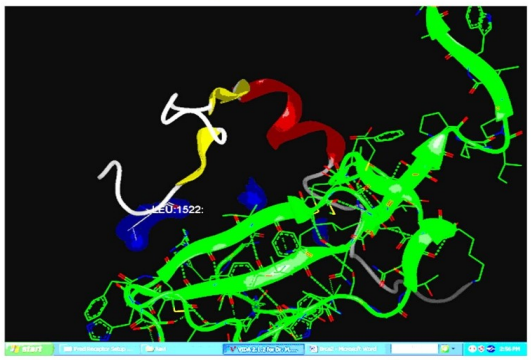
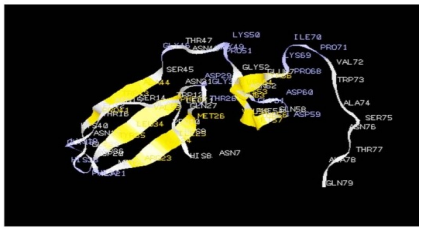


PLATE 2

3D structure of VSrc protein

3D structure of lectin protein



Molecular Docking between lectin and VSrc proteins

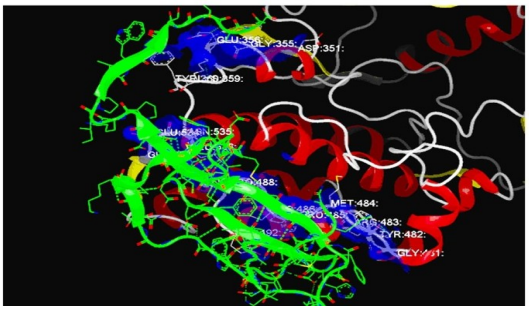
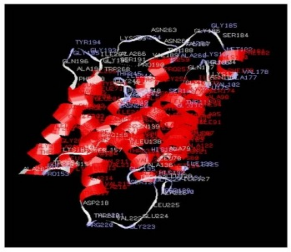
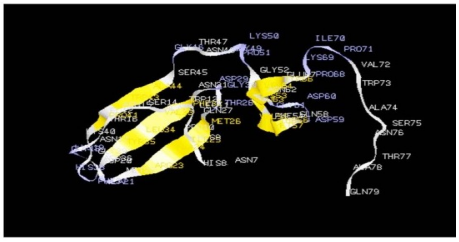


PLATE 3

3D structure of Nip4 protein



3D structure of lectin protein



Molecular Docking between lectin and Nip4 proteins

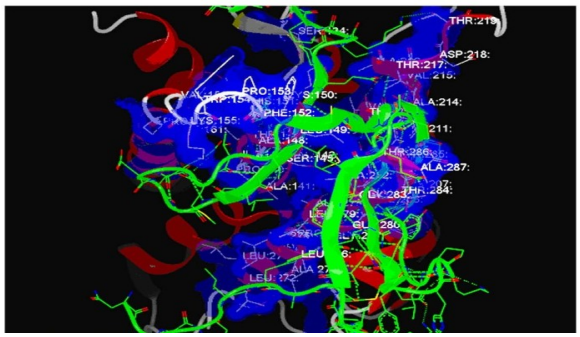
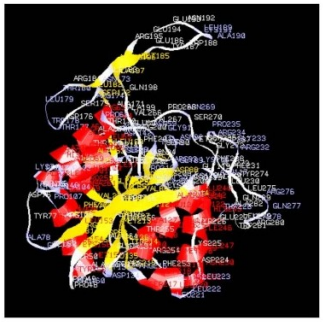
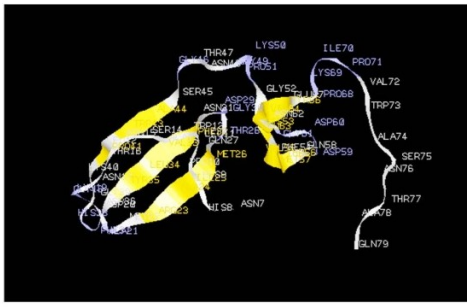


PLATE 4

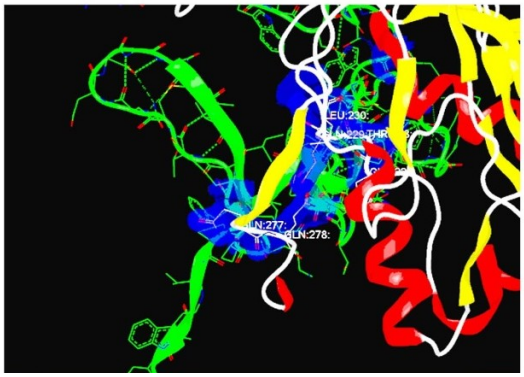
3D structure of Nodc protein



3D structure of lectin protein



Molecular Docking between lectin and Nodc proteins



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