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

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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 Å 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 PMID 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
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 E2F3 tumor marker.

MATERIALS AND METHODS

Sequence retrieval of E2F3 marker

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

Screening for best homologous templates

The target protein sequence was blasted using BLASTP 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 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 TOPPRED, and SMART tools.

In silico Comparative Modeling of E2F3 Protein

An alignment between the target and template is performed (Fig 2). An in silico comparative modeling of the E2F3 protein was carried out by the MODELLER 9v7. 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 and the backbone RMSD value was analysed. The modeled protein was visualized by PyMOL.

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, GROMOS and VERIFY3D. The parameters included the covalent bond distances and angles, stereochemical validation and atom nomenclature were validated using PROCHECK. The statistics of non-bonded interactions between different atom types were selected and value of the error function was analyzed by ERRAT. The modeled E2F3 Protein was deposited to the Protein Model Data Base.

Selection of Potential Drug Candidates against E2F3 protein

The Pubchem, KEGG and Drug Bank databases provides collection of drugs that help to treat breast and prostate cancer. 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.

Molecular Docking

A rigid body docking was performed with HEX 6.1 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敦煌, 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 amino acids 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).

Screening of Best homologous

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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 Å and R value is 0.242.

<table>
<thead>
<tr>
<th>Uniport ID</th>
<th>Name of protein</th>
<th>Number of amino acids</th>
<th>Number of functional domains</th>
</tr>
</thead>
<tbody>
<tr>
<td>O00716</td>
<td>Transcription factor E2F3</td>
<td>465</td>
<td>6</td>
</tr>
<tr>
<td>Q4905</td>
<td>E2F3 Protein</td>
<td>126</td>
<td>4</td>
</tr>
<tr>
<td>Q4QJ3</td>
<td>E2F3 Protein</td>
<td>224</td>
<td>3</td>
</tr>
<tr>
<td>Q96AR0</td>
<td>E2F3 Protein</td>
<td>133</td>
<td>2</td>
</tr>
</tbody>
</table>

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

**Table 2: Best Homologous Template Structures for E2F3 protein**

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Chain</th>
<th>Number of amino acids</th>
<th>Percentage of identity</th>
<th>Percentage of similarity</th>
<th>E-value</th>
<th>Organism</th>
</tr>
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<tbody>
<tr>
<td>2AZ</td>
<td>E</td>
<td>106</td>
<td>52</td>
<td>76</td>
<td>2e-25</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>1CF7</td>
<td>A</td>
<td>76</td>
<td>64</td>
<td>78</td>
<td>1e-19</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>2W6</td>
<td>A</td>
<td>218</td>
<td>31</td>
<td>47</td>
<td>4.1</td>
<td>Mus musculus</td>
</tr>
<tr>
<td>1SQ</td>
<td>B</td>
<td>453</td>
<td>26</td>
<td>49</td>
<td>8.0</td>
<td>Bos taurus</td>
</tr>
<tr>
<td>1QC</td>
<td>B</td>
<td>423</td>
<td>26</td>
<td>49</td>
<td>9.4</td>
<td>Bos taurus</td>
</tr>
<tr>
<td>1BG</td>
<td>B</td>
<td>439</td>
<td>26</td>
<td>49</td>
<td>8.3</td>
<td>Bos taurus</td>
</tr>
<tr>
<td>1LK</td>
<td>A</td>
<td>364</td>
<td>32</td>
<td>52</td>
<td>8.1</td>
<td>Salmonella enterica</td>
</tr>
<tr>
<td>1W4</td>
<td>X</td>
<td>542</td>
<td>41</td>
<td>72</td>
<td>0.84</td>
<td>Thermobifida fusca</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Drug ID</th>
<th>Name of the Drug</th>
<th>Binding energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB00570</td>
<td>Vinblastine</td>
<td>-4558.33</td>
</tr>
<tr>
<td>DB00541</td>
<td>Oncovin</td>
<td>-4493.85</td>
</tr>
<tr>
<td>DB00361</td>
<td>Navelbine</td>
<td>-4388.25</td>
</tr>
<tr>
<td>DB0129</td>
<td>Taxol</td>
<td>-4336.65</td>
</tr>
<tr>
<td>DB01248</td>
<td>Taxotere</td>
<td>-4082.99</td>
</tr>
<tr>
<td>DB00572</td>
<td>Flavopiridol</td>
<td>-1985.40</td>
</tr>
<tr>
<td>DB04115</td>
<td>Berberine</td>
<td>-1122.35</td>
</tr>
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<td>DB01206</td>
<td>Lomustine</td>
<td>-759.96</td>
</tr>
<tr>
<td>CID:528380</td>
<td>Mitoalactol</td>
<td>-178.18</td>
</tr>
<tr>
<td>CID:4208</td>
<td>Mitoalactol</td>
<td>-175.18</td>
</tr>
<tr>
<td>DB00665</td>
<td>Cisplatin</td>
<td>-124.40</td>
</tr>
<tr>
<td>CID:5288209</td>
<td>Fenretinide</td>
<td>-68.02</td>
</tr>
<tr>
<td>CID:1744</td>
<td>Fenretidine</td>
<td>-68.02</td>
</tr>
<tr>
<td>CID:42890</td>
<td>Idrabucin</td>
<td>-53.84</td>
</tr>
<tr>
<td>CID:4416</td>
<td>Nafosamide</td>
<td>-50.76</td>
</tr>
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<td>CID:11683</td>
<td>Megestrol acetate</td>
<td>-46.88</td>
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<td>CID:19090</td>
<td>Megestrol</td>
<td>-40.81</td>
</tr>
<tr>
<td>CID:6603872</td>
<td>Fenretinide</td>
<td>-37.89</td>
</tr>
<tr>
<td>CID:6540837</td>
<td>Testolactone</td>
<td>-35.33</td>
</tr>
<tr>
<td>CID:59693</td>
<td>Fadrozole</td>
<td>-23.35</td>
</tr>
</tbody>
</table>

**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 template 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.B999990001’. 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...
Fig. 1: KEGG Molecular pathways representing the role of E2F3 in Prostate cancer

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
Program: ERRAT2

Chain: 1
Overall quality factor*: 57.363

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