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Comparative Modeling of Human P-gp NBD2 and Docking and Binding Mode Analysis of 8-Geranyl Chrysin as a P-gp Modulator

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Abstract

The resistance of tumour cells against cytotoxic drug is significant limitation in successful chemotherapeutic treatment of cancer. To date, no crystal structure is available for human P-gp. We developed homology model for human P-gp NBD2 by using coordinates of transporter associated protein (TAP1). Docking study was performed for 8-geranyl-chrysin (Flavonoids) inhibitor in the NBD2 model. Ligand-protein interactions were determined which indicates that the 8-geranyl chrysin shares two overlapping sites in the cytosolic domains of P-gp, the ATP site and a hydrophobic steroid-binding site.

Key words : CCR2, CCR5, Dual Antagonists

1. Introduction

The resistance of tumour cells against cytotoxic drug is significant limitation in successful chemotherapeutic treatment of cancer. Although the drug efflux by transporter proteins is an underlying mechanism of multidrug resistance (MDR). In MDR tumour cells, various members of ATP-binding Cassette (ABC) family of proteins are simultaneously involved and expressed, among them P-glycoprotein (P-gp) is the most prominent[1]. This transporter uses energy from ATP hydrolysis, to transport a wide variety of substrates out of cells against concentration gradients. Active efflux of substrate from cells leads to decrease intracellular concentration of substrate and hence failure of chemotherapy. Flavonoid compounds have been shown to possess a wide spectrum of biological activities. Flavonoids are the most abundant poly phenols in our diet[2] and are believed to produce beneficial effects on some aspects of human health[3]. It is now well established that flavonoids bind at ATP sites in enzymes. Flavonoid compounds interact with other ATP-dependent enzymes, including the P-glycoprotein or P-gp, a drug transporter involved in chemo-resistance. P-gp is present in number of cells including apical membrane of epithelial cells of the jejunum and the colon and limits oral drug absorption by pumping drugs back to the intestinal lumen[4,5]. Treatment of cancer involves regular exposure of cells to cytotoxic drugs and often leads to overexpression of P-gp in the plasma membrane. This lowers the effectiveness of the cytotoxic drug in cancer cells since the transporter actively pumps the drug out of the cells. Since P-gp extrudes drugs at the expense of ATP hydrolysis, flavonoid compounds may competitively inhibit ATP binding and block the action of the transporter. Therefore the interaction of flavonoids with P-gp is interesting from both the physiological and the pharmacological points of view, and a number of studies have appeared[6,7].

Scientist putted lots of effort to find out potent, selective and specific P-gp inhibitors, also called MDR modulator. First and second generation modulators are removed from chemotherapy because of their lower efficacy and serious drug-drug interactions. In contrast to first (Verapamil, Cyclosporine-A) and second (Dexverapamil, PSC833) generation modulators the third generation modulators are not structurally related to or derived from existing one. Although they posses high efficacy, increased selectivity and low toxicity. Tariquidar (XR9576), elacridar (GF120918) zosuquidar (LY335979) and laniquidar (R1010933) are representatives of third generation modulators. Thus, to date the search for new non-toxic, potent modulator lacking pharmacokinetic

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interaction is still in progress. Current study was undertaken to know the binding mode of flavonoid (8-geranyl chrysin) in a P-gp NBD2.

2. Materials and Method

2.1. Homology Modeling

For human P-gp, there is no crystal structure available. So, homology model development of human P-gp is the only option left for us. Sequence of human P-gp (UniProtKB: P08183) was retrieved from the UniProtKB database (http://www.uniprot.org/uniprot/P08183). The NBD2 containing sequence (1031-1280) was taken and submitted for the BLAST (http://www.ebi.ac.uk/Tools/sss/ncbiblast/nucleotide.html) search to get appropriate template to derive homology model. Results show that 1JJ7 (2.4 Å) a transporter associated protein is appropriate with high resolution than the recently published mouse P-gp structure (PDB code: 3G5U, 3.8 Å). The next step in homology model development is sequence alignment with the template (1JJ7). Sequence alignment was done using ClustalW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Aligned sequence then imported into Modeller9v48 program to develop 3D-model of NBD2.

2.2. Molecular Docking

Molecular docking was performed with the SYBYL8.19 molecular modeling package. Surflex dock10 module of SYBYL was utilized to dock ligand in a putative binding pocket. Surflex dock works with the predefined protomol and dock ligands in active site. It utilizes empirical scoring function to score the docked ligand conformations. Protein structure for docking study was prepared by using protein preparation tool of SYBYL. 8-geranyl-chrysin was sketched by using SYBYL and minimized with Tripos force field with conjugate gradient minimizer and finally partial atomic charges were assigned by using Gasteiger-Huckel atomic charges. For docking of 8-geranyl chrysin the binding site was defined based on previous mutagenesis study.

3. Results and Discussion

3.1. Homology Modeling

To date, no crystal structure is available for human P-gp (UniProtKB ID: P08183). So, we developed a 3D model for NBD2 monomer by using 3D coordinates of human TAP1 (PDB code: 1JJ7 with resolution 2.4 Å).

First multiple sequence alignment (Fig. 1) was done to know the conserved regions of protein and sequence identity (45%), alignment was done by using ClustalW2.0 program. Homology model of human P-gp was generated by using Modeller9v4 program.8 Model was selected on the basis of lower MolPdf score and higher DOPE score values. The RMSD between template NBD2 and modeled human monomer NBD2 was 0.22 Å. Conserved regions consists of Walker-A loop, Walker-B loop, Q-loop and Signature region and shown in Fig. 2. Ramachandran plot was generated to validate developed model, it shows that 99.5% of total residues are in most favored and allowed region, indicates reliability of developed homology model.

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NBD2  --------------VTFGEVVFNYPTRPDIPVLQGLSLEVKKGQTLALVGSSGCGKSTVV 46
1JJ7  PPSGLLTPLHLEGLVQFQDVSFAYPNRPDVLVLQGLTFTLRPGEVTALVGPNGSGKSTVA 60
                  * * :* * **.***: *****:: :: *:. ****..*.*****.

NBD2  QLLERFYDPLAGKVLLDGKEIKRLNVQWLRAHLGIVSQEPILFDCSIAENIAYGDNSRVV 106
1JJ7  ALLQNLYQPTGGQLLLDGKPLPQYEHRYLHRQVAAVGQEPQVFGRSLQENIAYG-LTQKP 119
       **:.:*:* .*::***** : : : ::*: ::. *.*** :*. *: ******  ::

NBD2  SQEEIVRAAKEANIHAFIESLPNKYSTKVGDKGTQLSGGQKQRIAIARALVRQPHILLLD 166
1JJ7  TMEEITAAAVKSGAHSFISGLPQGYDTEVDEAGSQLSGGQRQAVALARALIRKPCVLILD 179
      : ***. ** ::. *:**..**: *.*:*.: *:******:* :*:****:*:* :*:**

NBD2  EATSALDTESEKVVQEALDKARE--GRTCIVIAHRLSTIQNADLIVVFQNGRVKEHGTHQ 224
1JJ7  DATSALDANSQLQVEQLLYESPERYSRSVLLITQHLSLVEQADHILFLEGGAIREGGTHQ 239
      :******::*:  *:: * :: *  .*: ::*:::** :::** *:.::.* ::* ****

NBD2  QLLAQKGIYFSMVSVQAGTKRQ 246
1JJ7  QLMEKKGCYWAMVQAPADAPE- 260
      **: :** *::**.. *.: .
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Fig. 1. Sequence alignment of template (1JJ7) and target (human NBD2). Alignment was performed using ClustalW program.

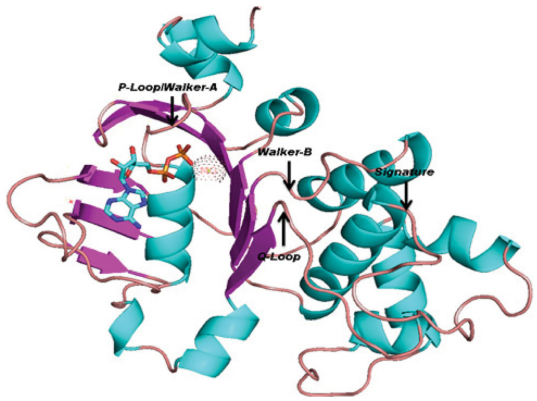

Fig. 2. Homology model structure of human NBD2 represented by ribbon-tube. ADP molecule was shown in capped stick model whereas magnesium ion is shown in dotted sphere.

3.2 Molecular Docking Analysis

In this work we performed docking study of one of the potent flavonoid inhibitor against homology model of human NBD2. Docked pose of 8-geranyl-chrysin is shown in Fig. 3.

It shows that inhibitor binds in flavonoids binding region and overlap ATP binding region as well as steroid binding region. ATP binding site was located inside NBD2 with cavity forming residues Tyr1044, P-loop and His1232. The α, β and γ phosphate groups of ATP were located within the P-Loop containing Gly1073, Cys1074, Gly1075, Lys1076, Ser1077, and Thr1078. Highly hydrophobic steroid binding site was identified with the residues Ile1050, Pro1051, Val1052 and Gly1249 residues, where geranyl part of the inhibitor is binding which is in line with the previous report.11 The ATP- and steroid-binding sites within ABCB1 NBD2 are predicted to overlap in the vicinity of the P-Loop where both ATP and steroids interact with Val1052, Gly1075 and Ser1077. Both ATP and steroids are also predicted to interact with Tyr1044. Hydrogen bond interactions were observed in ligand-protein. C=O at 4th position interacted through hydrogen bond with NH of Gln1079 and OH group of Tyr1044. Another hydrogen bond interaction was predicted for the OH of ligand at 7th position and main chain NH of Gly1075. Phenyl ring at 2nd position of ligand interacts hydrophobically

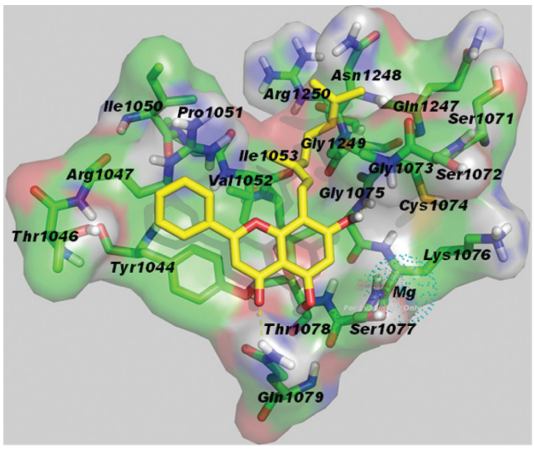

Fig. 3. Docked pose of 8-Geranyl-chrysin shown in active site of human NBD2 model. Active site residues are represented in green capped stick and ligand is shown in yellow capped stick. Hydrogen bond interactions were shown in dotted yellow lines, while magnesium ion is shown in cyan dotted sphere.

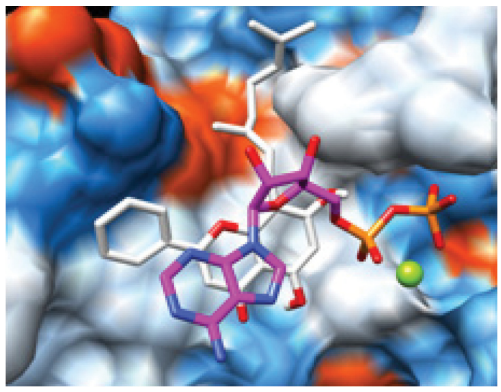

Fig. 4. Overlay of docked poses of 8-Geranyl chrysin (white carbon) with the cocrystalled ADP molecule (magenta carbon). Receptor is shown in hydrophobic surface.

with that of the Tyr1044 with π-π stacking interactions. Hydrophobic pocket was made to accommodate this phenyl ring by Val1052 and side chain of Arg1047. Geranyl side chain at 8th position of ligand interacts in a highly hydrophobic steroid binding region containing Val1051, Ile1053, Gly1073, Gly1075, Gly1249 and side chains of Asn1248 and Arg1250. The Mg2+ ion is shown in cyan dotted sphere. The overlapped pose of the cocrystalled ADP with the docked inhibitors is shown in Fig. 4.

The overlapped docked pose of ligand with that of the ADP shows that the bi-aryl ring of ligand (white carbon) overlap the adenine ring of ADP (magenta carbon).

4. Conclusion

The design of P-gp inhibitors is difficult in the absence of a three-dimensional structure. In addition, a number of known modulators of P-gp activity are acting as pseudo-substrates that are themselves transported by P-gp. Due to the interaction of flavonoids with the ATP-binding site of the NBD, they may block the action of P-gp through a different mechanism, by preventing ATP-binding. Since NBD function is critical for substrate transport, targeting these domains is an extremely attractive strategy for potentially counteracting drug efflux. Despite there being conserved motifs within the NBD, regions of sequence diversity are also apparent and represent potential targets for de novo drug design.

Therefore, understanding the structural requirements for, and mechanism of interaction of compounds with NBDs may prove useful in developing more effective modulators targeted to ABC transporter proteins.

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