Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(4): 254-260

Research Article ISSN 0975-248X

In Silico Investigation and Docking Studies of E2F3 Tumor Marker: Discovery and Evaluation of Potential Inhibitors for Prostate and Breast Cancer

Sinosh Skariyachan*, Rao Shruthi Krishnan, Usha B Biradar

Department of Biotechnology, Dayananda Sagar College of Engineering, Bangalore, India

ABSTRACT
E2F3 encodes a transcription factor important for cell cycle regulation and DNA replication. It plays a significant role in the development of various types of human cancer. Genomics and proteomics features of the tumor marker have a pronounced significance in the pharmainformatics studies. The crystal structure of E2F3 is not available in any structural database; hence a 3D structure is very essential for structural studies and discovery of potential inhibitors against tumour proteins. In this study we modelled a 3D structure of E2F3 by X-ray crystal structure of Bovine Bc1 with Azoxystrobin of *Bos taurus* (PDB ID: 1SQB, Chain B) used as the template. Our study found that E2F3 predominantly consists of α helix. The RMSD value of modelled protein was found to be 0.5 A^o and steriochemical validation shows 86. 1% residues are in allowed region of Ramachandran plot. Further validation was done by various empirical force fields. Overall quality factor of the model identified to be 57.36 and error values of individual residues are negligible. The modeled protein was submitted to Protein Model Database and can be downloaded with PMDID 0076554.With the help of docking studies the best ligand against E2F3 was found to be Vinblastine, an antitumor alkaloid isolated from *Vinca rosea,* with binding energy -4558.33.The ligand interacts with the modeled protein at residues Glu-432, Asp-433, Tyr-434, Leu-435 and 436.The other best inhibitors identified from our study were Oncovin, Navelbine, Taxol and Taxotere. The investigation concluded that these drugs could be used as the potential inhibitors against E2F3 tumor marker in prostate and breast cancer.

Keywords: E2F3, Tumor marker, Homology Modeling, Refinement, Docking, Vinblastine.

INTRODUCTION

Cancer is associated with multiple genetic and regulatory aberrations in the cell. It is a highly heterogeneous disease, both morphologically and genetically. [1] Analysis of cancer pathways shows a number of interrelated markers responsible for oncogenesis. Selection of a potential target for therapy is a daunting task. In this study the protein of interest was E2F3, member of the E2F family of transcription factors.E2F3 is an activator of transcription that is amplified or over-expressed in several tumors, including those of the bladder, prostate and lung (Fig. 1). E2F3 has been demonstrated to drive the expression of Oncomir- 1 *in vitro*. Oncomir-1 is an oncogenic cluster of microRNAs located on chromosome 13 that has been shown to play an important role in promoting tumor cell proliferation. [2] The E2F3 locus encodes two proteins, E2F3a and E2F3b, which differ only in their N-terminal sequences. E2F3a has been linked to the transcriptional activation of E2F-responsive genes. This family of transcription factors have several targets involved in cell cycle such as Cyclin E [3] and expression of some E2F family members have been associated with poor prognosis in breast carcinomas. [4]

In-silico modeling is a multidisciplinary method integrating mathematical models with experimental (*in vitro* and *in vivo*) and clinical data. [5] Homology or evolutionary relatedness represents a key concept in studying protein sequence, structure, and function. Homologs can be inferred by sequence similarity search tools such as the popular sequence-profile comparison method PSI-BLAST. [6] Basic Local Alignment Search Tool (BLAST) provides an "expect" value, statistical information about the significance of each alignment. [7] MACS (multiple alignments of complete sequences) are typically used to perform comparative analysis at the genome level, to define the phylogenetic relationships between organisms in evolutionary studies, to identify conserved functional residues, motifs or domains and to predict protein. [8] Comparative, or homology, modeling of

***Corresponding author: Mr. Sinosh Skariyachan,** Department of Biotechnology, Dayananda Sagar College of Engineering, Bangalore, India; **Tel.:** +91-9739654015; **Fax:** + 91-80-26635929; **E-mail:** sinoshskariya@gmail.com

protein structures is the most widely used prediction method when the target protein has homologues of known structure. [9] This study is aimed at the genomic and proteomic characterization, modeling and evaluating the verified structure of E2F3 as a potential drug target in cancer therapy. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. [10] A variety of experimental and computational techniques can be used to identify possible protein binding partners of protein. The prediction of putative protein-ligand interaction studied by computational docking methods is of increasing importance in the field of structure based drug designing. [11] In this study we have modeled a detailed 3D structure of E2F3 and docking studies were carried out to design and optimize novel and potential inhibitors for E2F3tumor marker.

MATERIALS AND METHODS

Sequence retrieval of E2F3 marker

The protein sequence of E2F3, Accession Number O00716 was retrieved from UNIPORT [12] database and the FASTA sequence is used for our studies.

Screening for best homologous templates

The target protein sequence was blasted using BLASTP [13-14] across Protein Data Bank to obtain the most identical structures based on the percentage of identity, similarity, expectation values and alignment scores which could be considered as templates in the modeling procedure.

Multiple sequence alignment and phylogenetic characterization

Multiple sequence alignment is the key step for the prediction of protein structure and identification of specific functional residue in protein. T-COFFEE [15] tool was used for alignment of query E2F3 with template sequences to analyze the conservation factor in the closely related proteins. The guide tree created from the best homologs was analyzed by NJ Plot.

Proteogenomic analysis of E2F3

In order to analyze various sites present in the query, the protein sequence was reverse translated to a 1395 base pair nucleotide sequence using the tool called Reverse Translate hosted in ExPASy server. The reverse translated sequence was submitted to ORF-Finder to obtain the various open reading frames. The proteomic analysis was carried out using Protparam, PSI-PRED [16] TOPPRED [17], and SMART [18] tools

In silico Comparative Modeling of E2F3 Protein

An alignment between the target and temple is performed (Fig 2). An *in silico* comparative modeling of the E2F3 protein was carried out by the MODELLER 9v7. [19] The best homolog identified earlier in the proteogenomic characterization was used to generate alignment, atom and the script files for modeling. The target and template was superimposed by DaliLite [20] and the backbone RMSD value was analysed. The modeled protein was visualized by PyMOL. [21]

Model refinement, validation and submission of modeled structure to PMDB

The modeled protein is validated by molecular dynamics and mechanics with the help of various empirical force fields such as ANOLEA [22], GROMOS [23] and VERIFY3D. [24] The parameters included the covalent bond distances and angles, steriochemical validation and atom nomenclature were validated using PROCHECK. [25] The statistics of non-bonded interactions between different atom types were detected and value of the error function was analyzed by ERRAT. [26] The modeled E2F3 Protein was deposited to the Protein Model Data Base. [27]

Selection of Potential Drug Candidates against E2F3 protein

The Pubchem [28], KEGG [29] and Drug Bank [30] databases provides collection of drugs that help to treat breast and prostate cancer. [31-35] The .sdf structure files of these drugs were obtained and converted into .pdb format using Open Babel software. The DrugBank database has a wide collection of plant alkaloids and the drugs were directly obtained in pdb format for docking based on the literature studies.

Molecular Docking

A rigid body docking was performed with HEX 6.1 [36] by SP Fourier Transform, FFT steric scan, FFT final search and MM refinement. The clustering histogram with the scoring function was generated to analyze the binding energy of each selected conformations. Based on the docking results, preferably in terms of E_{Total}, minimum energy value, the effectiveness of both receptor (E2F3 protein) and ligands (drug molecules) to interact with each other were studied and best inhibitor was selected. The docked complex is viewed and the interaction of aminoacids with the ligands was analyzed by PyMOL.

RESULTS AND DISCUSSION

Retrieval of E2F3 protein sequence

The protein sequence of E2F3 consists of 465 amino acids. Four sequences were obtained and the best selected based on their functional domains. (Table 1).

>sp|O00716|E2F3_HUMAN Transcription factor E2F3 OS=Homo sapiens GN=E2F3 PE=1 SV=1
MRKGIQPALEQYLVTAGGGEGAAVVAAAAAASMDK
RALLASPGFAAAAAAAAAAGAYIQILTTNTSTTSCSSSL
QSGAVAAGPLLPSAPGAEQTAGSLLYTTPHGPSSRAGL
LQPPALGRGGSGGGGGPPAKRRLELGESGHQYLSDGL
KTPKGKGRAALRSPDSPKTPKPSEKTRYDTSLGLLTKK
FIQLLSQSPDGVLDLNKAAEVLKVQKRRIYDITNVLEG
IHLIKKSKNNVQWMGCSLSEDGGMLAQCQGLSKEVT
ELSQEEKKLDELIQSCTLDLKLLTDSENQRLAYVTYQD
IRKISGLKDQTVIVVKAPPETRLEVPDSIESLQIHLASTQ
GPIEVLCPEETETHSPMKTNNQDHNGNIPKPASKDLAS
TNSGHSDCSVSMGNLSPASPANLLQTEDQIPSNLEGPF
VNLLPPLLQEDYLLSLGEEEGISDLFDAYDLEKLPLVE
DFMCS

Screening of Best homologous

The P- BLAST results were analyzed and the best protein hits were selected based on the percentage of identity, similarity and query coverage. The identity range was 26-64%, similarity range was 47-76% and E- value range was 2e-25 to 9.4 (Table 2).

MSA and phylogenetic characterization

The multiple sequence analysis was performed with T-COFFEE and the conservation present in the target protein was interpreted. The phylogram represents that query is closely related to templates 1CF7 and 2AZE. (Fig. 3) However, template 1SQB was selected due to its better quality and due to the fact that it is a eukaryotic protein. Hence the best template for homology modeling was

identified to be 1SQB-chain B, X-ray crystal structure of Bovine Bc1 with Azoxystrobin of *Bos taurus*. The Resolution factor of the structure is 2.69 A^o and R value is 0.242.

Table 1: Sequence data of E2F3 tumor marker available in Uniprot

Uniport ID	Name of protein	Number of amino acids	Number of functional domains
O00716	Transcription factor E2F3	465	6
Q499G5	E2F3 Protein	126	4
Q24JQ3	E2F3 Protein	224	3
Q96AR0	E2F3 Protein	133	2

Table 2: Best Homologous Template Structures for E2F3 protein

PDB ID	Chain	Number of amino acids	Percentage of identity	Percentage of similarity	E-value	Organism
2AZE	B	106	52	76	2e-25	*Homo sapiens*
1CF7	A	76	64	78	1e-19	*Homo sapiens*
2W60	A	218	31	47	4.1	*Mus musculus*
1SQB	B	453	26	49	8.0	*Bos taurus*
1QCR	B	423	26	49	9.4	*Bos taurus*
1BGY	B	439	26	49	8.3	*Bos taurus*
1LKC	A	364	32	52	8.1	*Salmonella enterica*
1W4X	B	542	41	72	0.84	*Thermobifida fusca*

Table 3: Docking result of E2F3 with various drugs selected from Pubchem and DrugBank

Drug ID	Name of the Drug	E_{Total} (Binding energy)
DB00570	Vinblastine	-4558.33
DB00541	Oncovin	-4493.85
DB00361	Navelbine	-4388.25
DB01229	Taxol	-4336.65
DB01248	Taxotere	-4082.99
DB00572	Atropine	-1985.40
DB03496	Flavopiridol	-1967.91
DB04115	Berberine	-1122.35
DB01206	Lomustine	-759.96
CID:5284380	Mitolactol	-178.18
CID:4208	Mitolactol	-175.18
DB00665	Cisplatin	-124.40
CID:5288209	Fenretinide	-68.02
CID:1744	Fenretinide 4-hydroxy phenyl retinamide	-68
CID:42890	Idarubicin	-53.84
CID:4416	Nafoxidine	-50.76
CID:11683	Megestrol acetate	-46.88
CID:19090	Megestrol	-40.81
CID:6603872	Fenretinide	-37.89
CID:6540837	Testolactone	-35.33
CID:59693	Fadrozole	-23.35

Proteogenomic analysis

Genomic analysis predicted an exon of length 1278 spanning from 97 to 1378. Proteomic analysis revealed the presence of 54 positively charged residues and 43 negatively charged residues. The ORF-Finder result shows it has 4 ORFs with their respective positions at 1-1394 (+1), 1-1293(-1), 311-439(+2) and 3-104(+3) in the sequence and corresponding length. The primary structural features were identified by PROTPARAM and it has predicted the presence of structures like leucine zipper at position 204-225 and a cyclin binding domain at 101-153. The molecular weight was found to be 49161.6 Daltons and theoretical isoelectric point was 5.9. Leucine was the predominant amino acid identified in E2F3 marker which constitutes 13.1% of the total content. The secondary structure of E2F3 was predicted by PSI-PRED. It has been noticed that E2F3 consists majorly of random coils. It was predicted that 31.4% were α helices, 14.62 % were extended strand, 5.38% were β turns and 48.6 % were random coils. The hydrophobicity profile of the query was predicted using TopPred. The transmembrane helices are mainly present in regions between 13 - 33 with a score of 1.181, 36 - 56 with a score of 1.278 and a putative sequence between 72-92 with a score of 0.697. The functional domain prediction with SMART predicted 7 low complexity regions along with a coiled coil region at location 252-285.The domains were also compared with Pfam databases and a certain domain shared similarity with Pfam: E2F_TDP with e value of 1.40e-37.The domain is starting from 178 and ends at position 243. This is a mammalian transcription factor E2F plays an important role in regulating the expression of genes that are required for passage through the cell cycle. Multiple E2F family members have been identified that bind to DNA as heterodimers, interacting with proteins known as DP - the dimerisation partners.

Comparative modeling

An alignment between the target and temple is performed (Fig 2). The Modeller program was executed and a series of files were generated and the final modeled protein was written as 'E2F3.B99990001' The superimposition was performed to analyze the structural alignment, backbone threading and fold recognition of modeled protein (Fig. 4). Almost 424 residues are aligned between the target and template with a Z score of 56.9. It has noticed that RMSD value of threaded structure to be 0.5 which indicate the backbone configuration of the protein is good. The modeled protein was visualized in PyMOL. It has observed that the random coils were predominant in the modeled protein and beta sheets were present in small proportion. (Fig. 5).The percentage of alpha helix was found to be 31.4 % accounting 146 residues of the sequence. The extended strand is 14.62% and random coil 48.6%.

Validation of the modeled protein

The modeled protein was validated by various empirical force fields such as ANOLEA, GROMOS, QMEAN, VERIFY 3D (Fig. 6) PROVE and DSSP. The score of each amino acid was obtained in the form of data along with a graphical output with a 3D averaged score. The scores of most residues were found to be within 0.0 and 0.73. Model validation with PROVE gave a total amount exterior surface accessible volume of E2F3 as 86214 cubic angstroms and accessible area was 22428 square angstroms. The corresponding volume and areas of different cavity surfaces was also analysed. The complete atomic description with z-mean and distribution values for each residue was studied by data and graphical output. The z-mean average score is 0.432. The overall quality of the modeled protein was found to be 57.363 and the error value of each residue was plotted (Fig. 7).The maximum error was found to be in at positions 320-335,425-440 and 445-460. The modeled protein is further validated by Ramachandran Plot generated by PROCHECK. The plot value was found to be 86.1% with

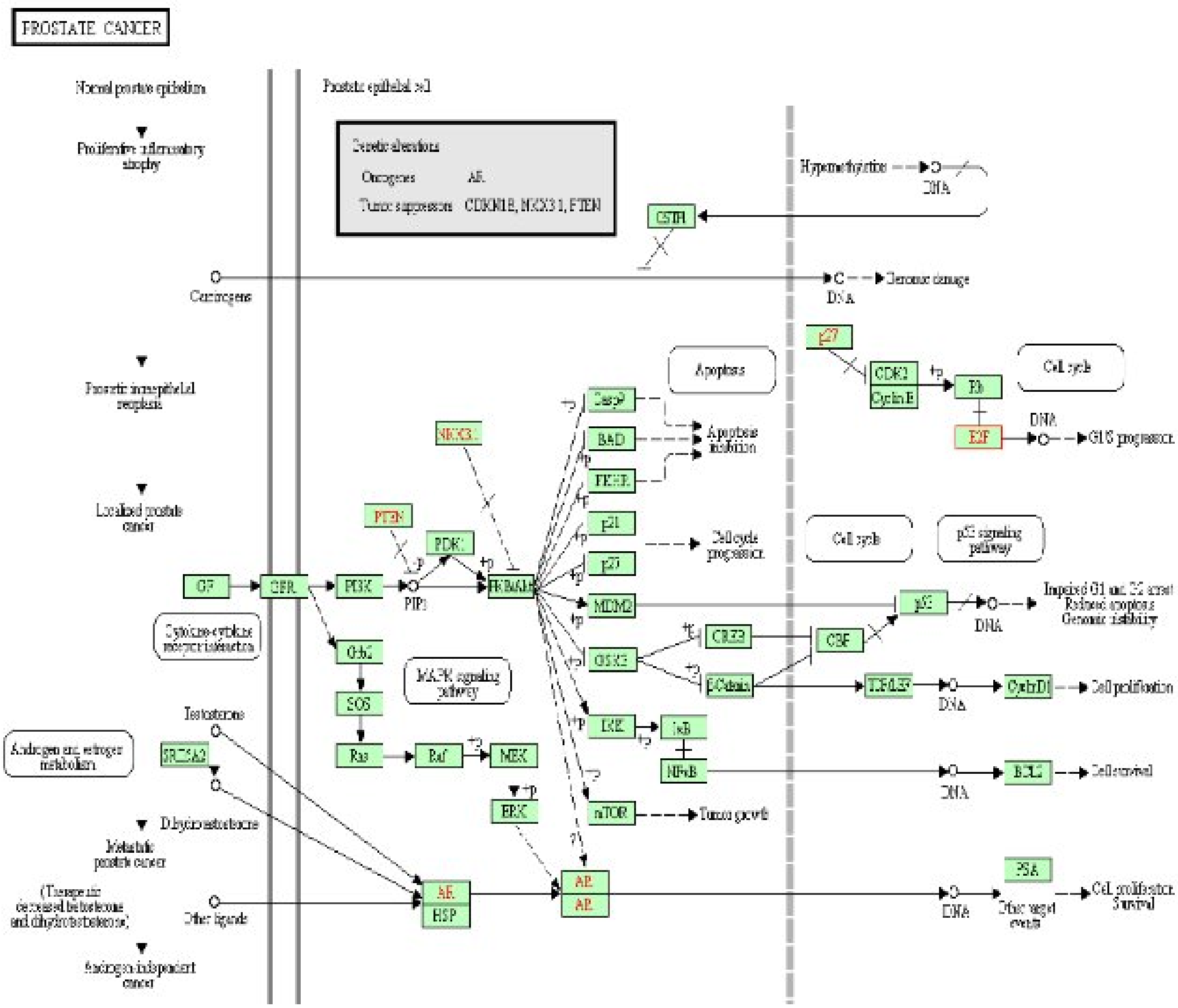
PROSTATE CANCER
Genetic alterations
Oncogenes AR
Tumor suppressors CDKN1B, NKX3.1, PTEN
Hypermethylation
DNA
GSTP
Carcinogens
Genomic damage
p27
CDK2
Cyclin E
Rb
E2F
G1/S progression
Cell cycle
Apoptosis
Casp9
BAD
FKHR
Apoptosis inhibition
NKX3.1
PTEN
PDK1
p21
p27
Cell cycle progression
p53 signaling pathway
GF
GFR
PI3K
PIP3
PKB/Akt
MDM2
p53
Cytokine-cytokine receptor interaction
Grb2
SOS
Ras
Raf
MEK
ERK
MAPK signaling pathway
GSK3
CREB
CBP
β-Catenin
TCF/LEF
CyclinD1
Cell proliferation
IKK
IκB
NFκB
BCL2
Cell survival
mTOR
Tumor growth
Testosterone
Androgen and estrogen metabolism
SRD5A2
Dihydrotestosterone
AR
HSP
PSA
Other ligands
Cell proliferation
Survival
Other target events
Metastatic prostate cancer
Androgen-independent cancer

Fig. 1: KEGG Molecular pathways representing the role of E2F3 in Prostate cancer

```
1SQB_B                 MKLLTRAGSLSRFYSLKVAPKVKATEAPAGVPPHPQDLEFTRLPNGLVIASLENYAPASR 60
O00716|E2F3_HUMAN      LTTNTSTTSCSSSLQSCAVAACPLLPSAPCAEQTACSLLYTTPHCPSSDACLLQQPPALC 60

1SQB_B                 IGLFIKAG----SRYENSNNLGTSHLLRLASSLTTKGASSFKITRGIEAVGGKLSVTSTR 116
O00716|E2F3_HUMAN      RGGSGGGGGPPAKRRLELGESGHQYLSDGLKTPKGKGRAALRSPDSPKTPKSPSEKTRYD 120

1SQB_B                 ENMAYTVECLRDDVDILMEFLLNVTTAPEFRRWEVAALQPQLRIDKAVALQNPQAHVIEN 176
O00716|E2F3_HUMAN      TSLGLLTKKFIQLLSQSPDGVLDLNKAAEVLKVQKRRIYDITNVLEGIHLIKKKSKNNVQ 180

1SQB_B                 LHAAAYRNALANSLYCP--DYRIGKVTPVELHDYVQNHFTSARMALIGLGVSHPVLKQVA 234
O00716|E2F3_HUMAN      WMGCSLSEDGGMLAQCQGLSKEVIELSQEEKKLDELIQSCILDLKLLIEDSENQRLAYVI 240

1SQB_B                 EQFLNIRGGLGLSGAKAKYHGGEIREQNGDSLVHAALVAESAAIGSAEANAFSVLQHVLG 294
O00716|E2F3_HUMAN      YQDIRKISGLKDQTVIVVKAPPETRLEVPDSIESLQIHLASTQG------PIEVYLCPEE 294

1SQB_B                 AGPHVKRGSNATSSLYQAVAKGVHQPFDVSAFNASYSDSGLFGFYTISQAASAGDVIKAA 354
O00716|E2F3_HUMAN      TETHSPMKTNNQD---HNGNIPKPASKDLASTNSGHSDCSVS-MGNLSPLASPANLLQQT 350

1SQB_B                 YNQVKTIAQGNLSNPDVQAAKNKLKAGYLMSVESSEGFLDEVGSQALAAGSYTPPSTVLQ 414
O00716|E2F3_HUMAN      EDQIPSNLEG----PFVNLLPPLLQEDYLLSLGEEEGISDLFDAYDLEKLPLVEDFMCS- 405

1SQB_B                 QIDAVADADVINAAKKFVSGRKSMAASGNLGHTPFIDEL 452
O00716|E2F3_HUMAN      ---------------------------------------
```

Fig. 2: Alignment between E2F3 and its best template

Fig. 3: Evolutionary analysis of E2F3 and best homologous sequences

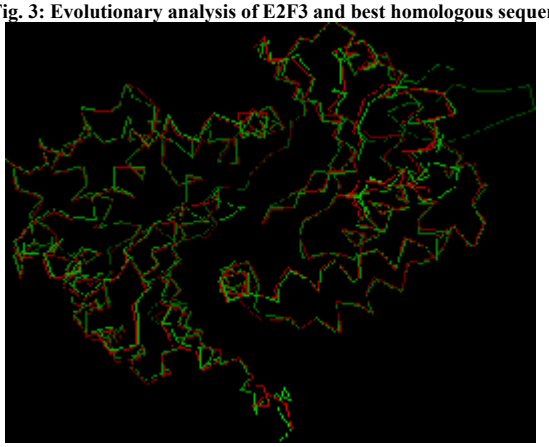

Fig. 4: Superimposed structure of E2F3 and template

Fig. 5: Visualization of modeled protein using PyMOL depiction of secondary structures like helix (red), sheet (yellow) and loop (green).

Fig. 6: Verify 3D plot result

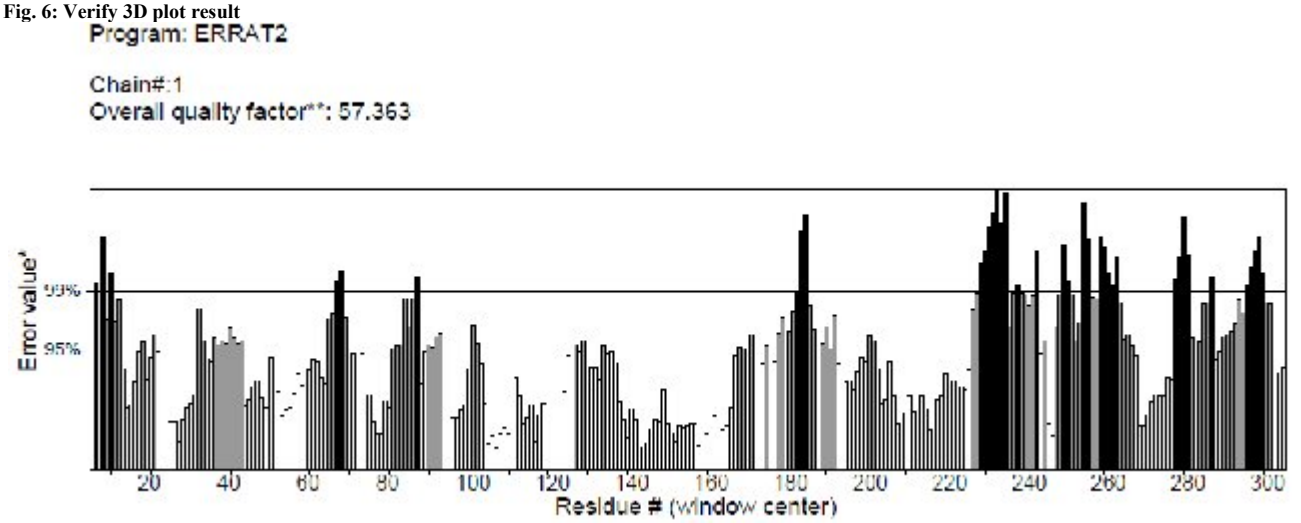

Fig. 7: ERRAT result depicting overall quality factor of modeled protein as well as individual residue factors

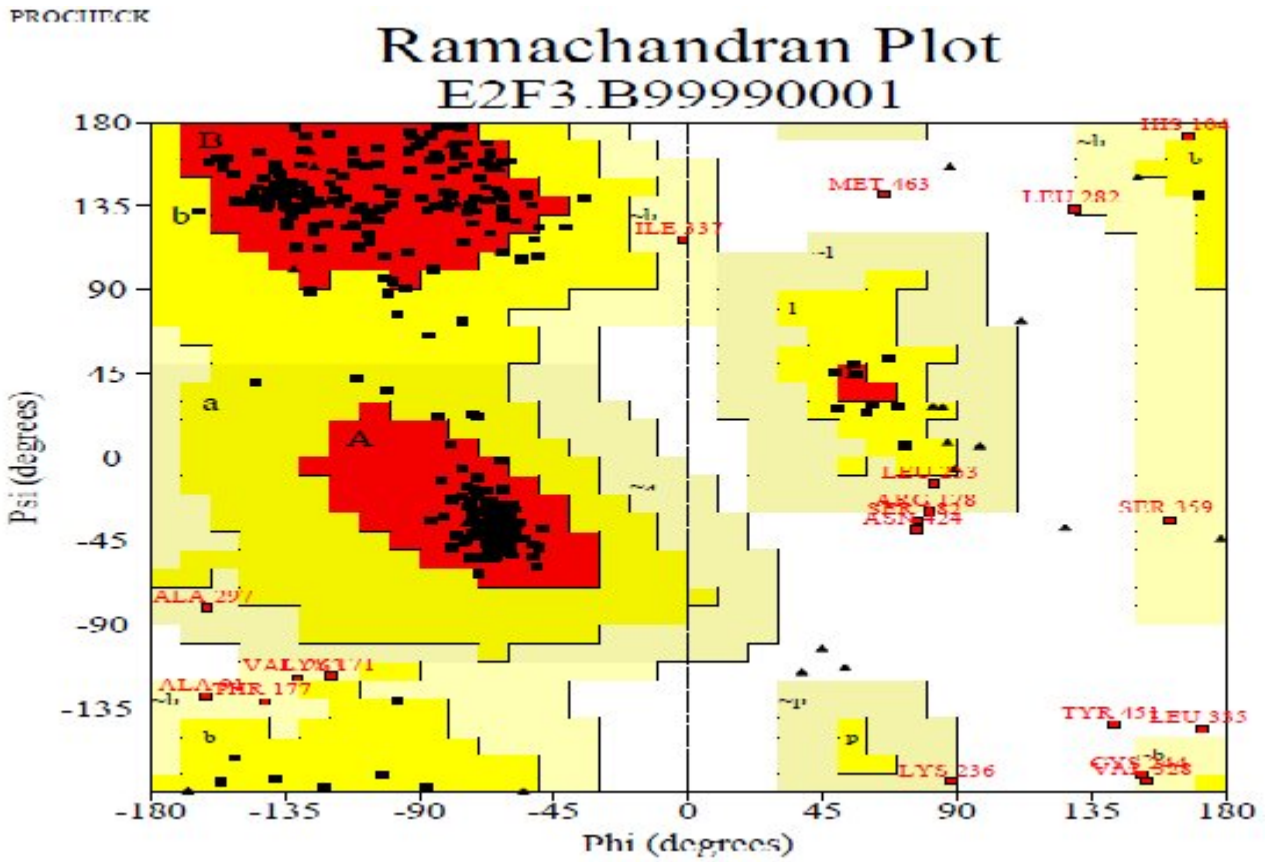

Fig. 8: Ramachandran plot generated by PROCHECK

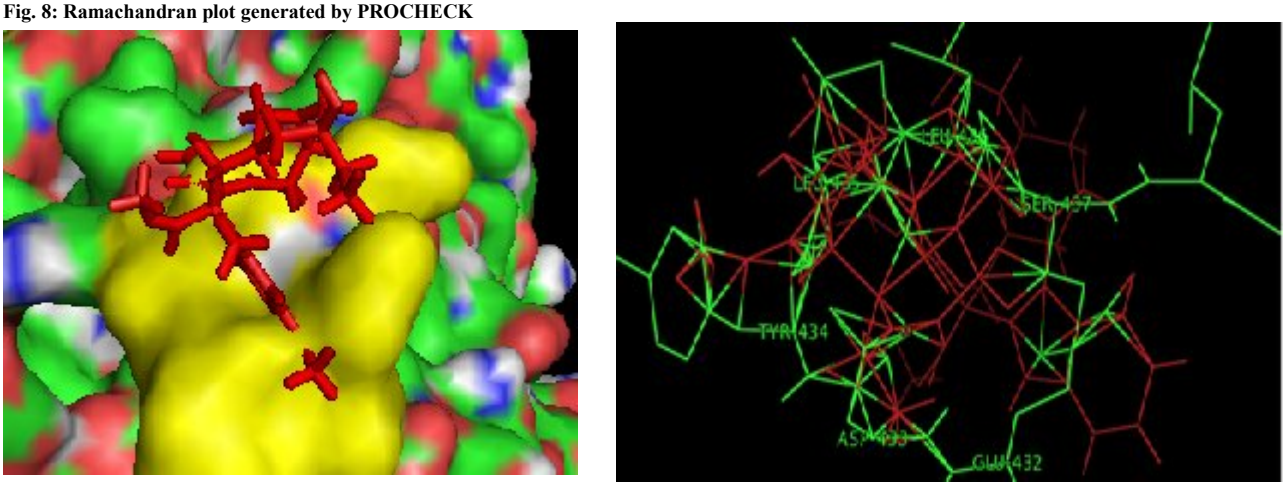

Fig. 9: Interaction of Vinblastin with modeled E2F3 protein

335 residues in the favored region.9% of the residues lie in additional allowed region and 3.3% in the generously allowed region. Only about 1.5% of the residues were located in the disallowed region. The number of glycine residues are 41 and proline residues are 33 (Fig. 8).

Submission of the modeled structure in to Protein model database

The modeled E2F3 protein was submitted to the Protein Model Database and information regarding the sequence, methods used for modeling and docking were provided. The structure of the protein can be downloaded by general pubic using the provided ID 'PM0076554'.

Docking E2F3 with best inhibitors

The modeled protein was docked against a range of drugs that are used to treat breast and prostate cancer. The matched and docked complexes were analyzed by bindng energy obtained from clustering histogram. The drug Vinblastine was found to have the best binding energy value of -4558.33.It is an antitumor alkaloid isolated from *Vinca rosea*. The antitumor activity of vinblastine is thought to be due primarily to inhibition of mitosis at metaphase through its interaction with tubulin. Vinblastine binds to the microtubular proteins of the mitotic spindle, leading to crystallization of the microtubule and mitotic arrest or cell death. Vinblastin interacts with the modeled protein at sites Glu-432, Asp-433, Tyr-434, Leu-435 and Leu-436. The other ligands with good binding energy values are Oncovin, Navelbine, Taxol and Taxotere. The results with other drugs were tabulated. (Table-3) The docked complex was visualized with PyMOL (Fig. 9).

ACKNOWLEDGMENT

The authors would like to acknowledge the support and facilities provided by the R & D Centre, Department of Life Sciences and Engineering, Dayananda Sagar Institutions, Shavige Malleswara Hills, Kumaraswamy Layout, Bangalore, Karnataka, India

REFERENCES

1. Yan Lu, Yijun Yi, Pengyuan Liu, Weidong Wen, Michael James, Daolong Wang, Ming You. Common Human Cancer Genes Discovered by Integrated Gene-Expression Analysis. PLoS ONE. 2007; 2: e1149.
2. Eric J. Kort, Leslie Farber, Maria Tretiakova, David Petillo, Kyle A. Furge, Ximing J. Yang, Albert Cornelius, Bin T. The E2F3—Oncomir 1 axis is activated in Wilms Tumor. Cancer Res. 2008; 68: 4034-4038.
3. Tsantoulis PK, Gorgoulis VG. Involvement of E2F transcription factor family in cancer. Eur J Cancer. 2005; 41:2403-2414.
4. Rakha EA, Pinder SE, Paish EC, Robertson JF, Ellis IO. Expression of E2F-4 in invasive breast carcinomas is associated with poor prognosis. J Pathol. 2004; 203:754-761.
5. Sandeep Sanga, Hermann B. Frieboes, Xiaoming Zheng, Robert Gatenby, Elaine L.Bearer, Vittorio Cristini. Predictive oncology: multidisciplinary, multi-scale in-silico modeling linking phenotype, morphology and growth. Neuroimage. 2007; 37: 120-134.
6. Bong-Hyun Kim,Hua Cheng, Nick V. Grishin, Hor A. Web server to infer homology between proteins using sequence and structural similarity. Nucleic Acids Res. 2009; 37: 532-538.
7. Christiam Camacho, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos, Kevin Bealer, Thomas L Madden. BLAST+: Architecture and applications. BMC Bioinformatics. 2009; 10: 421 doi: 10.1186/1471-2105-10-421.
8. Anne Friedrich, Raymond Ripp, Nicolas Garnier, Emmanuel Bettler, Gilbert Deléage, Olivier Poch, Luc Moulinier, Blast sampling for structural and functional analyses. BMC Bioinformatics 2007; 8: 62 doi: 10.1186/1471-2105-8-62.
9. David Piedra, Sergi Lois, Xavier De La Cruz. Preservation of protein clefts in comparative models. BMC Struct Biol. 2008; 8: 2. doi:10.1186/1472-6807-8-2.
10. Martin Zachairias. Protein–protein docking with a reduced protein model accounting for side-chain flexibility. Protein Sci. 2003; 1271-1282.
11. Lyskov S, Gray JJ. The Rosetta Dock server for local protein-protein docking, Nucleic Acids Res. 2008; 36: 233-238.
12. Rolf Apweiler. UniProt: the Universal Protein knowledgebase. Nucleic Acids Res. 2004; 32: 115-119.
13. Mark Johnson. NCBI BLAST: a better web interface. Nucleic Acids Res. 2008; 36: 5-9.
14. Sagl B Needleman, Christus D, Wuksch A. General Method Applicable to the Search for Similarities in the Amino Acid Sequence of Two Proteins. J. Mol. Bwl. 1970; 48: 443-453.
15. Notredame C, Higgins DG, Heringa J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol. 2000; 302:205-17.
16. McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. Bioinformatics. 2000; 16: 404-5.
17. Claros MG, von Heijne G. TopPred II: An improved software for membrane protein structure predictions. Comput Appl Biosci 1994; 10: 685-6.
18. Ivica Letunic, Richard R. Copley, Birgit Pils, Stefan Pinkert, Jörg Schultz, Peer Bork. SMART 5: domains in the context of genomes and networks. Nucleic Acids Res. 2006; 1: 257-260.
19. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol. 1993; 234:779-81.
20. Holm L, Park J. DaliLite workbench for protein structure comparison. Bioinformatics. 2000; 16: 566-7.
21. Daniel Seeliger, Bert L. De Groot. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des. 2010; 24: 417-422.
22. Melo F, Devos D, Depiereux E, Feytmans E, ANOLEA: a www server to assess protein structures. Proc Int Conf Intell Syst Mol Biol. 1997; 5:187-90.
23. Markus Christen. The GROMOS Software for Biomolecular Simulation: GROMOS05. J Comput Chem 26. 1719-1751, 2005.
24. David Eisenberg, Roland Lüthy, James U. Bowie VERIFY3D: Assessment of protein models with three dimensional profile. Methods in Enzymology. 1997; 277: 396-404
25. Laskowski, R.A. PROCHECK: a program to check the stereiochemical quality of protein structures. J. Appl. Cryst. 1993; 26:283–291.
26. Colovos C, Yeates TO. Verification of protein structures: patterns of non-bonded atomic interactions. Protein Sci. 1993; 2:1511-1519.
27. Tiziana Castrignanò. The PMDB Protein Model Database. Nucleic Acids Res. 2006; 34: 306–309.
28. Yanli Wang. An overview of the PubChem BioAssay resource. Nucleic Acids Res. 2010; 38: 255-266.
29. Minoru Kanehisa, Susumu Goto, Miho Furumichi, Mao Tanabe, Mika Hirakawa. KEGG for representation and analysis of molecular networks involving diseases and drugs. Nucleic Acids Res. 2010; 38: 355-360.
30. David S. Wishart, Craig Knox, An Chi Guo, Dean Cheng, Savita Shrivastava, Dan Tzur, Bijaya Gautam, Murtaza Hassanali. DrugBank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res. 2008; 36: 901-906.
31. Gary Macindoe, Lazaros Mavridis, Vishwesh Venkatraman, Marie-Dominique Devignes, David W. Ritchie. HexServer: an FFT-based protein docking server powered by graphics processors. Nucleic Acids Res. 38: 445-449.
32. Lingling Duan, Kristen Sterba, Sergey Kolomeichuk, Heetae Kim, Powel H. Brown, Timothy C. Chambers. Inducible over expression of c-Jun in MCF7 cells causes resistance to vinblastine via inhibition of drug-induced apoptosis and senescence at a step subsequent to mitotic arrest. Biochem Pharmacol. 2007; 73: 481-90.
33. Johnson I S, Armstrong JG, Gorman M, Burnett J P, The Vinca alkaloids: a new class of oncolytic agents. Cancer Res. 1963; 23: 1390-427.
34. Marty M, Fumoleau P, Adenis A, Rousseau Y, Merrouche Y, Robinet G, Senac I, Puozzo C: Oral vinorelbine pharmacokinetics and absolute bioavailability study in patients with solid tumors. Ann Oncol. 2001; 12:1643.
35. Fuchs DA, Johnson RK. Cytologic evidence that taxol, An antineoplastic agent from Taxus brevifolia, acts as a mitotic spindle poison. Cancer Treat Rep. 1978; 62:1219-22.
36. Ritchie DW. Evaluation of protein docking predictions using Hex 3.1 in CAPRI rounds 1 and 2. Proteins. 2003; 98-106.