RESEARCH ARTICLE

A Potential Target of Tanshinone IIA for Acute Promyelocytic Leukemia Revealed by Inverse Docking and Drug Repurposing

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Abstract

Tanshinone IIA is a pharmacologically active ingredient extracted from Danshen, a Chinese traditional medicine. Its molecular mechanisms are still unclear. The present study utilized computational approaches to uncover the potential targets of this compound. In this research, PharmMapper server was used as the inverse docking tool andnd the results were verified by Autodock vina in PyRx 0.8, and by DRAR-CPI, a server for drug repositioning via the chemical-protein interactome. Results showed that the retinoic acid receptor alpha (RARα), a target protein in acute promyelocytic leukemia (APL), was in the top rank, with a pharmacophore model matching well the molecular features of Tanshinone IIA. Moreover, molecular docking and drug repurposing results showed that the complex was also matched in terms of structure and chemical-protein interactions. These results indicated that RARα may be a potential target of Tanshinone IIA for APL. The study can provide useful information for further biological and biochemical research on natural compounds.

Keywords: Acute promyelocytic leukemia - chemical-protein interactome - drug repurposing - inverse docking

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Introduction

Tanshinone IIA (Figure 1) is the most abundant active compound isolated from Danshen (Salvia miltiorrhiza), a traditional Chinese medicine, which has been used in preventing and treating cardiovascular diseases for more than 2000 years in China and other Asian countries (Gao et al., 2012; Xu et al., 2013). Moreover, new pharmacological roles of this compound have recently been reported. Interest in its versatile protective effects on cancers has been growing over the last decade (Dong et al., 2011; Xu et al., 2013). The anti-tumor effect of Tanshinone IIA includes enhancing apoptosis of advanced cervix carcinoma CaSki cells (Pan et al., 2013), inhibiting invasion and metastasis of human colon carcinoma cells (Shan et al., 2009), suppressing angiogenesis in human colorectal cancer (Zhou et al., 2012), down-regulating epidermal growth factor receptors in hepatocelluar carcinoma cell (Zhai et al., 2009), and inhibiting Stat3 in breast cancer stem cells (Lin et al., 2013). Although substantial progress has been made in elucidating the cellular and molecular targets of Tanshinone IIA, it remains unknown which are direct targets as well as the mechanism of its multi-targeted actions (Xu et al., 2013). Drug discovery and development is a time-consuming and expensive process (Zhang, 2011). Therefore, identifying new indications or new targets for existing drugs is an efficient way of maximizing their potential (Luo et al., 2011). Computer-aided approaches have been widely used in drug research to improve the efficiency of the drug discovery and development pipeline, depending on the purpose and systems of interest (Zhang, 2011). Ligand-protein inverse docking approach is to find potential protein targets of a small molecule by the computer-automated docking search of a protein cavity database (Chen et al., 2001). Recently, we reported that GTPas HRas may be a potential anticancer target of Danshensu by PharmMapper, an inverse docking approach (Chen et al., 2014). Computational repurposing (or repositioning) is the process of designing and validating automated workflows that can generate hypotheses for new indications for a drug candidate (Hurle et al., 2013). Now, identifying new indications or new targets for existing drugs via computer tool is becoming a new hotspot in the drug research.

The aim of the present study was to identify new targets

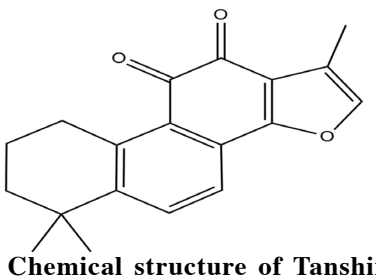

Figure 1. Chemical structure of Tanshinone IIA (PubChem CID: 164676)

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for Tanshinone IIA, and to facilitate an understanding the molecular mechanisms underlying. To do this, PharmMapper was used to identify the potential targets of Tanshinone IIA. Furthermore, the results were checked by autodock vina in PyRx 0.8 and DRAR-CPI, a server for chemical-protein interactome (CPI). The objective was to find a way to illustrate the molecular mechanism in a visual manner.

Materials and Methods

Protein target prediction by PharmMapper

Pharm Mapper server is a web server for potential drug target identification using pharmacophore mapping approach (Liu et al., 2010). It is designed to identify potential target candidates for the given small molecules (drugs, natural products, or other newly discovered compounds with unidentified binding targets) (Liu et al., 2010). PharmMapper is freely available at http://59.78.96.61/pharmmapper. The server hosts a large, in-house repertoire of pharmacophore database annotated from all the targets information in TargetBank, BindingDB, DrugBank and potential drug target database, including over 7000 receptor-based pharmacophore models (Liu et al., 2010). PharmMapper automatically finds the best mapping poses of the query molecule against all the pharmacophore models in PharmTargetDB and lists the top N best-fitted hits with appropriate target annotations, as well as respective molecule's aligned poses are presented (Liu et al., 2010). The server was employed for targets searching in a previous research paper on compound Danshen Formula for cardiovascular disease treatment (Li et al., 2012). In addition, it was used to identify potential targets of saffron (Bhattacharjee et al., 2012), and to discover targets of essential oils in cardamom (Bhattacharjee et al., 2013).

This step was performed as described previously (Chen et al., 2014). Briefly, 2D SDF file of Tanshinone IIA (PubChem CID: 164676) was downloaded from PubChem database, and converted into a mol2 format in Openbabel soft. Then, the mol2 file was submitted to the PharmMapper server. During the procedure, the maximum generated conformations was set to 300, targets set was set to all 7302, and the number of reserved matched targets was set to 300. Other parameters were kept as default.

Molecular docking by autodock vina in PyRx 0.8

Molecular docking was performed via Autodock vina in PyRx0.8 in this study. AutoDock Vina in PyRx0.8, an open-source program for doing molecular docking, significantly improves the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading (Trott et al., 2010). The soft was performed on virtual screening of Indonesian herbal database as HIV-1 reverse transcriptase inhibitor (Syahdi et al., 2012). It was also used in a research on testicular receptor 4 (Deshmukh et al., 2013), as well as in a report focused on essential oils in cardamom (Bhattacharjee et al., 2013). Accordingly, PyRx 0.8 with easy-to-use user interface offers a valuable tool for computer-aided drug design (Trott et al., 2010).

Protein or ligand was prepared as described in our previous paper (Chen et al., 2014). Briefly, target protein was pretreated by the protein preparing tool in TCM database@taiwan (http://dock.cmu.edu.tw/ligand.php) that can extract ligand from binding site, protonate protein structure, and show ligand coordinate (listed in Table 2) and radius information. Ligand (Tanshinone IIA) was processed via software OpenBabel in PyRx0.8. During the docking procedure, the gird 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 centre coordinate was shown in Table 2. Other parameters were kept as default.

Chemical-protein interactome by DRAR-CPI

Drug repurposing of Tanshinone IIA was performed in DRAR-CPI server (freely available at http://cpi.bio-x.cn/drar/). It is a web server for identifying drug repositioning potential and adverse drug reactions via the CPI (Luo et al., 2011). Using the server, Luo et al had successfully predicted the connections between anti-psychotics and anti-infectives, indicating the underlying relevance of anti-psychotics in the potential treatment of infections (Luo et al., 2011). The server will visualize the drug-protein interactions, with amino acid residue around 6 Å of the molecule highlighted (Luo et al., 2011). Therefore, it will be another useful tool for prediction of drug-target interactions.

Tanshinone IIA molecule in mol2 format with charges and hydrogens added was pretreated following the web instructions (Luo et al., 2011). Then, the file was submitted to the server. Parameters were set to default values.

Visualization

The visualizations of the complex structure were performed using soft PyMol, and the diagrams of chemical-protein interactions were shown by soft Ligplot.

Results

Protein target prediction by PharmMapper

Sorted by fit score in descending order, the top 10 disease-related targets are listed in Table 1. According to disease information, leukemia-related targets correspond to retinoic acid receptor alpha (RARα) and proto-oncogene tyrosine-protein kinase LCK (Lck). In addition, the pharmacophore of RARα had five hydrophobic, three acceptors (Figure 2). The pharmacophore of Lck had five hydrophobic, one positive, three donors and two acceptors (Figure 2). Moreover, the details of each pharmcophore model candidate and a 3D interactive visualization of molecule-pharmacophore alignment poses are accessible in Figure 2.

Molecular docking

RARα was chosen for further investigation in this research. The binding energy of the lowest state of Tanshinone IIA- RARα was -7.5 kcal/mol (Table 2). As shown in Figure 3, Tanshinone IIA, colored in yellow, bound to RARα ligand-binding domains (LBDs). This compound can form one hydrogen bond with Leu269

Table 1. The Top 10 Potential Diseases-related Targets of Tanshinone IIA with High Fit Score

PDB ID	Name	Fit Score	Disease
1RLB	Transthyretin	5.494	Amyloidosis type 1
1RBP	Retinol-binding protein 4	4.64	Retinol-binding protein deficiency
1DKF	Retinoic acid receptor alpha	4.315	Acute promyelocytic leukemia
1I9Q	Carbonic anhydrase 2	3.839	Autosomal recessive osteopetrosis type 3
1S9J	Dual specificity mitogen-activated protein kinase kinase 1	3.815	Cardiofaciocutaneous syndrome
1RDT	Peroxisome proliferator-activated receptor gamma	3.751	Type 2 insulin-resistant diabetes; hypertension
3CH6	Corticosteroid 11-beta-dehydrogenase isozyme 1	3.747	Cortisone reductase deficiency
2IL6	Aldose reductase	3.737	Diabetes; galactosemia
2OG8	Proto-oncogene tyrosine-protein kinase LCK	3.736	leukemias
2QXW	Aldose reductase	3.721	Diabetes; galactosemia;

Table 2. The Center Coordinates of Binding Site and the Lowest Binding Energy

PDB ID	Name	Center (x×y×z)	Binding Energy (kcal/mol)
1DKF	RARα	-20.62×84.28×0.79	-7.5

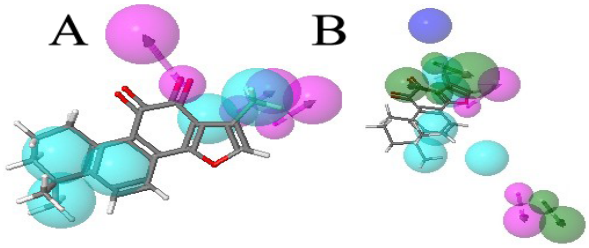

Figure 2. Alignment of Tanshinone IIA and Pharmacophore Model of Targets. A) 1DKF. **B)** 2OG8. Note: Pharmacphore features are schemed by color: Hydrophobic, cyan; Positive, blue; Negative, red; Donor, green; Acceptor, magenta

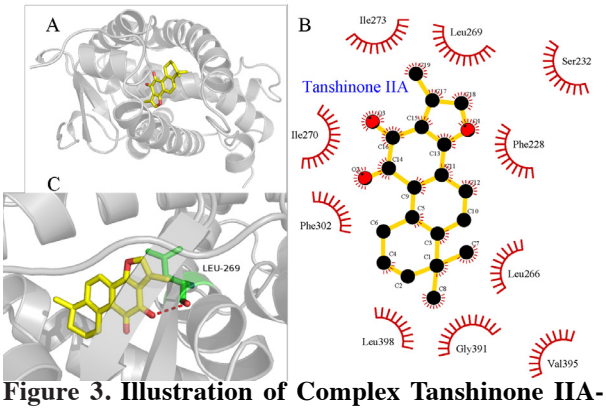

Figure 3. Illustration of Complex Tanshinone IIA-RARα. A) Overview. **B)** 3D interaction diagram by PyMOL. Tanshinone IIA: yellow; hydrogen bond: red dash line. **C)** 2D schematic of interactions by ligplot. Danshensu: yellow; C, N, O atoms are represented in black, blue and red; Hydrophobic contacts are presented in brick red

(Figure 3B). A number of hydrophobic interactions are also observed in Figure 3C. Residues Phe228, Ser232, Leu269, Ile270, Ile273, Phe302, Leu398, Gly391 and Val395 formed hydrophobic contacts with Tanshinone IIA. The differences in Figure 3B and Figure 3C were probably due to the different analytic tools using in this experiment.

Chemical-protein interactome by DRAR-CPI

Interactomes of drug across the targetable proteins was ranked using Z'-scores (Luo et al. 2011). The results of Tanshinone IIA-RARα by DRAR-CPI, including affinity score, Z-score and Z'-score are given in Table 3. In addition, the binding pattern is visualized in Figure 4.

Table 3. Results of Tanshinone IIA-RARα Interactome by DRAR-CPI

PDB ID	Affinity Score	Z-score	Z'-score
1DKF	-33.906	-0.802367	-0.581015

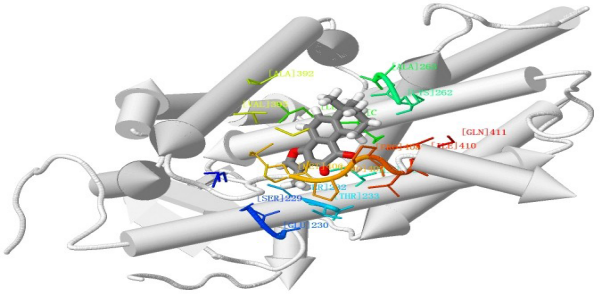

Figure 4. Bind Pattern of Tanshinone IIA-RARα Illustrated by DRAR-CPI. Note: Protein chain: rocket; Drug: stick; Key residues: colorful line

Discussion

In silico methodologies have advanced as a valuable technique in early drug discovery and as more and more target structures, structure bioactivity data and, therefore, optimized chemoinformatic tools come available they are likely to expand impact within drug development (Achenbach et al., 2011). Inverse docking, first proposed in 2001, refers to computationally docking a specific small molecule of interest to a database of protein structure (Chen et al., 2001; Chen et al., 2014). In this study, we found the RARα may be the potential disease-related target of Tanshinone IIA via inverse docking (Table 1). The pharmacophore models aligned to molecule features of Tanshinone IIA (Figure 2), which further support the inverse docking results.

Chemical-protein interactions information may help us in-depth understanding the complex structures. Then we investigated the interaction of Tanshinone IIA-RARα complex by software PyMOL and Ligplot. Original ligand BMS614 interacted with the RARα LBDs via many key residues, including Leu269 (Bourguet et al., 2000). This supported the result that Tanshinone IIA can interact with Leu269 (Figure 3B). Hydrophobic interactions are also

critical for complex stabilization (Chen et al., 2014). As shown in the interactions diagram of RARα with origin ligand BMS614, many hydrophobic amino acids can be observed, including Phe228, Ser232, Cys235, Ile236, Leu266, Phe302, Val395 and Met406 (Bourguet et al., 2000). Tanshinone IIA was surrounded by hydrophobic residues, Phe228, Ser232, Leu269, Ile270, Ile273, Phe302, Leu398, Gly391 and Val395 (Figure 3C). These results mean that Tanshinone IIA, same as BMS614, can interact with RARα LBDs. Moreover, Ser232, Leu266, Ile270 and Val395 correspond to those residues that determine the RARα, β, γ isotype selectivity of synthetic retinoids (Bourguet et al., 2000). Thus, these results indicate that Tanshinone IIA may play a pharmacological role on RARα selectivity via these key residues.

Drug repurposing is being used to systematically identify novel indications for drugs already known or discontinued in clinical development in recent years (Achenbach et al., 2011). CPI, an approach to drug repurposing, is an interaction strength matrix of drugs across multiple human proteins aiming at exploring the unexpected drug-protein interactions, with a variety of computational strategies, including docking, chemical structure comparison and text-mining etc (Yang et al., 2011). Yang et al. discovered that estrogen receptor and histone deacetylase may be two new therapeutic targets of Alzheimer's disease via CPI (Yang et al., 2010).

DRAR-CPI has a representative collection of drug molecules and targetable human proteins for identifying drug repositioning potential and adverse drug reactions via the CPI (Luo et al., 2011). In a DRAR-CPI job, targets towards the uploaded drug with a Z'-score <-0.5 were treated as the favorable targets and those with Z'-score>0.5 as unfavorable targets (Yang et al., 2009; Luo et al., 2011). According the Z'-score listed in Table 3, RARα may be the favorable targets of Tanshinone IIA. As shown in Figure 4, more residues interacted with Tanshinone IIA, including many key residues Ser232, Ile236, Leu266, Val395, Met406 (Figure 4). These CPI results are in agreement with the molecular docking results (Figure 3C), and with the results of BMS614. All these indicate that Tanshinone IIA can fit RARα LBDs well.

Disease information is also summarized in Table 1. Acute promyelocytic leukaemia (APL) is a rare disease, but it has considerable importance as one of the best understood malignancies and the only one that has been cured through target therapies (de The et al., 2010). As a potential anticancer compound, Tanshinone IIA plays roles in APL treatments. Liu et al reported that Tanshinone IIA exhibited induction of apoptosis by activation of caspase-3, downregulation of anti-apoptotic protein bcl-2 and bcl-xl and upregulation of pro-apoptotic protein bax, as well as disruption of the mitochondrial membrane potential in APL NB4 cells, suggesting that Tanshinone IIA may serve as an effective adjunctive reagent for the treatment of APL (Liu et al., 2006). And The APL cell line HL-60s' cell growth inhibition and apoptosis-induced rates were found higher in the group Tanshinone IIA plus Salvianolic Acid B than other single groups (Guo et al., 2008). In addition, Wang et al reported that tetraarsenic tetrasulfide, indirubin, and Tanshinone IIA combination yielded synergy in the treatment of a murine APL model *in vivo* and in the induction of APL cell differentiation *in vitro* (Wang et al., 2008). But its molecular targets are still unclear.

RARα plays a central role in the biology of the myeloid cellular compartment (Parrado et al., 2000). Translocation and the consequent fusion promyelocytic leukaemia (PML) –RARα, the only constant genomic abnormality in APL cells and often the only detectable one, probably represent the malignant initiating events in APL (Parrado et al., 2000; Akagi et al., 2009; de The et al., 2010). Notable, Wang et al reported that tetraarsenic tetrasulfide, indirubin, and tanshinone IIA combination causes intensified ubiquitination/degradation of PML–RARα oncoprotein, stronger reprogramming of myeloid differentiation regulators, and enhanced G1/G0 arrest in APL cells through hitting multiple targets (Wang et al., 2008). These results are in keeping with the identification of RARα as a potential target of Tanshinone IIA for APL.

Another leukemia-related target of Tanshinone IIA is proto-oncogene tyrosine-protein kinase LCK (Lymphocyte-specific kinase, Lck) (Table 1). Lck is a cytoplasmic kinase of the Src family expressed in T cells and natural killer (NK) cells (DiMauro et al., 2006). But there has no report about the effects of Danshen, or Tanshinone IIA on Lck. Whether it is another direct target, further studies are needed.

In this study, the potential targets of Tanshinone IIA were screened via PharmMapper, verified by autodock vina in PyRx 0.8 and CPI. The results suggest that RARα may be a potential target of Tanshinone IIA for APL. The study provides a guide for further biological and biochemical research, and a novel molecular mechanism of Tanshinone IIA actions.

Acknowledgements

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