Research Article

Revealing potential drug targets against Proto-oncogene Wnt10B by comparative molecular docking

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Abstract

Wingless type mouse mammary tumor virus (MMTV) integration site-10B (Wnt10B) is an important member of the Wnt protein family that functions as cellular messenger in paracrine manner. Aberrant Wnt10B activity is the cause of several abnormalities including cancers of breast, cervix, liver, gastric tract, esophagus and pancreas as well as physiological problems like obesity and osteoporosis. The objective of this study was to determine the possible inhibitors against aberrant expression of Wnt10B in order to prevent and treat the physiological disorders associated with it. Wnt10B3D structure was predicted by using comparative modeling and then analyzed by PROCHECK, Verify3D and Errat. The model having 84.54 % quality value was selected and acylated to satisfy the hydrophobic nature of Wnt10b. For search of inhibitors virtual screening was performed on Natural Products (NP) database. The compounds were filtered and Ligand based screening was performed using the antagonist for mouse Wnt-3a. This resulted in a library of 272 unique compounds having most potent drug like activities for Wnt-4. Out of the 271 molecules analyzed three small molecules ZINC35442871, ZINC85876388 and ZINC00754234 having activity against Wnt4 aberrant expression were found common through docking experiment of Wnt10B. Therefore, the three molecules ZINC35442871, ZINC85876388 and ZINC00754234 can be considered as lead compounds for performing further drug designing experiments against aberrant Wnt expressions.

Keywords: Comparative Computational Studies; Molecular Docking; Proto-Oncogene; Wnt10B inhibitors

Introduction

Wingless type MMTV Virus Integration site-10B (Wnt10B) signaling protein belongs to Wnt-family and is considered as Proto-oncogene due to its higher expressions in endometrial cancer cells leading to gastric cancer, pancreatic cancer, esophageal cancer, cervical cancer and breast cancer [1]. Wnts have hydrophobic nature that makes

them a difficult target for experimentation whereas, liposomally packed antagonists are the only example to directly inhibit wnts (mouse Wnt3a) [2]. Wnt inhibitors can be designed with the help of these antagonists, which can directly inhibit aberrant wnt expressions.

Wnt10B is comprised of 389 amino acids where first 28 amino acids behave as signal

peptide which is dispatched from mature protein with loss of 361 (29–389) amino acids [3]. The human *Wnt10B* is localized at 12q13.1 and shows 95% identity to the mouse *Wnt10b*. Wnt10B mature peptide contains 24 cysteine residues having eminent fellow-based tendency to make cysteine-cysteine disulfide bonds. There are two Asn-linked glycosylation sites and one Ser-linked acylation site [4]. Acylation not only adds extra hydrophobicity but also a significant character of wnt-frizzled interaction. Inhibition or restriction of acyl compound by another inhibitor may cause unusual behavior of wnts, resulting in disturbance of wnt-frizzled interaction. Such inhibitions may also cause partial or complete loss of activity of wnts [5]

The highly subtle, intricate, and hydrophobic nature of wnt proteins had rendered their isolation and thus crystallization till Janda and his coworkers functionally developed first crystal structure (4F0A) in 2012 followed by another (4KRR) in 2013 [6]. According to crystal structure analysis, wnts extends a thumb from the NTD (N-Terminal Domain) and an index finger from the CTD (C-Terminal Domain) to grasp the globular Frizzled CRD (Cysteine Rich Domain) [7]. CRD serves as a binding target for wnt protein incorporating palmitoleic acid as interaction developer. The presence of palmitoleic acid at thumb domain of wnts is important for wnt-frizzled interactions [8]. The post translational modifications of wnts include glycosylations and fatty acylations. Lipidation of conserved serine residues is important for intracellular processing, secretion and activity of all wnt proteins [9]. For inhibiting aberrant wnt palmitoylation and secretion, a class of compounds has been defined as Inhibitors of Wnt Protein (IWPs) [10]. IWPs successfully inhibit wnt activity by inhibiting other members of wnt signaling cascades or even by targeting

RNA interferences[11]. The mouse Wnt-3a inhibitors can act as antagonists for identifying Inhibitors for human wnt family in order to stabilize their aberrant expressions. This study is aimed to scrutinize direct inhibitors of Wnt10B in its acylated form homology modeling and comparative molecular docking. Utilization of antagonist of mouse Wnt-3a to perform inhibitor searching for human Wnt4 has been reported earlier and 272 Natural Products (NP) based small molecules were scrutinized [12]. These 272 compounds were verified by running test simulation of 10 ns on the highest ranked inhibitor previously identified for human protein Wnt-4. This set of 272 NPs has been used in the present study against human Wnt10B for identifying the potential inhibitors. The lead compounds found from this work can be further used in drug designing against aberrant wnt expressions.

Materials and methods

This study was conducted from January to June 2015 in the bioinformatics lab at COMSATS Sahiwal. The information about Wnt10B and its canonical amino acid sequence comprising of 389 amino acids was retrieved from Uniprot Knowledge Base (KB) with Uniprot ID O00744. Basic Local Alignment Search Tool (BLAST) was employed for Wnt10B sequence alignment against Protein Data Bank (PDB) entries to find a suitable template [13]. The sequence and co-ordinate file of top prioritized template (4F0A_B) was retrieved from RCSB web browser (www.rcsb.org). The Physicochemical properties were also determined for Wnt10B through protparam tool [14].

Structure acquisition

MODELLER (version 9.15) tool was employed for model building by aligning Wnt-10B with the selected template (4F0A_B). The predicted models obtained from MODELLER were ranked according

to their evaluation score [15]. Web-based modeling was performed for acquisition of comparatively most optimal and reliable model. Six web servers including RaptorX, I-TASSER, Phyre2, HHpred, M4T and IntFOLD2 were utilized for prediction of Wnt10B3D structure [16].

The predicted protein structures were evaluated through PROCHECK, Verify3D and Errat for overall quality assessment including stereochemical quality, G-factor values, and Ramachandran plots [17]. The model was visualized through UCSF Chimera to check any structural anomaly in terms of secondary structure elements. Afterwards, the stereochemistry of selected model was improved through energy minimization step while using AMBER parameters for standard protein residues [18].

Molecular docking

For search of inhibitors against Wnt-4, screening was performed on Natural Products (NP) database downloaded from ZINC database [19]. The compounds were filtered through the program FILTER and Ligand based virtual screening was performed through ROCS and EON of openeye's module using the antagonist for mouse Wnt-3a. This resulted in a library of 272 unique compounds having most potent drug like activities for Wnt-4. To verify results of the experiment, the top ranked inhibitor for Wnt-4 was subjected to 10 nanoseconds (ns) Molecular Dynamics (MD) simulation. The compounds that were once passed through the pipeline can be used for Wnt10B inhibitor identification.

Palmitoylation is important for interaction of Wnt10B with frizzled receptor, therefore, manual addition of palmitoleic acid at Ser253 of Wnt10B was achieved through UCSF Chimera (Figure 1). Comparative molecular docking was performed through Genetic Optimization for Ligand Docking (GOLD) and AutoDock-VINA (AD-VINA).

The docked compounds were sorted according to Goldscore and Chemscore of GOLD and binding energy values of AD-VINA. LigPlot was employed to analyze the interactions between compounds and Wnt10B receptor.

Figure 1. Attachment of Palmitoleic acid at Ser253 of Wnt10B. A covalent bond between OG atom of Ser253 and C16 of Palmitoleic acid was added through 'CONNECT' records in coordinate file

Results

Wnt10B properties

The Wnt10B amino acid sequence with Uniprot ID O00744 was retrieved from Uniprot KB. The physiochemical properties of Wnt10B was determined through Protparam tool (Table 1). It was observed that Wnt10B has high instability index (51.60) and high aliphatic index (70.75) while it has lower GRAVY index (-0.421) suggesting that Wnt10B has high ratio of hydrophobic amino acids. Previously Molecular Dynamics (MD) Simulation studies of Wnt4 highlighted importance of cysteine residues as structure shaper of wnt proteins. It was observed that all cysteine residues were arranged nearer to loops of thumb and index finger domains and therefore, could be involved in cysteine-cysteine disulfide bridges which is a structural and functional property of wnt family that can add more hydrophobicity to wnts.

Table 1. Protparam results for Wnt10B: The physiochemical properties of Wnt10B demonstrate that it is a highly hydrophobic protein

Property	Value
Molecular Weight (Daltons)	43000.4
No. of Amino acid residues	389
Isoelectric Point (pI)	9.37
Extinction Co-efficient	41480-42980 M ⁻¹ cm ⁻¹
Instability Index (II)	51.60
Aliphatic Index (AI)	70.75
GRAVY	-0.421

Structure acquisition

While aligning Wnt10B with PDB entries, PDB identifiers 4F0A showed highest alignment scores of 175 with 33% identity level and up to 80% query coverage. It is inferred from the similarity results that conserved residues have conserved structural features and share conserved functional properties.

Comparative model building approach was employed to generate reliable model for Wnt10B. All predicted models were evaluated through PROCHECK, Verify3D, and Errat and it was observed that I-TASSER model 3 superseded all other models. PROCHECK most favored and allowed region value was 92.3, Errat quality factor value was 84.54 %, and Verify3D value was 73.26 %. UCSF Chimera was employed to minimize energy of selected model by utilizing AMBER ff99SB force field with AMBER standard residues to enhance backbone conformation of selected model.

Comparative molecular docking

Detailed *in silico* analyses of selected library revealed that the compounds were efficiently bound at the palmitoleic acid region of the protein. Docking analyses were done against selected library by utilizing GOLD software and cross validated the results by utilizing AD-VINA. Both the utilized docking tools showed effective binding energies and also showed the same binding domain. The results from GOLD

were ranked according to goldscore values and docked complexes were generated for Wnt10B through GOLD graphical interface. The top 20 compounds were compared in terms of the goldscore of GOLD and binding energy of AD-VINA and found that they may have potential to inhibit the aberrant expression of Wnt10B. It was observed that the three compounds ZINC00754234 (goldscore value: 50.27 and binding energy value: -5.9), ZINC35442871 (goldscore value: 43.64 and binding energy value: -6.4), and ZINC85876388 (goldscore value: 42.96 and binding energy value: -6.4) interacted with Wnt10B and palmitoleic acid and found common in both the docking programs among top 20 compounds (Table 2). The selected top 20 compounds by both the utilized tools were analyzed through ligplot and interaction studies between compounds and protein with bound palmitoleic acid confirmed that inhibitors interacted with protein and palmitoleic acid substantiating the idea to block wnt-frizzled interaction. The interactions of ZINC00754234 from ligplot and UCSF Chimera (Figure 2 & 3), was critically analyzed and visualized for molecular interactions. The interacting residues Phe258, Asp155, Gly251, His250, Thr252, Gln257, Gly254, and Ser253 were involved in interactions with inhibitor and palmitoleic acid among all complexes and considered as potential residues. Mostly hydrophobic interactions were observed throughout the

molecular docking experiment whereas, palmitoleic acid also supports hydrophobic interactions. The 2D structures of three common compounds are shown (Figure 4) while 2D structures of top 20 selected inhibitors were also drawn.

Discussion

Bioinformatics analyses have shown success in research methodologies to design numerous novel computer-aided molecules against neurological disorders and cancers [20]. Protein structure predictions through

X-Ray Crystallography and Nuclear Magnetic Resonance methods are very expensive and time consuming. Moreover, currently no single biological or computational method exists to predicts errors in 3D structures consistently and accurately [21]. Analysis of the X-ray crystallographic template used in this study (4F0A) also ascertained that some of its residues (22 residues) were missing due to limitations of techniques available (Figure 5).

Table 2. The top 20 compounds selected from AD-VINA and GOLD with binding energy values from AD-VINA and gold score values from GOLD

Compound Name	Goldscore	Compound Name	Binding Energy
ZINC00754234*	50.27	ZINC03843220	-6.6
ZINC70454311	48.36	ZINC00489027	-6.5
ZINC85875407	46.93	ZINC12296700	-6.4
ZINC85879688	45.24	ZINC35442871*	-6.4
ZINC03843146	44.88	ZINC85876388*	-6.4
ZINC03843264	44.33	ZINC00095316	-6.3
ZINC35442871*	43.64	ZINC03843242	-6.3
ZINC13692706	43.42	ZINC03843255	-6.3
ZINC20112056	43.18	ZINC02120567	-6.2
ZINC85876388*	42.96	ZINC31158121	-6.2
ZINC85876563	42.71	ZINC04029462	-6.1
ZINC49543724	42.55	ZINC35442852	-6.1
ZINC00754306	42.44	ZINC00489019	-6
ZINC14512189	41.5	ZINC00491326	-6
ZINC06092865	41.33	ZINC03851815	-6
ZINC01645309	41.28	ZINC33830801	-6
ZINC27415691	41.27	ZINC00095299	-5.9
ZINC05141262	41.13	ZINC00095310	-5.9
ZINC04090656	41.13	ZINC00725541	-5.9
ZINC79189966	40.83	ZINC00754234*	-5.9

*compounds that are common through docking experiments

Figure 2. Interaction diagram for ZINC00754234 from AutoDockVINA and GOLD showing inhibitor ZINC00754234 interaction with protein residues as well as with palmitoleic acid. The receptor is covalently attached with palmitoleic acid

Figure 3. The docked complex of protein Wnt10B showing interactions of inhibitor with palmitoleic acid and Wnt10B.

Figure 4. Structures in 2D format for three compounds ZINC00754234, ZINC35442871, and ZINC85876388 that were common in GOLD and AutoDock-VINA

Figure 5. X-ray crystallography structure of Template (4F0A) showing missing residues between 223 to 234 producing broken chains of proteins

Using *in vivo* and/or *in vitro* methods to discover intrinsic details between folding and unfolding including important atomic level details of given protein is currently inconceivable [22]. Therefore, homology modeling techniques are used to predict 3D structures of proteins whereas molecular docking techniques establishes interactions between large molecules and small molecules and classical MD simulation demonstrate real-time dynamics of molecules under Newtonian equations. The bifunctional behavior of Wnt10B including β -catenin/Tcf activation and down-regulation of cell growth makes the

study of Wnt10B significant [23]. The aberrant expression of Wnt10B in any of these pathways may lead to different developmental diseases as well as cancers. The 3D structure of Wnt10B was comparatively predicted and minimized to remove any energy restraints that may affect accurate folding of protein. *In silico* approaches including homology modeling, threading and *abinitio* tend to solve structure prediction problems in given circumstances [24]. The 3D structure of Wnt10B was necessary to be predicted for molecular docking studies. Computational drug designing utilizing computationally derived

3D structure is an effort to analyze the receptor-ligand interactions [25].

IWPs used in earlier experiments are either proteins or molecules that inhibit proteins in wnt-signaling cascade; they may invoke unnatural processes effecting overall functioning of the cell. Such IWPs may produce different side effects leading to cancer or even death of cell in extreme cases which is not a wiser option until other options are void. First time, liposomally packed inhibitors were designed against aberrant mouse wnt3a expression such that the antagonists directly inhibit mouse wnt3a expression. The binding patterns and locations of these antagonists are still unknown but earlier studies reveal that they may have interacted with acyl compound for inhibiting possible wnt-frizzled interaction [26]. Computational technology has led to develop new algorithms for molecular docking which have enabled researchers to search large libraries of compounds. Comparative docking studies not only provides binding patterns but also helps to determine the docking mode and better conformation analysis under equilibrium conditions [27]. The library of compounds used in this study was selected from earlier reports that tallies only one large scale virtual screening of compounds based on small molecular antagonists for wnts [28]. It was suggested that using same antagonists for ligand based virtual screening of NP produce same results. Therefore, library of 272 unique NP compounds was selected for docking with Wnt10B for possible small molecule inhibitors of wnt proteins (IWPs) against aberrant Wnt10B. Site-directed molecular docking of Wnt10B with bound palimitoleic acid, was performed. The X-ray crystal structure of wnt deacylase notum (4UZQ) evidences same result where wnt-7a was deacylated to inhibit wnt-7a expression [29].

Top 20 compounds from docking through AD-VINA (with respect to binding energy values) and GOLD (goldscore values) were selected and critically analyzed. The interactions were drawn through Chimera and Ligplot tools. The analysis of interaction diagrams substantiated our idea and showed interactions with protein and bound palimitoleic acid. It was also observed that hydrophobic interactions were dominant in all the interaction diagrams for the highest ranked selected inhibitors. From top 20 compounds, three compounds were found common in both docking programs. It was also observed that out of three common compounds from both tools including GOLD and AD-VINA, the compound with ID ZINC85876388 was also common in the previously reported experiment on Wnt4. According to the results the efficiency of the compound ZINC85876388 has increased due to its evaluation as top compound in both experiments. Therefore, it is suggested that selected inhibitors may act as IWPs and can be used in further studies as lead compounds for screening of compounds, inhibitor designing, and experimentation with protein. It is also suggested that selected inhibitors may interrupt the wnt-frizzled cascades by interacting with Wnt10B and bound palimitoleic acid when supplied under aberrant conditions.

Conclusion and recommendation

It is concluded that from the conservation point of view the interacting molecules ZINC35442871, ZINC85876388 and ZINC00754234 may be considered as lead compounds for performing further drug designing experiments against aberrant wnt expressions.

Author's Contribution

Conceived the idea and concept: HU Mubeen, Literature search, data collection and analysis: Z Khalid, Data analysis, data interpretation, drafting and critical review: S Mannan.

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