RESEARCH ARTICLE

Identification of a Potential Anticancer Target of Danshensu by Inverse Docking

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Abstract

Objective: To study potential targets of Danshensu via dual inverse docking. Method: PharmMapper and idTarget servers were used as tools, and the results were checked with the molecular docking program autodock vina in PyRx 0.8. Result: The disease-related target HRas was rated top, with a pharmacophore model matching well the molecular features of Danshensu. In addition, docking results indicated that the complex was also matched in terms of structure, H-bonds, and hydrophobicity. Conclusion: Dual inverse docking indicates that HRas may be a potential anticancer target of Danshensu. This approach can provide useful information for studying pharmacological effects of agents of interest.

Keywords: Danshensu - GTPas HRas - IdTarget -inverse docking - PharmMapper - pharmacological agents

Asian Pac J Cancer Prev, **15 (1)**, 111-116

Introduction

Danshensu is a prominent water-soluble constituent purified from Danshen (Salvia miltiorrhiza), one of the most widely used traditional Chinese medicine (TCM) in China (Zhou et al., 2005). Danshensu has been shown to dilate basilar arteries (Tang et al., 2011), improve cerebral microcirculation (Li et al., 2012), and impart cardiovascular protection (Husna et al., 2007; Li et al., 2012). In addition, it has been found to be able to scavenge oxygen-free radicals (Zhou et al., 2012), suppress cardiomyocytes apoptosis (Yin et al., 2013), protect the endothelial cells against homocysteine-induced dysfunction (Chan et al., 2004), and inhibit the proliferation and activation of hepatic stellate cell-T6 (Zhang et al., 2012). Now, Danshen, Danshensu or other active constitute from Danshen is being the hotspot of the TCM research.

Hot to get the right target is the bottleneck in the molecular mechanism researches of TCM which philosophy, framework and technologies are quite different from those of western medicine (Li et al., 2010). To meet this challenge, some basic chemoinformatics techniques, including molecular similarity searching, virtual screening and inverse docking, have been utilized in an attempt to gain a deep understanding of TCM and to accelerate the TCM-based drug discovery (Li et al., 2010).

Ligand-protein inverse docking is first proposed in 2001, and refers to computationally docking a specific small molecule of interest (natural product, lead or synthetics compound) to a database of protein structure (Chen et al., 2001). This database is developed from protein 3D structure in the protein data bank (PDB), and the docking is conducted with a procedure involving multiple-conformer shape-matching alignment of a molecule to a cavity followed by molecular-mechanics torsion optimization and energy minimization on both the molecule and the protein residues at the binding region (Chen et al., 2001; Chen et al., 2001). The application of this approach may facilitate the prediction of unknown and secondary therapeutic target proteins and those relate to the side effects and toxicity of a drug or drug candidate (Chen et al., 2001; Chen et al., 2001). So, as a small molecule from traditional medicine, Danshensu is well suited as the subject of an inverse docking study.

The aim of the present study is to identify the potential molecular targets of Danshensu via PharmMapper or idTarget, an inverse docking approach, and to verify by autodock vina in PyRx 0.8. The objective is to find a way to illustrate the potential mechanism of TCM.

Materials and Methods

Protein target prediction by PharmMapper

PharmMapper Server (http://59.78.96.61/pharmmapper), first appeared in 2010, is a freely accessed web-server designed to identify potential target candidates for the given probe small molecules (drugs, natural products or other newly discovered compounds with binding target unidentified) using pharmacophore mapping approach (Liu et al., 2010). PharmMapper hosts a large, in-house repertoire of pharmacophore database annotated from all the targets information in TargetBank,

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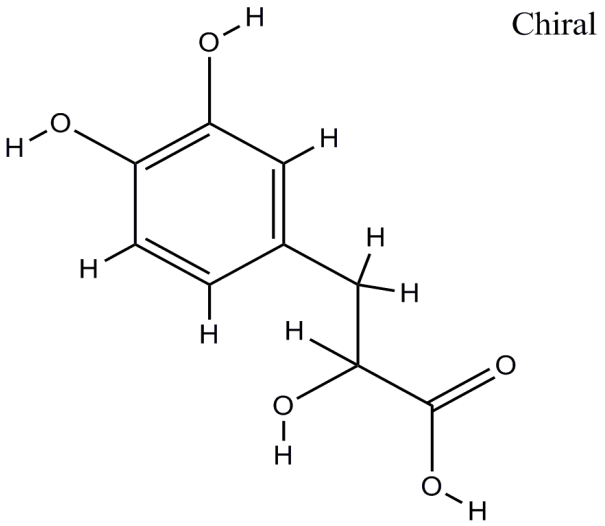

Figure 1. Chemical Structure of Danshensu Downloaded from Pubchem (CID 439435)

BindingDB, DrugBank and potential drug target database, including over 7000 receptor-based pharmacophore models (covering over 1500 drug targets information) (Liu et al., 2010). A comparative inverse screening approach using idTarget and PharmMapper was done to identify the potential receptors for Saffron bioactive substance such as Croctein, Picrocrocinand Safranal (Bhattacharjee et al., 2012). And tyrosine kinase might be the potential targets of a novel series of acenaphtho[1,2-b]pyrrole derivatives via PharmMapper (Chen et al., 2011). It was also used to identify the proapoptic, anti-inflammatory, anti-proliferative, anti-invasive and anti-angiogenic targets of essential oils in cardamom that is also known as "Queen of Spices" (Bhattacharjee et al., 2013).

2D SDF file of Danshensu (also known as 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid, CID 439435) was download form Pubchem database(http://pubchem.ncbi.nlm.nih.gov/) (Figure 1). SDF file format was converted into a mol2 format in Openbabel soft (freely accessible from web http://openbabel.org/wiki/Main_Page). This step is a prerequisite for submission of the bioactive compounds in PharmMapper (Liu et al., 2010; Bhattacharjee et al., 2012). Then, the mol2 file was submitted to the server. During the procedure, yes of Generate Conformers, 300 of maximum generated conformations, All 7302 targets of targets set, 300 of number reserved matched targets. Other parameters were set to the default values.

Protein target prediction by idTarget

idTarget is a freely web-based server (http://idtarget.nchc.org.tw/idtarget/) for identifying protein targets of small chemical molecules with robust scoring functions and a divide-and-conquer docking approach (Wang et al., 2012). It was used to identify potential anti-neoplastic targets of Saffron functional components (Bhattacharjee et al., 2012).

The file of Danshensu was submitted in idTarget server. Fast mode of Protein set, AM1-BCC/Amber PAR M99SB of charge models for ligand/protein. Other parameters were set to the default values.

Molecular docking

Docking calculations were carried out using Autodo vina in PyRx 0.8 (freely downloaded from http://pyrx.sourceforge.net/downloads) in the experiment. Autodock vina, a new program for molecular docking and virtual screening, was reported to achieve an approximately two orders of magnitude speed-up compared to Autodock 4, while also significantly improving the accuracy of the binding mode predictions (Trott et al., 2010). PyRx0.8 includes autodock vina wizard with easy-to-use user interface and chemical spreadsheet-like functionality and powerful visualization engine, which makes it a valuable tool for molecular docking (Trott et al., 2010; Abreu et al., 2012). It was used to identification small molecules inhibitor of p53-mortalin complex for cancer drug (Utomo et al., 2012), and to dock the essential oils in cardamom to protein targets (Bhattacharjee et al., 2013).

The energy form of Danshensu was minimized and converted to pdbqt format by OpenBabel in PyRx 0.8 as ligand for molecular docking. The target protein structure was downloaded from protein Data Bank database. Protein preparing tool in TCM database@taiwan (http://dock.cmu.edu.tw/ligand.php) was used to extract ligand from binding site and protonate the protein structure, and to show ligand coordinate and radius information on target protein PDB file downloaded from the PDB website (Chen, 2011; Tsai et al., 2011).

During the docking procedure, the grid box was centered to cover the binding site residues and to accommodate ligand to move freely. The box was set to 10×10×10 nm, and the center coordinate was shown in Table 4. Other parameters were set to default values.

Visualization

The visualizations of the structure were done using PyMol molecular graphics system, and the diagrams of protein-ligand interaction were shown by ligplot soft.

Results

Protein targets predicted by PharmMapper

PharmMapper extracted all the pharmacophore model's ligands from PDB and stored them as a library dataset and calculate these ligand's fit score, and the output of a PharmMapper run is demonstrated in the form of a ranked list of hit target pharmacophore models that are sorted by fit score in descending order (Liu et al., 2010). Among the top 20 targets (Table 1), GTPase HRas (PDB ID 1P2S, Rank 14, PDB ID 5P21, Rank15, respectively) is related to diseases reported, which may be the cause of Costello syndrome, the cause of congenital myopathy with excess of muscle spindles, the cause of susceptibility to Hurthle cell thyroid carcinoma, and are implicated in a variety of human tumors including bladder cancer and oral squamous cell carcinoma. The text about diseases were extracted and summarized in Table 1.

The details of each pharmacophore model candidate, including the number of each pharmacophore feature, a 3D interactive visualization of molecule-pharmacophore alignment pose can be accessible after a PharmMapper running (Liu et al., 2010). The pharmacophore of HRas

Table 1. The Danshensu Potential Targets with High Fit Score Screened by PharmMapper

Rank	PDB ID	Target name	Fit score	Diseases
1	1P60	Deoxycytidine kinase	5.244	None
2	1C1L	Congerin-1	4.679	None
3	1YQT	RNase 1 inhibitor	4.384	None
4	1PI3	Benzoylformate decarboxylase	4.374	None
5	1R0Z	Cystic fibrosis transmembrane conductance reguator	4.273	None
6	1O25	Thymidylate synthase thyX	4.225	None
7	1I52	2-C-methyl-D-erythritol 4-phosphate cytidylytransferase	4.216	None
8	1XC7	Glycogen phosphorylase, muscle form	4.194	None
9	1NWK	Actin, alpha skeletal muscle	4.159	None
10	1H72	Homoserine kinase	4.081	None
11	1GU1	3-dehydroquinate dehydratase	4	None
12	1REJ	cAMP-dependent protein kinase catalytic subunit alpha	3.945	None
13	1RE8	cAMP-dependent protein kinase catalytic subunit alpha	3.866	None
14	1P2S	GTPase HRas	3.79	Costello syndrome; congenital Myopathy;
15	5P21	GTPase HRas	3.766	Cancer.
16	1GTE	Dihydropyrimidine dehydrogenase	3.75	None
17	1ADO	Fructose-bisphosphate aldolase A	3.749	None
18	1SEH	Deoxyuridine 5-triphosphate uncleotidohydrolase	3.707	None
19	1F81	Isocitrate lyase	3.699	None
20	1JF7	Tyrosine protein phosphatase non-receptor type 1	4.637	None

Table 2. Features of Danshensu and Pharmacophore Model

PDB ID	Name	Hydrophobic	Positive	Negative	Donor	Acceptor	Aromatic
1P2S	HRas	0	0	1	4	10	0
5P21	HRas	0	0	2	5	8	0

Table 3. Top 10 Potential Targets Screened by IdTarget

Rank	PDB ID	Protein name	ΔGpred (kcal/mol)	Kipred	Z-score
1	1UMG	Putative uncharacterized protein ST0318	-10.21	32.8nM	1.75
2	2D2F	SufC protein	-9.99	47.5nM	0.05
3	1N6K	Ras-related protein Ral-A	-9.72	74.9nM	0.5
4	2A78	Ras-related protein Ral-A	-9.7	77.5nM	0.51
5	2ZTS	Putative uncharacterized protein PH0186	-9.64	85.7nM	-0.18
6	1W4R	Thymidine kinase	-9.62	88.7nM	0.57
7	3BBP	Ras-related protiein Rab-6A	-9.62	88.7nM	0.57
8	1Z0D	Ras-related protein Rab-5C	-9.61	90.2nM	0.57
9	1G17	Ras-related protein SEC4	-9.58	94.9nM	0.59
10	3LBI	GTPase HRas	-9.56	98.1nM	0.61

Table 4. The Center Coordinates of Binding Site

PDB ID	Name	Center (x×y×z)	Binding Affinity (kcal/mol)
1P2S	GTPase HRas	11.65×33.35×19.69	-6

(1P2S) had one negative, four donors, and ten acceptors (Table 2). The pharmacophore of HRas (5P21) had two negative, five donors, and eight acceptors (Table2). The 3D interactive visualization of molecule-pharmacophore alignment is also shown in Figure 2.

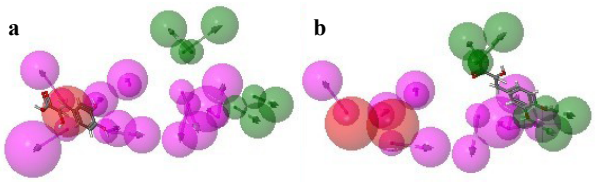

Figure 2. Alignment of Danshensu and Pharmacophore Model of HRas (a) 1P2S; (b) 5P21. Note: The pharmacophore features are schemed by color: Negative, red; Donor, green; Acceptor, magenta

Protein target predicted by idTarget

Among the all 7531 protein targets, GTP HRas (PDB ID 3LBI) is at the top 10 (Table 3).

Molecular docking

Autodock vina in PyRx 0.8 was used as the tool to verify the binding model of Danshensu with HRas. We chose HRas (PDB ID 1P2S) as the research target. In this experiment, the active binding site was extracted from origin ligand location, which coordinate was showed in Table 4. After docking procedure, superimposition of Danshensu conformations bound HRas (1P2S) is shown in Figure 3. And the lowest energy binding of complex was -6.0kcal/mol (Table 4).

Danshensu, colored in yellow, bound to HRas in the active cavity composed of residues such as Gly13, Val14, Gly15, Lys16, Ser17, and Glu31 (Figure 4b). More details can be seen in Figure 4c. Gly13, Gly15, Lys16, Ser17

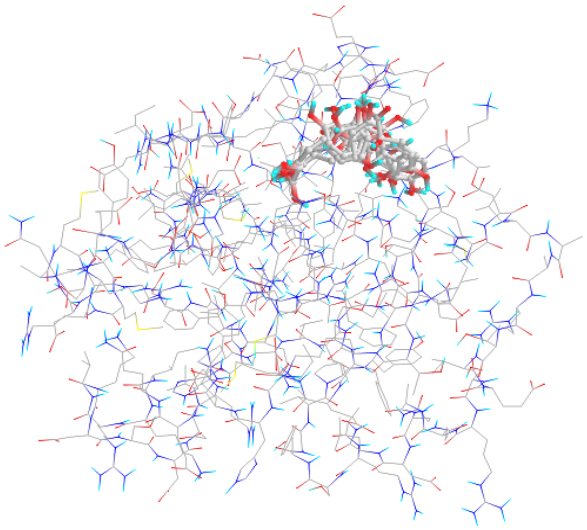

Figure 3. Docking Conformations of Danshensu Bound HRas (1P2S) written by PyRx 0.8. Danshensu: stick; HRas protein: line

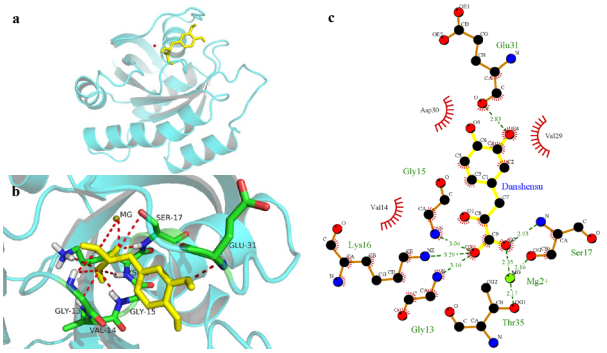

Figure 4. Diagrams Illustrating HRas-danshensu Interaction. (a) Overview of the complex of danshensu (yellow stick) - HRas (cyan cartoons, PDB ID 1P2S) structure. (b) 3D interaction diagram by PyMOL. Danshensu: yellow; hydrogen bond: red dash line. (c) 2D schematic of the key interaction diagrams in the complex. Danshensu is presented in yellow, C, N, O, and Mg atoms are represented in black, blue, red and green, respectively. Hydrogen bond and its length are presented in Olive green; hydrophobic contacts are presented in brick red

formed hydrogen bonds with N…O-H, having bond distance 3.16Å, 3.06Å, 3.29Å, 2.93Å, respectively. And Glu31 formed bonds with O…O-H with Danshensu, having bonding distance 2.83 Å. C9-OH in Danshensu interacted with Mg^{2+} in HRas, coordination bonding distance 2.35 Å.

A number of hydrophobic interactions are also observed in Figure 4c. Residues Val14, Val29, Asp30 formed hydrophobic contacts with Danshensu. Because of the different analytical tool, a few differences existed in Figure 4b and 4c.

Discussion

Inverse docking is a relatively new technique to find the potential protein targets of small molecules, which may be applied to the identification of unknown and secondary therapeutic targets of drugs, drug leads, natural products and other ligands (Chen et al., 2001). Several specific inverse docking strategies have been employed in academic and industrial researches, including GOLD, FlexX, Tarfisdock, PharmMapper (Kamper et al., 2006; Hui-fang et al., 2010; Liu et al., 2010). Oxidosqualenen cyclase, which is part of the cholesterol synthetic pathway, may be a new target for developing anticancer therapies by MDock, an inverse docking approach (Grinter et al., 2011). And it is effective to predict potential toxicity and side effect protein targets of small molecule such as Aspirin, Penicillin G, and Vitamin C by an inverse docking approach (Chen et al., 2001).

The chemical constituents of Danshen have been studied since the early 1930s, however, the molecular mechanism of action still be obscure (Zhou et al., 2005). So, the inverse docking approach can be very useful and have been used to find targets of the active chemical components from Danshen. Epidermal growth factor receptor was predicted to be the most possible direct protein target of Salvianolic acid B, another natural compound from Danshen, by INVDOCK, a ligand-protein inverse-docking algorithm (Feng et al., 2011). We found that HRas may be the potential disease-related target of Danshensu via PharmMapper (Table 1). It can be further confirmed by the details of each HRas (1P2S, 5P21) pharmacophore model which align to molecule features of Danshensu (Table 2, Figure 2). The result of HRas was also verified by idTarget (Table 3).

The 'classical' Ras proteins (HRas, NRas and KRas) are monomeric guanine nucleotide binding proteins that mediate a wide variety of cellular processes by converting a multitude of extracellular stimuli into specific biological response including proliferation, differentiation and survival (Silvius, 2002; Omerovic et al., 2007). HRas play key roles in cellular regulation and that are frequently mutated in human cancers (Silvius, 2002). The G4-decoys repressed HRAS transcription and caused a strong antiproliferative effect, mediated by apoptosis, in T24 bladder cancer cells where HRAS is mutated (Membrino et al., 2011). HRAS (G12V) transformed Ink4a/Arf-/- mouse mammary cells formed lethal tumors in vivo and had intratumoral heterogeneity (Kai et al., 2013). So, HRas may be an appropriate anticancer target of TCM. Natural chemical class, terpenoids, phenolics, quinones, lactones, polycarboxylic acids, alkaloids and cyclopentenediones, isolated form terrestrial as well as marine sources, show ability to modulated these KRas post-translational targets and their promising as potential anticancer agents (Bharate et al., 2012).

Danshensu are reported to have anticancer effects. Danshensu had anti-metastatic properties possibly via its anti-angiogenesis induced by down-regulation of VEGF and suppression of the invasion ability of cancer cells mediated by down-regulation of MMP-2 and MMP-9 and VEGF in B16F10 melanoma cell (Zhang et al., 2010). Danshensu might have the effect on treating non-small cell lung cancer by scavenging ROS and inhibiting phosphorylation of Akt, ERK1/2 and down-regulation Nrf2 expression in A549 cells (Tao et al., 2012).

The anticancer effect of Danshen or its active constituents may be through the Ras signaling pathway. A synthetic intermediate of Salvianolic acid A that is one of the active components from Danshen can suppress the

overexpression of c-myc oncogene, inhibit the function of Ras oncoprotein, increase the expression of P53 tumor suppressor gene to suppress the growth of mouse Lewis lung carcinoma, S180 sarcoma and H22 hepatic carcinoma in a dose-dependent manner (Li et al., 2002). Tanshinones, another bioactive compounds of Danshen, exhibited anticancer effect, both in vitro and in vivo, that were mediated at least partly through the interleukin-8, Ras-mitogen-activated protein kinase, and Rac 1 signaling pathway (Lee et al., 2008). From Table 1 or Table 3, we can say that HRas may be the potential anticancer target of Danshensu.

Information on other related-diseases of HRas is also shown in Table1. Defects in HRAS are the cause of Costllo syndrome, a distinctive rare multisystem disorder comprising a characteristic coarse facial appearance, intellectual disabilities, and tumor predisposition (Gripp et al., 2012), and activating HRAS mutations cause syndrome of congenital myopathy with excess of muscle spindles, hypertrophic cardiomyopathy and variable Noonan syndrome like facial features(van der Burgt et al., 2007). But there was no report about the effects of Danshen, Danshensu or other active components from Danshen on such diseases.

Protein-organic compound interactions are governed by hydrogen bonds, van der Waals contacts, and covalent bonds (Chen et al., 2009). Then we evaluated interaction of Danshensu-HRas complex by docking followed with PyMOL and Ligplot. Danshensu formed several H-bonds with residues 13-17 and Gly31 (Figure 4b, 4c), which is corresponding to the research that hydrogen bonds donors included the main chain NH groups of residues 13-18, 35 and 60, the hydroxyl group of Ser17 and Thr35 and the phenolic hydroxyl of Tyr32 from a neighboring molecule of p21 in the crystal lattice which made contact with the γ-phosphate (Pai et al., 1990).

Mg^{2+} plays a critical role in the stability and folding of HRas. The removal of both GDP and Mg^{2+} completely released the side chain packing but left a substantial fraction of the secondary structure intact in human P21H-Ras protein (Zhang et al., 1998). Protein-metal ion interactions are dominated by electrostatic force and coordination bonds (Chen et al., 2009). Mg^{2+} in the complex was coordinated to one oxygen each of the β- and γ-phosphates, and to the side chain hydroxyl groups of Ser17 and Thr35 in p21, respectively (Pai et al., 1990). Mg^{2+} appeared to be coordinated to one oxygen ion in Danshensu, to one in Ser17, to one in Thr35, forming three coordination bonds (Figure 4c). It means that danshensu may play a role on in the folding of HRas via Mg^{2+}.

Hydrophobic interactions are also critical for complex stabilization. Danshensu was surrounded by hydrophobic amino acids, including residues Gly13, Val14, Lys16, Ser17, Val19, Val29, Asp30, and Gly31 in HRas (Figure 4c). From the interaction diagram of HRas (1P2S) with origin ligand GNP shown in PBD web, more hydrophobic contacts can be found with residues Gly13, Lys16, Ser17, Ala18, Val29, Thr35, Gly60 et al (Buhrman et al., 2003). The ability of residues Gly13, Lys16, Ser17, Val29 to form hydrophobic contacts with GNP or Danshensu implied that they may play important roles in ligand-HRas complex stability. These results suggest that Danshensu has a lot of stabilizing interactions with HRas. Taken together, GTPase HRas may be the anticancer target of Danshensu.

In this study, the potential receptors of Danshensu were screened via PharmMapper and idTarget approach and verified by autodock vina in PyRx 0.8. Results suggest that GTPase HRas may be the potential anticancer targets of Danshensu. It illustrates that the inverse docking can be an important tool to prediction target of TCM active ingredients, providing an alternative method for rapid identification of therapeutic targets in the potential pharmacology theory to the clinical use of Danshensu or other traditional medicine.

Acknowledgements

This work was supported by Foundation of Zhejiang Province educational Committee (Y201330180).

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