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Virtual Screening and Pharmacophore Design for a Novel Theoretical Inhibitor of Macrophage Stimulating Factor as a Metastatic Agent

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ABSTRACT

Introduction: Metastasis is a crucial aspect of cancer. Macrophage stimulating protein (MSP) is a single chain protein and can be cleaved by serum proteases. MSP has several roles in metastasis. In this *in silico* study, MSP as a metastatic agent was considered as a drug target. ***Methods:*** Crystallographic structure of MSP was retrieved from protein data bank. To find a chemical inhibitor of MSP, a library of KEGG compounds was screened and 1000 shape complemented ligands were retrieved with FindSite algorithm. Molegro Virtual Docker (MVD) software was used for docking simulation of shape complemented ligands against MSP. Moldock score was used as scoring function for virtual screening and potential inhibitors with more negative binding energy were obtained. PLANS scoring function was used for revaluation of virtual screening data. ***Results:*** The top found chemical had binding affinity of -183.55 based on MolDock score and equal to -66.733 PLANTs score to MSP structure. ***Conclusion:*** Based on pharmacophore model of potential inhibitor, this study suggests that the chemical which was found in this research and its derivate can be used for subsequent laboratory studies.

Introduction

Metastasis is the darkest aspect of cancer, as it accounts for 90% of cancer-associated mortalities, and the least understood component of its pathogenesis. Once the cancer spreads all throughout the body, it is literally impossible to eradicate it neither by surgical nor by nonsurgical techniques.[1] However, metastasis is not a single step process: cells have to free themselves from where they have originally arisen, access blood stream by invading local tissues and vessels (intravasation), extravasate and establish new cellular colonies at distant sites.[2] Hence, it wouldn't be surprising that many factors should intervene in such a convoluted process. Among the possible candidates we put spotlight on Macrophage-stimulating protein.

As its name implies, Macrophage-Stimulating Protein (MSP) was first known as a chemotactic and activating serum agent for macrophage.[3] MSP is a member of Kringle domain-containing proteins which have diverged from an ancient family of serine proteases involved in blood coagulation and fibrinolysis.[4] During evolution, by some substituting mutation, MSP has turned into an inactive serum agent; however, this single-chain protein can still be cleaved by other serum serine proteases to form its active disulfide-linked heterodimer.[5] The main cells to synthesize MSP are hepatocytes, which secrete it into the circulation and maintain it in the serum at a relatively high concentration. MSP exerts its multiple biological effects on peritoneal resident macrophages in various ways such as induction of shape change and motility, direct chemotactic attraction, stimulation of ingestion of complement-coated erythrocytes, inhibition of endotoxin- or cytokine-induced expression of inducible nitricoxide synthase mRNA, induction of interleukin-6 production and differentiation of megakaryocytes , suppressing the colony formation of human bone marrow cells induced by Steel factor plus granulocyte macrophage-stimulating factor and stimulation of the bone resorbing activity of osteoclasts.[6-8] However, Macrophages are not the sole target of this protein; in particular, MSP can promote migration of various epithelial cell lines, a possible trigger for initiation of metastasis.[9-11] Up to date, Its role

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in metastasis of some cancers such as breast cancer has been confirmed.[12,13] Therefore it is expected that down regulation or inhibition of this protein leads to reduction of metastatic tumors in certain cancers. Having these in mind, we have dedicated this study to investigate MSP as a metastatic agent and to perform virtual screening for finding potential inhibitors of this protein and finally to design a pharmacophore model for inhibiting this protein. Since MSP is involved in metastasis, we aimed to target it in an *in silico* model. By application of this *in silico* modeling, further pharmacological approaches will be facilitated.

Material and methods

Protein structure and ligands

Crystallographic structure of human MSP was retrieved from protein data bank (http://www.rcsb.org/) with PDB ID: 2ASU. The model quality was X-RAY diffraction with resolution of 1.85 Å. This model was used for further virtual screening purpose. Also for finding proper theoretical inhibitors of MSP, we screened a library of KEGG.[14] To do this, FindSite webserver was used.[15] The algorithm which is provided by this server finds shape complemented ligands. One thousand MSP specific shape complemented chemicals were obtained from FindSite and used for further simulation docking analysis study.

Molecular docking study

Molegro Virtual Docker (MVD) software version 5.5 2012.5.5.0 by CLC bio Company was used for computer simulated docking analysis. Before initiation of docking operation, structure of protein and ligands were prepared. In preparation process, charge was calculated by MVD and assigned to the models, and flexible torsions in ligands were detected. Also probable explicit hydrogens were created and possible missing bonds were assigned. Finally, side chain minimization of MSP model was performed. During minimization process, only torsion angles in the side chains of amino acids were modified and other properties including bond lengths and backbone atom positions were held fixed. For simulating *in vivo* solvent condition, we performed molecular docking operation in the presence of H_2O and metal ions. Water box and neutralizing ions were added to MSP model by web-based CHARMM algorithm (http://www.charmm-gui.org/).

Docking parameters

Because MSP is a small protein and has 228 amino acids, we used a docking radius to cover the entire protein structure. To do this, docking radius was set on 26. In the next step of docking process, MolDock score with a grid resolution of 0.30 Å was used as scoring function for virtual screening.[16] we used MolDock as scoring function for virtual screening because it is a fast and accurate scoring function. During virtual screening process, internal electrostatic interaction and hydrogen bond between ligand and protein were permitted. MolDock SE was used as the docking algorithm and ten runs for each ligand with 1500 times iterations were carried out. By 1500 times iterations the least minimized energy of poses was reached. After simulated docking operation, minimization of energy and optimization of hydrogen bonds were performed for each pose. Also we used PLANTS score as an alternative scoring function for revaluation of docking data.[17]

Results

Molecular docking

Molegro Virtual Docker performs flexible ligand docking so that the optimal geometry of the ligand is determined during the docking. MVD includes MolDock and PLANTS scoring functions for evaluating docking results. The MolDock scoring function is based on a piecewise linear potential (PLP) and considers the directionality and charges of hydrogen bonding. MolDock scoring function is defined as:[16,18]

$$E_{score} = E_{inter} + E_{intra}$$

Where E_{inter} is ligand- protein interaction energy and is defined by:

$$E_{inter} = \sum_{i \in ligand} \sum_{j \in protein} \left[E_{PLP}(r_{ij}) + 332.0 \frac{q_i q_j}{4r_{ij}^2} \right]$$

The summation encompasses all heavy atoms in the protein and the ligand as well as any cofactor atoms and water molecule atoms. The second term points up the electrostatic interactions between charged atoms. E_{intra} describes the internal energy of the ligand:

$$E_{intra} = \sum_{i \in ligand} \sum_{j \in ligand} E_{PLP}(r_{ij}) + \sum_{flexible\ bonds} A[1 - cos(m.\theta - \theta_0)] + E_{clash}$$

The double summation contains all atom pairs in the ligand except those which are connected with two bonds or less. Second term is a torsional energy and θ is the torsional angle of the bond. E_{clash} term assigns penalty of 1000 provided that the distance between two heavy atoms is less than 2.0 Å.

Moreover, PLANTS scoring function which is used in this research is defined by:

$$E_{plantsscore} = f_{PLP} + f_{clash} + f_{tors} + _{csite} - 20$$

The PLP potential is similar to the one used by MolDock Score but here more interaction types (repulsive, buried, nonpolar, hydrogen bonding and metal) are taken into account. The ligand clash and torsional potentials, f_{clash} and f_{tors} take into account internal ligand clashes and torsional contributions for the flexible bonds in the ligand. The c_{site} term specifies a penalty that is calculated if a ligand conformation (pose) is located outside the binding site. The -20 energy offset was originally needed for the PLANTS search algorithm and is included here in order for PLANTS scores to be comparable with the original PLANTS implementation. Table 1 shows docking energy of each scoring function for top 3 poses.

Table 1. Binding affinity of chemical inhibitor to MSP based on PLANTS, MolDock and Reranking scores

Rerank Score	MolDock Score	PLANTS score	Pose Number
-81.8368	-183.55	-66.7331	Pose 1
-68.874	-159.693	-63.8119	Pose 2
-66.480	-142.119	-61.2327	Pose 3

Discussion

To find a chemical compound with high binding affinity to MSP, we selected 1000 shape-complemented chemicals from a library of KEGG compounds. Because MolDock is a fast algorithm, virtual screening was performed by this scoring function. The found chemical from virtual screening was used for next step docking analysis in the same condition with PLANTS as scoring function (Table 1). The screening data suggested that one chemical compound can bind to MSP with affinity of -183.55 based on MolDock score and equal to -66.733 binding energy based on PLANTS score. These scores indicate that our found potential inhibitor can bind to the structure of the MSP efficiently. Fig. 1 indicates best pose of the chemical inhibitor in contact with MSP structure. Furthermore, interactions between three top poses and MSP are depicted in Fig. 2. In this figure contact residues are determined. The structure of potential chemical inhibitor is depicted in Fig. 3. In the pharmacophore model of potential chemical inhibitor hydrophobic interactions, hydrogen donors and hydrogen acceptors are main pharmacophore classes. Table 2 describes pharmachophore coordinates in found potential inhibitor. Based on top 3 interactions between the potential inhibitor and MSP, it seems that the entire pharmacophore properties (Table 2 and Fig. 2 part D) are not included in interaction with MSP. In the structure of chemical inhibitor, the largest aromatic ring established minimum interaction with MSP. It is expected that change in the number and nature of the atoms which are present in this aromatic ring will lead to change in binding affinity to MSP. Resizing and replacement of atoms in this ring can be considered for further experimental studies.

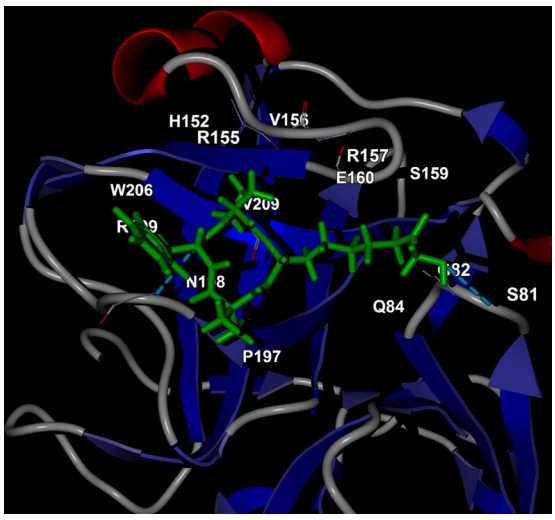

Fig.1. Top pose of potential inhibitor in contact with MSP

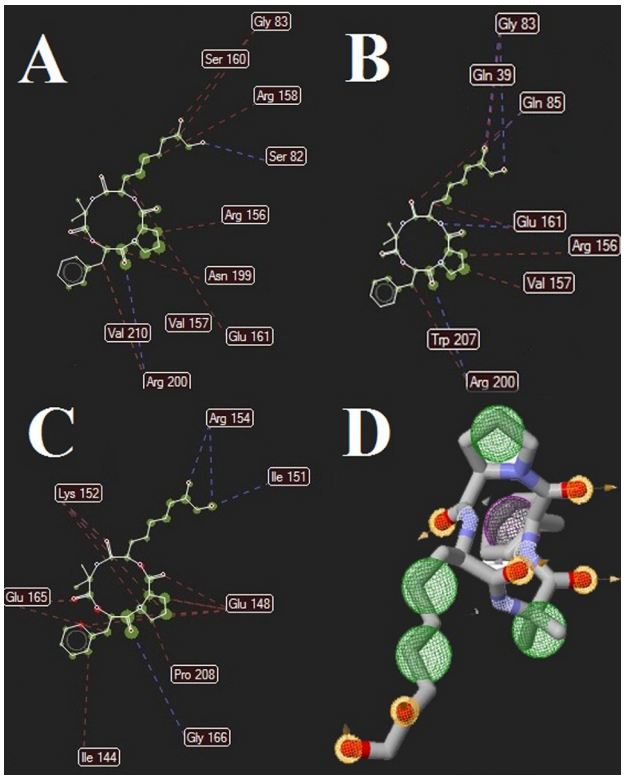

Fig. 2. Interactions between pose 1 (A), pose 2 (B) and pose 3 (C) with MSP and pharmacophore model of chemical inhibitor (D)

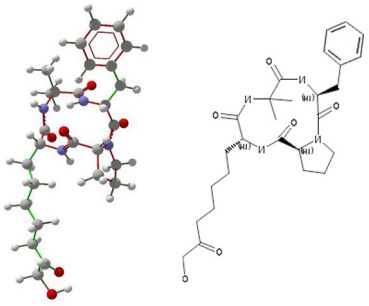

Fig. 3. The structure of chemical inhibitor. This structure can bind to MSP protein with avidity of -183.55 based on MolDock scoring function.

Conclusion

The preliminary aim of virtual screening trials is finding a pharmacophore model which could be used for further drug design purposes. Therefore finding an appropriate pharmacophore model is the most important step of *in silico* drug discovery. In this study we used x-ray crystallographic structure of MSP with resolution of 1.8 Å. This quality leads to more accurate simulation operations.

Table 2. pharmacophore properties of found chemical inhibitor

Pharmacophore Class	x	y	z	Radius
Aromatic	70.05	83.93	-4.85	1.10
Hydrogen Acceptor	71.66	79.23	-2.37	0.50
Hydrogen Acceptor	70.03	75.91	-4.30	0.50
Hydrogen Acceptor	68.50	79.55	-1.73	0.50
Hydrogen Acceptor	68.39	79.51	-6.97	0.50
Hydrogen Acceptor	72.62	70.81	-9.01	0.50
Hydrogen Acceptor	73.66	71.86	-11.42	0.50
Hydrogen Donor	70.32	79.59	-4.21	0.50
Hydrogen Donor	71.28	77.49	-5.47	0.50
Hydrogen Donor	68.19	77.56	-5.74	0.50
Hydrogen Donor	73.66	71.86	-11.42	0.50
Hydrophobic	70.05	83.93	-4.85	1.00
Hydrophobic	72.71	77.90	-4.34	1.00
Hydrophobic	71.57	73.69	-8.21	1.00
Hydrophobic	65.63	78.61	-4.06	1.00
Hydrophobic	69.58	74.97	-7.53	1.00

Also for molecular docking analysis we used Moldock, a fast and accurate scoring function. By using PLANTS as second scoring function, we tried to increase the accuracy of docking process. Moreover, docking operation was performed in solvent condition. As the result, the potential chemical inhibitor found in this study can be used for subsequent laboratory studies.

Ethical issues

Not applicable.

Competing interests

The authors declare no conflict of interests.

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