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Mechanism of dihydrotanshinone I in the treatment of helicobacter pylori infection based on network pharmacology and molecular docking technology

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Competing interests

The authors declare no conflicts of interest.

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Abbreviations

Hp, helicobacter pylori; DHT, dihydrotanshinone I; PPI, protein protein interaction; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological processes; MF, molecular functions; CC, cellular components; PDB, protein data bank; IGHG1, Ig gamma-1 chain C region; KDM4B, Lysine Demethylase 4B; RXRA, Retinoic acid X receptor alpha; STAT1, Signal transducerand activator of transcription 1; STAT3, Signal transducerand activator of transcription 3; PTGS2, Prostaglandin G synthase 2; HSP90AB1, Heat shock protein HSP 90-beta; PTGS1, Prostaglandin G synthase 1; SCN5A, Sodium channel protein type 5 subunit alpha; ADRA1A, Recombinant adrenergic receptor alpha 1A; ADRA1B, Alpha-1B adrenergic receptor; GABRA1, Gamma-aminobutyric acid receptor subunit alpha-1; PIK3CG, Phosphoinositide-3-kinase; MAPT, Microtubule Associated Protein Tau; ADRB2, Beta 2 adrenergic receptor; HTR3A, 5-hydroxytryptamine receptor 3A; IDO1, Indoleamine 2, 3-dioxygenase 1; Th17 cell, T helper cell 17; JAK, janus kinase; STAT, signal transducer and activator of transcription; IL-6, Interleukin-6; TFG-β, transforming growth factor-β; TNF-α, tumor necrosis factor-alpha; IFN-γ, Interferon-γ; mRNA, messenger RNA; CagA, cytotoxin-associated gene A protein; eEF1A1, eukaryotictranslation elongation factor 1 alpha 1; p-STAT3, phospho-STAT3; NF-kB, Nuclear Factor- Kappa B.

Citation

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Abstract

Objective: Based on the network pharmacology approach and molecular docking technology, the core targets of dihydrotanshinone I (DHT) for the treatment of helicobacter pylori (Hp) infection were searched and the potential mechanisms of drug therapy were explored. **Methods:** The TCMSP database and Swiss Target Prediction database were employed to identify drug targets. To mine disease targets based on GeneCards, OMIM, DrugBank, DisGeNET, and TTD databases. Then the two were intersected to obtain common targets. The protein protein interaction (PPI) network map of common targets was constructed on the basis of the String network platform and Cytoscape software, and the targets with degree values over 1/2 maximum degree value were selected as core targets. Molecular docking verification of DHT and core targets were performed using AutoDock and PyMOL software. Finally, gene ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the common targets were carried out using the Metascape database and R-4.0.2-win software. **Results:** A total of 13 targets of DHT was extracted for the treatment of Hp, and five core targets, including Signal transducerand activator of transcription 1 (STAT1), Signal transducerand activator of transcription 3 (STAT3), Prostaglandin G synthase 2 (PTGS2), Signal transducerand activator of transcription 4 (STAT4) and Indoleamine 2, 3-dioxygenase 1 (IDO1), were screened according to their degree values. Molecular docking indicated that DHT had an excellent binding to the core target. 29 pathways were yielded by KEGG enrichment analysis, and a total of 48 biological processes, 7 cellular components and 13 molecular functions were derived from GO enrichment analysis. **Conclusion:** DHT may decrease pro-inflammatory factor expression and immune cell infiltration to treat Hp infection via the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway regulated by STAT1, STAT3, STAT4, etc.

Keywords: dihydrotanshinone; helicobacter pylori; mechanism of action; network pharmacology; molecular docking

Background

Hp is a Gram-negative microaerobic bacterium that colonizes the human gastric mucosa and is classified as a class I carcinogen by the World Health Organization, and more than 60% of gastric cancers are caused by Hp [1, 2]. Epidemiological studies have shown that the global prevalence of Hp is 44.3% worldwide, 34.7% in developed countries, 50.8% in developing countries, and up to 56% in China [3–4]. Hp infection is a risk factor for chronic gastritis, gastric ulcer, gastric mucosa-associated lymphoid tissue lymphoma and gastric cancer, and eradication of Hp significantly reduces the incidence of Hp-associated gastrointestinal diseases [5–6]. The American College of Gastroenterology guidelines recommend proton pump triplex or bismuth quadruplex as a first-line regimen for eradication of Hp [7]. However, with the increasing resistance rate of Hp in recent years, the eradication rate of proton pump triple therapy has been less than 70%, and the eradication rate of bismuth quadruple method has been decreasing and the adverse effects such as diarrhea are becoming more prominent, which urgently needs the intervention of new drugs [8–9].

A recent study revealed that DHT combined with omeprazole was more effective than standard triple therapy in killing Hp in vivo in a mouse model of multi-drug resistant Hp infection, while in vitro assays showed strong time-dependent bactericidal activity of DHT, which is expected to be an emerging drug for eradication of multi-drug resistant Hp [10]. However, the mechanism of action of DHT for the eradication of Hp has not been elucidated, and its long-term efficacy and safety are unclear. Therefore, this study aims to predict the core targets and major pathways of DHT for the treatment of Hp infection based on network pharmacology and molecular docking techniques, so as to provide a reference for subsequent studies.

Methods

Source of drug targets

We searched DHT and reviewed the targets of DHT using the Systematic Pharmacology Database and Analysis Platform for Traditional Chinese Medicine (TCMSP，https://tcmsp-e.com/). The target genes corresponding to each target protein were then matched against the Uniprot database (https://www.uniprot.org/). At the same time, the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) was used to predict the targets of DHT. The targets obtained from the two databases were pooled to obtain the drug targets of DHT.

Source of disease targets

The disease related targets were obtained by searching for “Hp” using GeneCards (https://www.genecards.org/), OMIM (https://mirror.omim.org/), DrugBank (https://go.drugbank.com/), DisGeNET (https://www.disgenet.org/), TTD (http://db.idrblab.net/ttd/) databases, and the disease targets of Hp infection were summarized from the five databases. The common targets were derived by taking the intersection of drug targets and disease targets, and the Venn diagram of drug-disease common targets was drawn using R-4.0.2-win software.

Construction of PPI

The common targets of drugs and diseases were imported into the String network platform (https://string-db.org/). The protein species was set to “Homo sapiens”. The confidence level was set to > 0.9. The free targets were removed and the PPI was constructed. The node relationship information was loaded into Cyroscape 3.8.0 to calculate the degree values. The targets with degree value over 1/2 maximum degree value were selected as the core targets of DHT for the treatment of Hp. And Cyroscape 3.8.0 software was used to map the PPI.

GO functional enrichment analysis and KEGG pathway enrichment analysis

The common targets of compounds and diseases were imported into the Metascape database (http://metascape.org/). The species was defined as “Homo sapiens”, and $P < 0.01$ was set. GO functional enrichment analysis and KEGG pathway enrichment analysis of biological processes (BP), molecular functions (MF) and cellular components (CC) were performed on common targets. And the R-4.0.2-win software was used to draw the histogram of the GO enrichment analysis chart and the KEGG enrichment analysis bubble chart.

Drug component-common target molecular docking

The protein data bank (PDB) IDs of the common targets are searched through the String network platform and the corresponding protein structures were downloaded from the PDB database (https://www.rcsb.org/) based on the PDB IDs. If multiple PDB IDs existed for the same target, the best protein crystal structure was selected based on the root mean square deviation of the resolution. The proteins were processed using AutoDockT4.2.6 and PyMOL2.4 software to remove small molecule ligands and all water molecules. The location of the docking box was determined based on the small molecule composition provided by the PDB database. If the PDB database did not provide the small molecule composition, the POCASA platform (http://g6altair.sci.hokudai.ac.jp/g6/service/pocasa/) was used to predict the position of the docking box.

Results

Drug targets

The TCMSP showed a Mol ID of MOL007101 for DHT with a bioavailability of 45.04% and a drug-like property of 0.36. A total of 29 potential DHT targets were predicted using TCMSP and the Swiss Target Prediction database covering Prostaglandin G synthase 1 (PTGS1), Sodium channel protein type 5 subunit alpha (SCN5A), PTGS2, 5-hydroxytryptamine receptor 3A (HTR3A), Retinoic acid X receptor alpha (RXRA), Recombinant adrenergic receptor alpha 1A (ADRA1A), Alpha-1B adrenergic receptor (ADRA1B), Beta 2 adrenergic receptor (ADRB2), Gamma-aminobutyric acid receptor subunit alpha-1 (GABRA1), Heat shock protein HSP 90-beta (HSP90AB1), Phosphoinositide-3-kinase (PIK3CG), Ig gamma-1 chain C region (IGHG1), Nuclear receptor coactivator 2, Nuclear receptor coactivator 1, Microtubule Associated Protein Tau (MAPT), Lysine Demethylase 4A (KDM4A), Lysine Demethylase 4B (KDM4B), Lysine Demethylase 4C (KDM4C), Dual specificity tyrosine-phosphorylation-regulated kinase 1A, STAT3, STAT1, STAT2, STAT4, Muscleblind-like protein 1, Muscleblind-like protein 2, Muscleblind-like protein 3, IDO1, IDO2, Lysine Demethylase 4E (KDM4E) and others.

Disease targets and common targets

Using GeneCards, OMIM, DrugBank, DisGeNET, TTD database, a total of 1,969 disease targets related to Hp infection were obtained. After taking the intersection with the drug targets, a total of 13 common targets, namely DHT targets for the treatment of Hp infection, are obtained, which are PTGS1, PTGS2, HTR3A, RXRA, ADRB2, HSP90AB1, IGHG1, MAPT, KDM4B, STAT3, STAT1, STAT4, and IDO1 (Figure 1).

PPI

Thirteen common targets were imported into the String database. The PPI showed that IGHG1 targets were shed in the human species, KDM4B and RXRA were free targets, and the remaining 10 targets interacted with each other (Figure 2). The relevant data was loaded into Cytoscape 3.8.0 software for calculation. The results displayed that the highest degree values of STAT1, STAT3 and PTGS2 were 5, the degree values of STAT4 and IDO1 were 4, the degree value of HSP90AB1 was 3, and the degree values of PTGS1, MAPT, ADRB2 and

HTR3A were 1. Targets with STAT1, STAT3, PTGS2, STAT4 and IDO1 degree values > 3 may be the core targets of DHT for the treatment of Hp infection.

GO functional enrichment analysis and KEGG pathway enrichment analysis

The 13 common targets were imported into the Metascape database for GO enrichment analysis and KEGG enrichment analysis. GO enrichment results demonstrated the following: 48 BP of DHT for Hp infection (mainly related to interleukin-21 (IL-21) mediated signaling pathway, response to IL-21, cellular response to IL-21, interleukin-35 (IL-35) mediated signaling pathway and positive regulation of reactive oxygen species metabolic process, etc.); 7 CC (mainly related to receptor complexes, axons, neuronal cell bodies, cell bodies and dendrites, etc.); and 13 MF (mainly involving dioxygenase activity, heme binding, tetrapyrrole binding, oxidoreductase activity and oxidoreductase activity, etc.) (Figure 3).

KEGG enrichment results displayed a total of 12 pathways for DHT treatment of Hp infection, involving cancer pathways, T helper cell 17 (Th17 cell) differentiation, necrotizing ptosis, regulation of adipocyte lipolysis, inflammatory bowel disease, 5-hydroxytryptaminergic synapses, hepatitis C, JAK-STAT signaling pathway, nonalcoholic fatty liver, hepatitis B, Kaposi's sarcoma-associated herpesvirus infection, and Alzheimer's disease (Figure 4). The network relationship between drugs, targets, and pathways is shown in Figure 5.

Active ingredient-common target molecular docking

Ten common targets that exist interconnected were molecularly docked to the active ingredient DHT. When the binding energy of the ligand to the receptor is lower, the more stable the binding conformation of the two, and the greater the possibility of interaction, which is generally evaluated at -5kcal/mol [11]. The results indicated that DHT had a relatively high potential to bind to core targets such as STAT1, STAT3, PTGS2, STAT4 and IDO1, suggesting that they may be potential targets of DHT for the treatment of Hp infection. The possibility of DHT binding to other targets such as HSP90AB1, PTGS1, MAPT and ADRB2 are also relatively high, as shown in Table 1. Among them, the molecular docking patterns of DHT and the core targets STAT1, STAT3 and PTGS2 are shown in Figure 6, Figure 7 and Figure 8.

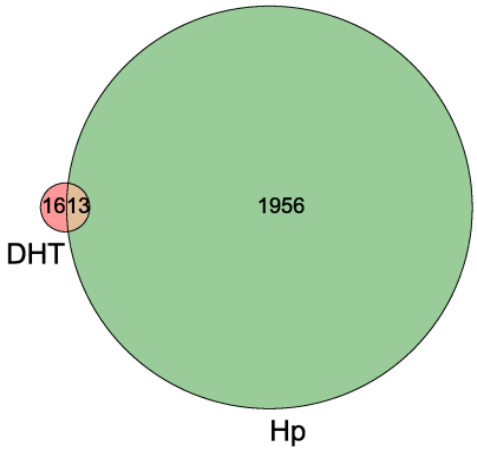

Figure 1 Venn DHT and Venn diagram of common targets of Hp infection. DHT, dihydrotanshinone I; Hp, helicobacter pylori.

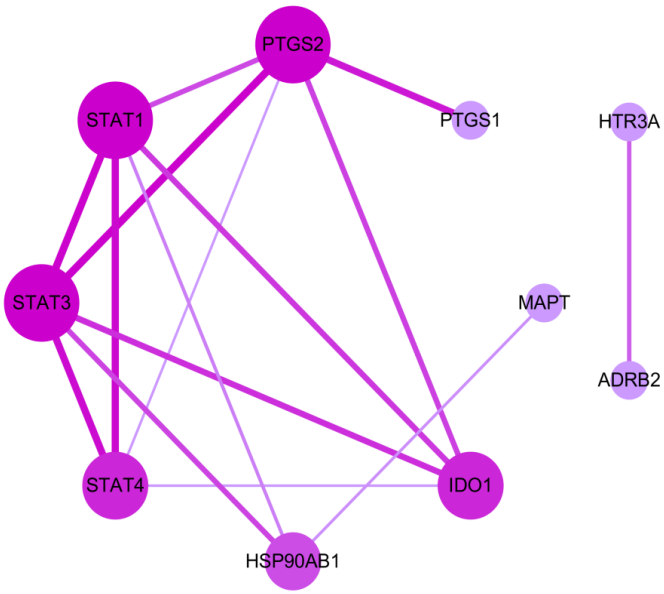

Figure 2 PPI diagram of DHT for Hp infection. PPI, protein protein interaction; DHT, dihydrotanshinone I; Hp, helicobacter pylori; STAT1, Signal transducerand activator of transcription 1; STAT3, Signal transducerand activator of transcription 3; STAT4, Signal transducerand activator of transcription 4; PTGS2, Prostaglandin G synthase 2; IDO1, Indoleamine 2, 3-dioxygenase 1; HSP90AB1, Heat shock protein HSP 90-beta; PTGS1, Prostaglandin G synthase 1; MAPT, Microtubule Associated Protein Tau; ADRB2, Beta 2 adrenergic receptor; HTR3A, 5-hydroxytryptamine (serotonin) receptor 3A.

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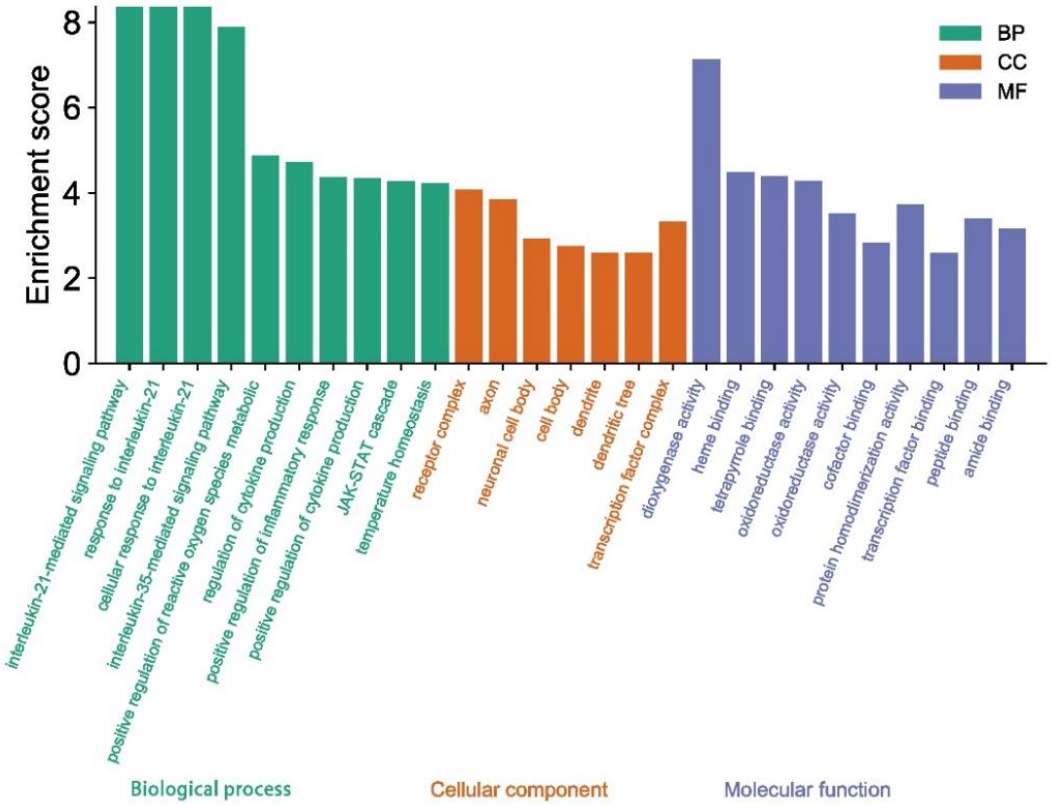

Figure 3 GO functional enrichment analysis of DHT treatment for Hp infection. GO, gene ontology; DHT, dihydrotanshinone I; Hp, helicobacter pylori; JAK-STAT, janus kinase-signal transducer and activator of transcription; BP, biological processes; MF, molecular functions; CC, cellular components.

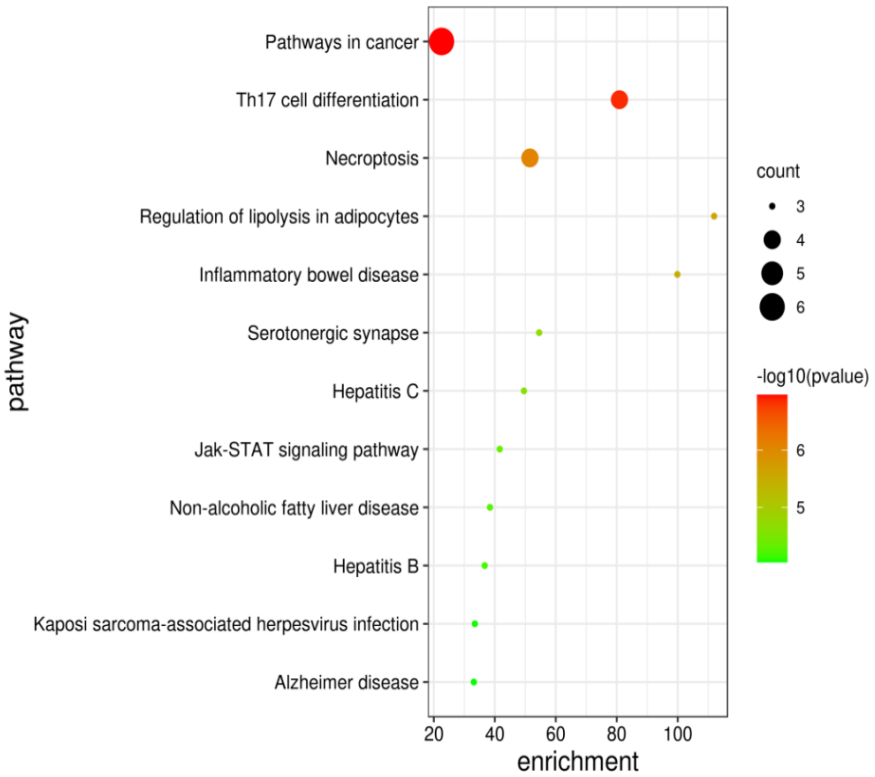

Figure 4 KEGG pathway enrichment analysis of DHT for the treatment of Hp infection. KEGG, Kyoto Encyclopedia of Genes and Genomes; DHT, dihydrotanshinone I; Hp, helicobacter pylori; Th17 cell, T helper cell 17; JAK-STAT, janus kinase-signal transducer and activator of transcription.

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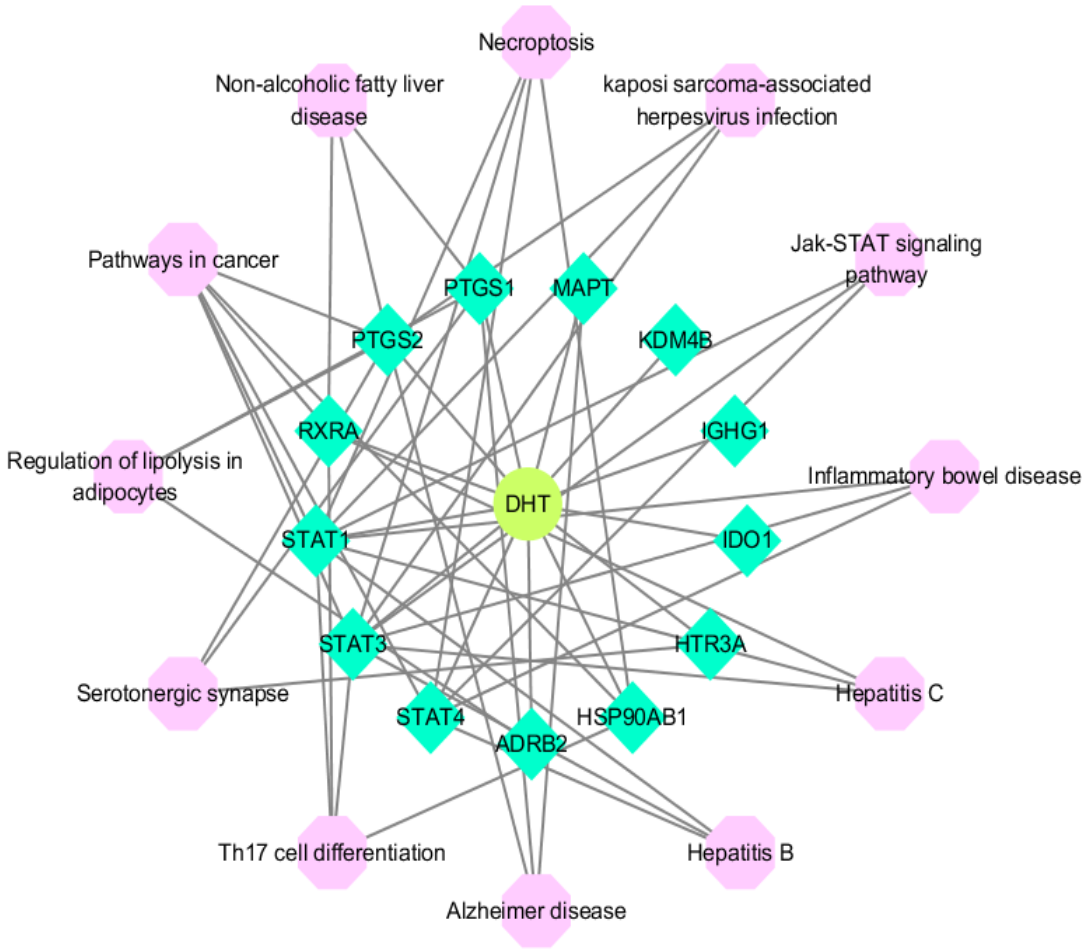

Figure 5 Target-pathway reticulation of DHT for Hp infection. DHT, dihydrotanshinone I; Hp, helicobacter pylori; Th17 cell, T helper cell 17; JAK-STAT, janus kinase-signal transducer and activator of transcription; STAT1, Signal transducerand activator of transcription 1; STAT3, Signal transducerand activator of transcription 3; STAT4, Signal transducerand activator of transcription 4; PTGS1, Prostaglandin G synthase 1; PTGS2, Prostaglandin G synthase 2; IDO1, Indoleamine 2, 3-dioxygenase 1; HSP90AB1, Heat shock protein HSP 90-beta; MAPT, Microtubule Associated Protein Tau; ADRB2, Beta 2 adrenergic receptor; HTR3A, 5-hydroxytryptamine (serotonin) receptor 3A; KDM4B, Lysine Demethylase 4B; RXRA, Retinoic acid X receptor alpha; IGHG1, Ig gamma-1 chain C region.

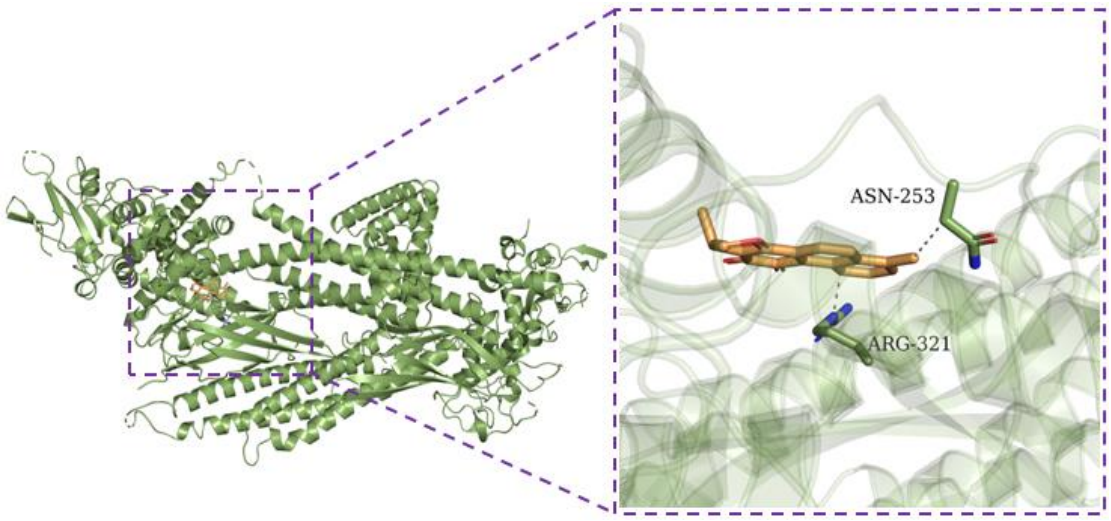

Figure 6 Molecular docking pattern of DHT and STAT1. DHT, dihydrotanshinone I; STAT1, Signal transducerand activator of transcription 1.

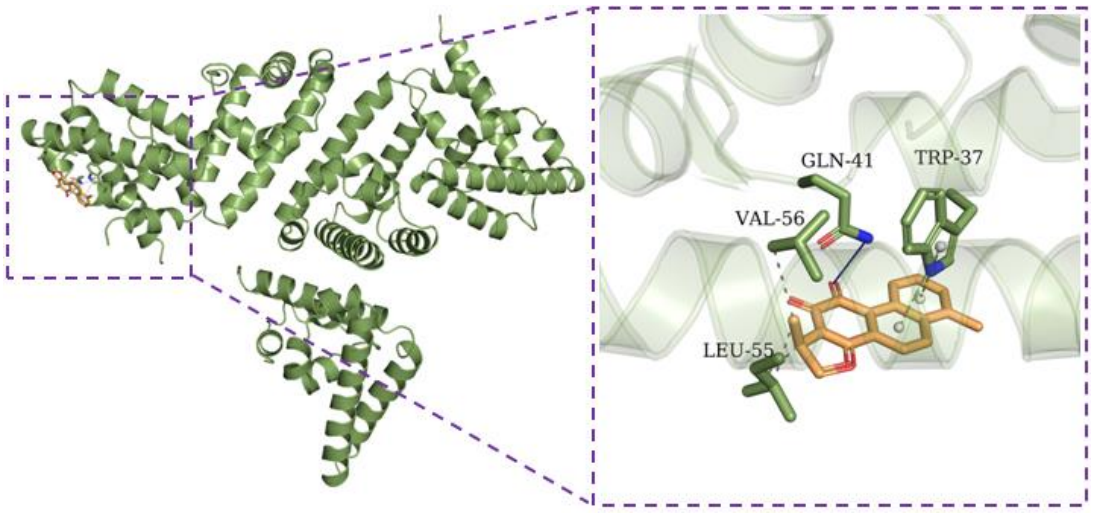

Figure 7 Molecular docking pattern of DHT and STAT3. DHT, dihydrotanshinone I; STAT3, Signal transducerand activator of transcription 3.

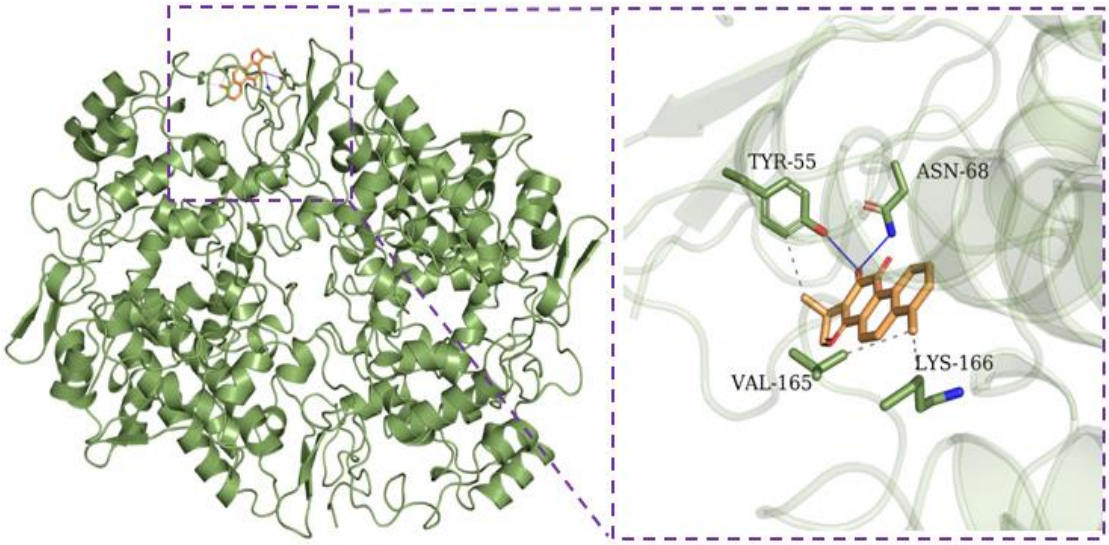

Figure 8 Molecular docking pattern of DHT and PTGS2. DHT, dihydrotanshinone I; PTGS2, Prostaglandin G synthase 2.

Table 1 Virtual docking of common targets and active ingredients

Target	PDB ID	Compound	Mol ID	Combination of energy kcal/mol
STAT1	1 yvl	DHT	MOL007101	-5.9
STAT3	4 zia	DHT	MOL007101	-5.2
PTGS2	5 fl9	DHT	MOL007101	-5.5
STAT4	1bgf	DHT	MOL007101	-7.6
IDO1	5 ek4	DHT	MOL007101	-10.0
HSP90AB1	5 ucj	DHT	MOL007101	-8.3
PTGS1	6 y3c	DHT	MOL007101	-7.2
MAPT	5 dmg	DHT	MOL007101	-5.4
HTR3A	4 pir	DHT	MOL007101	-4.2
ADRB2	3 sn6	DHT	MOL007101	-7.6

STAT1, Signal transducerand activator of transcription 1; STAT3, Signal transducerand activator of transcription 3; PTGS2, Prostaglandin G synthase 2; STAT4, Signal transducerand activator of transcription 4; IDO1, Indoleamine 2, 3-dioxygenase 1; HSP90AB1, Heat shock protein HSP 90-beta; PTGS1, Prostaglandin G synthase 1; ADRB2, Beta 2 adrenergic receptor; MAPT, Microtubule Associated Protein Tau; HTR3A, 5-hydroxytryptamine (serotonin) receptor 3A; DHT, dihydrotanshinone I.

Discussion

DHT is one of the active ingredients of Salvia miltiorrhiza and is a red lipophilic powder with a molecular weight of 278.3 [12]. DHT has also been shown to have moderate antibacterial activity against certain Gram-positive bacteria, such as bacillus subtilis and staphylococcus aureus [13]. Studies have shown that DHT has excellent bactericidal activity in vivo and in vitro and is effective in killing strains of drug-resistant to Hp [10]. The bactericidal activity of DHT is closely related to its extremely low resistance rate, which may result from the presence of multiple targets of DHT that affect Hp. Although DHT has been demonstrated to have strong time-dependent bactericidal activity against Hp and a very low resistance rate, the mechanism by which DHT antagonizes Hp remains to be elucidated. A total of 13 potential targets of DHT for the treatment of the Hp infection were obtained from the network pharmacological analysis, except for KDM4B and RXRA as free targets and IGHG1 target shedding, the remaining 10 targets had interactions. Among them, STAT3, STAT1 and PTGS2 had the highest degree values, followed by STAT4 and IDO1, which may be the core targets of DHT for the treatment of Hp infection. The above targets are highly enriched in the JAK-STAT signaling pathway (STAT3, STAT1, STAT4), and molecular docking also confirmed the possibility of DHT binding to STAT3, STAT1, and STAT4 targets, so the JAK-STAT signaling pathway may be an important pathway for DHT treatment of Hp infection. It has been proven that DHT can attenuate helper T-cell responses and reduce pro-inflammatory factor expression and immune cell infiltration by inhibiting STAT1 and STAT3, providing a theoretical basis for the relevance of DHT to the JAK-STAT signaling pathway [14].

The JAK-STAT signaling pathway is involved in major signaling pathways for a range of physiological and cellular processes, such as cell proliferation, stem cell self-renewal, and immune response [15]. In the state of Hp infection, cytokines such as Interleukin-6 (IL-6), Interleukin-17 (IL-17), and transforming growth factor-β (TFG-β) are released by helper and regulatory T cells within the gastric environment [16–18]. IL-6 and TGF-β are considered to be central mediators of the pathogenesis associated with Hp and are able to directly influence the JAK/STAT signaling cascade [19–20]. Many cytokines such asInterleukin-1 (IL-1), Interleukin-2 (IL-2), IL-6, Interleukin-10 (IL-10), IL-17, Interleukin-23 (IL-23), tumor necrosis factor-alpha (TNF-α), TGF-β, and Interferon-γ (IFN-γ) are secreted at the site of infection and in the blood circulation, and specific membrane receptors expressed by gastric epithelial cells can assist different cytokines in transmitting the signals they carry to the cell interior [21, 22]. They bind to cognate membrane receptors and regulate downstream effectors in a signaling cascade, inducing JAK phosphorylation, and phosphorylated JAK provides a docking point for STAT [23, 24]. After phosphorylation on tyrosine and serine residues, STAT forms a dimer and translocates to the nucleus, where it acts intracellularly after binding directly to the promoter regions of specific target genes involved in the immune response [24].

Cytotoxin-associated gene A protein (CagA) is an important virulence agent for Hp, and CagA in the cytoplasm after Hp infection significantly increased IL-6 messenger RNA (mRNA) and protein levels in gastric epithelial cells [25–27]. This is associated with CagA-eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) affecting phospho-STAT3 (p-STAT3) activity mediating IL-6 expression [20, 28]. IL-6 has been shown to be an important factor in Hp gastric carcinogenesis, and over expression of IL-6 and TGF-β in gastric cancer models leads to increased STAT3 activity, suggesting that abnormal activation of the JAK-STAT signaling cascade is a key event in gastric carcinogenesis [24, 29]. In addition, IFN-γ can also lead to STAT3 phosphorylation, which binds to components of the gastric epithelium and induces the expression of inflammatory genes [30–32]. Substances such as reactive oxygen species produced in its inflammatory response can damage DNA, damage cells and even promote cancer [32]. It has been reported that JAT/STAT3 is an upstream signaling pathway activated by Nuclear Factor- Kappa B (NF-kB) in Hp-infected gastric epithelial cells [33]. Agitated NF-kB is able to mediateInterleukin-8 (IL-8) expression [34]. IL-8 associated with Hp infection-mediated gastritis and gastric cancer is elevated in both gastric epithelial cells [35]. And elevated IL-8 levels are associated with increased risk of atrophic gastritis and diffuse gastric cancer [36]. It has been shown that glutamine supplementation may reduce IL-8 production by inhibiting hydrogen peroxide-mediated JAK1/STAT3 activation, thereby contributing to the prevention of gastric inflammation, corroborating the role of JAK-STAT-mediated IL-8 in gastric mucosal inflammation [37]. Furthermore, agonism of STAT1 has also been reported to promote IL-8 secretion, and STAT1 may also play a role in Hp-infected gastritis [38]. It is noteworthy that although the JAK-STAT signaling pathway may be a key pathway for DHT to treat Hp infection, the JAK-STAT signaling pathway needs to be in a phosphorylated state to perform its function [39–40]. And the pathway of DHT activation of JAK-STAT phosphorylation is unknown, so the predicted results remain to be validated by subsequent studies.
In summary, DHT may reduce pro-inflammatory factor expression and immune cell infiltration to treat Hp infection through the JAK-STAT signaling pathway regulated by STAT1, STAT3, and STAT4. This study predicted the potential targets and mechanisms of action of DHT for the treatment of Hp infection and provided a scientific basis for further studies on DHT for the treatment of Hp infection.

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