

Research Article

The Potential Mechanism of Dihydroartemisinin in the Treatment of Thyroid Cancer Through Network Pharmacological Analysis

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Abstract

Background: Thyroid cancer (THCA) has recently become one of the most common endocrine malignancies worldwide. Dihydroartemisinin (DHA) exhibits well-documented antitumor activity, but its therapeutic potential in THCA remains largely unclear. **Methods:** The relative mRNA and protein expression levels of the apoptosis-related markers Bax, Bcl-2, and Caspase-3 in the human THCA cell line TPC-1 were evaluated by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and Western blotting. Potential active targets of DHA were obtained from the TCMSP, SwissTargetPrediction and SuperPred databases. The THCA-related targets were collected from TCGA, DisGeNET and GeneCards. Protein-protein interaction (PPI) network construction, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to predict key targets and pathways of DHA against THCA. **Results:** DHA treatment significantly modulated the expression of apoptosis-related markers, including upregulation of Bax and Caspase-3 and downregulation of Bcl-2, suggesting that DHA may promote apoptosis in TPC-1 cells. A total of 230 potential targets were identified from the overlap between DHA- and THCA-related targets. Indeed, *STAT3*, *PIK3R1*, *PIK3CA*, *PIK3CD*, *MAPK1*, *MAPK3*, *EGFR*, *HSP90AA1*, *ESR1*, *PTPN11* were identified as the top 10 hub genes. In total, 2214 biological processes, 118 cellular components, and 220 molecular functions were enriched. KEGG analysis indicated that DHA may suppress THCA primarily via the chemical carcinogenesis-receptor activation pathway. **Conclusion:** These findings suggest that DHA is a promising candidate for THCA treatment and warrant further investigation, including direct apoptosis assays and *in vivo* validation.

Keywords: thyroid neoplasms; dihydroartemisinin; network pharmacology; apoptosis; carcinogenesis; G-Protein-Coupled Receptors

1. Introduction

Thyroid cancer (TC), also referred to as thyroid carcinoma (THCA), represents the most prevalent malignancy of the endocrine system. Based on histopathological characteristics, TC is generally classified into three major categories: differentiated thyroid cancer (DTC), which includes papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC); medullary thyroid cancer (MTC), encompassing both sporadic and hereditary forms; and undifferentiated thyroid cancer, comprising poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC) [1]. Recent reports indicate a rising incidence of advanced THCA, with females being significantly more susceptible than males at a ratio of approximately 7:1 [2]. In China, the standardized incidence rate of THCA increased markedly from 1.4 per 100,000 person-years in 1990 to 14.65 per 100,000 person-years in 2016 [3]. Although current therapeutic approaches, including surgery, radioactive iodine (I-131, RAI, the most effective non-surgical therapy), hormone replacement therapy (HRT), external beam radiotherapy, targeted therapy and immunotherapy have substantially improved clinical outcomes for most patients with THCA, it remains a challenge for the treatment of advanced

or poorly differentiated and dedifferentiated THCA patients on account of their unfavorable prognosis and limited survival outcomes [4,5]. Therefore, the identification of novel therapeutic agents is essential to improve clinical efficacy and patient prognosis.

Dihydroartemisinin (DHA), a derivative and the primary active metabolite of artemisinin (ART), is widely used to treat malaria and is renowned for its excellent efficacy and favorable safety profile. Accumulating evidence indicates that DHA exerts potent antitumor activity through diverse mechanisms, including cell cycle inhibition, apoptosis induction, antiangiogenesis, ferroptosis, and vascular suppression [6,7]. DHA has been reported to be a potent agent in managing the initiation and development of colitis-associated colorectal cancer without obvious side effects [8]. In pancreatic cancer cells, DHA induces ferroptosis by disrupting iron metabolism and promoting lipid oxidative damage [9]. Compared with free DHA, ZIF-encapsulated DHA nanoparticles exhibit superior antitumor activity in several ovarian cancer cell lines [10]. Peng et al. [11] found that DHA promoted the effectiveness of oxaliplatin in colon cancer by enhancing PDGFR/RAF mediated signal transduction. DHA potentiated the therapeutic effect of doxorubicin

bicin, leading to significantly improved anticancer activity in breast cancer cells [12]. DHA could target PKM2-mediated glycolysis to enhance the antitumor effect of photodynamic therapy in oesophageal cancer cells [13]. Moreover, DHA can inhibit IL-6 production, which in turn affects the Hippo pathway and leads to the suppression of EMT in medullary thyroid carcinoma (MTC) [14]. These findings collectively highlight DHA as a promising candidate for THCA therapy. However, the exact molecular mechanism underlying the effects of DHA on THCA remains poorly understood and requires comprehensive investigation.

Network pharmacology is an established approach for investigating the molecular associations between active pharmaceutical ingredients and diseases, serving to uncover novel drug targets and clarify pharmacological mechanisms [15]. By simultaneously analyzing genes, drugs, pathways, and targets, this approach has shifted the traditional paradigm from “one drug–one target” to a systems-level model of multi-target therapeutics. In this study, we applied network pharmacology to systematically predict the shared and intersecting targets of DHA and THCA, aiming to identify their potential synergistic pathways. To further validate these *in silico* findings, we integrated the network predictions with experimental assays in cellular and animal models. This combined strategy provides a robust framework for elucidating the joint pharmacological actions of DHA and THCA and offers new insights into combination therapy development.

2. Material and Methods

2.1 Cell Culture

The human thyroid cancer cell line TPC-1 was obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin–streptomycin solution (Beyotime Biotechnology, Shanghai, China). Cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ using a Thermo Fisher Scientific 371 incubator (Waltham, MA, USA). To evaluate the effects of dihydroartemisinin (DHA; Shanghai Institute of Materia Medica, Shanghai, China), cells were exposed to 6.25, 25, or 100 µM DHA for 24 h, corresponding to low-, medium-, and high-dose treatment groups, respectively. Cells were seeded in 6-well plates at a density of 2×10^5 cells/well and cultured overnight before DHA treatment. Cell line authentication was confirmed by short tandem repeat (STR) analysis, and the obtained profile was consistent with the corresponding record in the ATCC database. In addition, mycoplasma contamination was excluded using a PCR-based detection assay (Beyotime, China).

2.2 Real-Time Quantitative PCR (qRT-PCR)

RNA extraction from TPC-1 cells was performed using a commercial total RNA isolation kit (Beyotime Biotechnology, China). RNA yield and quality were assessed using a NanoDrop One Spectrophotometer (Thermo Scientific, USA). First-strand cDNA was synthesized using a reverse transcription kit (Takara, Kyoto, Japan). The qRT-PCR analysis was conducted on an ABI 7900HT Fast Real-Time PCR platform (Applied Biosystems, USA). β -actin was used as the endogenous control, and gene expression was quantified using the $2^{-\Delta\Delta Ct}$ approach. The primers used for Bax, Bcl-2, Caspase-3, and β -actin are provided as follows:

Bax:	forward	sequence,	5'-
		GCCCTTTTGCTTCAGGGTTT-3'	
	Reverse	sequence, 5'-TCCAATGTCCAGCCTTTG-	3'
Bcl-2:	forward	sequence,	5'-
		CGGAGGCTGGGATGCCTTTG-3'	
	Reverse	sequence,	5'-
		TTTGGGGCAGGCATGTTGAC-3'	
Caspase-3:	forward	sequence,	5'-
		GAGCAGCTTTGTGTGTGTGA-3'	
	Reverse	sequence,	5'-
		GGCAGGCCTGAATGATGAAG-3'	
β -actin:	forward	sequence,	5'-
		TGAGGCCAGAGCAAGAGA-3'	
	Reverse	sequence,	5'-
		TGCAGTGCCACCAGTTGGTGACGAT-3'	

2.3 Western Blotting

Total protein was extracted from treated cells using RIPA lysis buffer (Beyotime, China) supplemented with 1 mmol/L PMSF. Protein concentrations were determined using a BCA Protein Assay Kit (Beyotime, China), followed by separation via SDS-PAGE. The primary antibodies used in this study included Anti-Bax antibody (1:5000, 60267-1-Ig, Proteintech, Rosemont, IL, USA), anti-Bcl-2 antibody (1:1000, sc-7382, SANTA CRUZ BIOTECHNOLOGY, INC, Dallas, TX, USA), anti-Caspase-3 antibody (1:2000, 66470-2-Ig, Proteintech, USA), and mouse anti-GAPDH antibody (1:10,000, 60004-1-Ig, Proteintech, USA). Proteins separated by SDS-PAGE were transferred to PVDF membranes and blocked in 5% BSA-containing PBST for 60 min. Membranes were incubated with specific primary antibodies overnight at 4 °C, followed by incubation with HRP-conjugated secondary antibodies after three PBST washes. Chemiluminescent signals were developed using an ECL detection system and quantified using ImageJ. Experimental data were generated from three independent biological replicates, each comprising triplicate technical measurements. Western blotting and qRT-PCR experiments were performed independently three times.

2.4 Database

The databases used in this study include: traditional Chinese medicines integrated database (TCMSP, <https://ngdc.cnmb.ac.cn/databasecommons/database/id/437>), SwissTargetPrediction (<http://www.swisstargetprediction.ch/>), SuperPred (<https://prediction.charite.de/index.php>), Universal Protein Resource database (UniProt, <https://www.uniprot.org/>), GeneCards (<https://www.genecards.org/>), Disease Gene Network (DisGeNET, <https://www.disgenet.org/>), The Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>), Search Tool for the Retrieval of Interaction Gene/Proteins (STRING, <https://cn.string-db.org/>), Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://davidbioinformatics.nih.gov/>), Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn/>), Kyoto Encyclopedia of Genes and Genomes database (KEGG, <https://www.genome.jp/kegg/>).

2.5 The Collection of Bioactive Compounds of DHA and the Prediction of DHA Targets

Information regarding DHA and its putative targets was retrieved from the TCMSP database. Relevant publications concerning DHA were additionally searched through PubMed and Google Scholar. These target proteins were subsequently converted into standardized gene symbols using the UniProt database, with the species restricted to *Homo sapiens*. After merging the datasets and removing duplicates, a final pool of potential DHA targets was established.

2.6 The Collection of Disease Target Genes of THCA

THCA-associated therapeutic target genes were identified by querying the GeneCards and DisGeNET databases with the keyword “thyroid cancer”. GeneCards and DisGeNET provide comprehensive curated disease-gene associations, while TCGA offers transcriptomic data from actual THCA patients. Duplicate targets were removed, and low-confidence targets (score <0.5 in DisGeNET or relevance score <5 in GeneCards) were excluded. Differentially expressed genes (DEGs) from TCGA were defined as $\text{adj. } p\text{-Val} < 0.05$ and $|\log_2 \text{fold change}| > 1$ (i.e., expression >2-fold or <0.5-fold). Genes were ranked by $\log_2\text{FC}$, and the top 10% (by absolute $\log_2\text{FC}$) were selected for intersection.

2.7 The Construction of Protein-Protein Interaction (PPI) Network and the Identification of Core Targets

The common targets between DHA and THCA were screened through an online Venn analysis platform. These intersecting targets were then submitted to the STRING database for the establishment of a PPI network to explore their potential interactions. The resulting interaction data (.tsv format) were visualized using Cytoscape (version

3.10.4, San Diego, CA, USA) to select core hub genes based on their degree values, where a higher degree denotes a greater number of protein-protein interactions, signifying increased biological importance within the network.

2.8 The Enrichment Analysis Via Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)

To investigate the biological functions and signaling pathways associated with the core targets, GO and KEGG enrichment analyses were carried out using DAVID. The resulting datasets were downloaded in Excel format, and significantly enriched terms ($p < 0.05$) were further selected and visualized as bubble plots.

2.9 Statistical Analysis

All statistical analyses were conducted using SPSS version 23.0 (IBM, Armonk, NY, USA) and GraphPad Prism 8 (GraphPad Software, Boston, MA, USA). Results are expressed as mean $\pm$ SD. Student's *t*-test was applied for comparisons between two groups, and one-way ANOVA was used for comparisons among multiple groups. Statistical significance was defined as $p < 0.05$.

3. Results

3.1 DHA-Induced TPC-1 Cell Apoptosis

To evaluate the pro-apoptotic effects of DHA in TPC-1 cells, the expression levels of Bax, Bcl-2, and Caspase-3 were examined at the mRNA and protein levels (Fig. 1). The results (Fig. 1C–I) showed that DHA treatment markedly enhanced Bax and Caspase-3 expression, whereas Bcl-2 was significantly suppressed in a dose-dependent manner relative to the control group. Furthermore, the increases of Bax and Caspase-3 levels were positively correlated with the doses of DHA. Conversely, Bcl-2 mRNA and protein levels decreased progressively with increasing doses of DHA. These findings indicate that DHA effectively promotes apoptosis in THCA cells, suggesting its critical role in suppressing thyroid tumor cell viability.

3.2 Intersection Targets Between DHA and THCA

To explore the potential mechanism driving the antitumor action of DHA in THCA, we first mapped the shared targets of the drug and the disease. In total, 243 DHA-associated potential active ingredient targets were identified based on the SwissTargetPrediction (100 targets) and SuperPred (161 targets) databases. According to the GeneCards (11,714 targets), TCGA (6406 targets) and DisGeNET (55 targets) databases, all together there were 14,830 THCA-associated targets were attainable after the removal of 3345 repeated targets. Venn diagram analysis performed using R software identified 230 overlapping targets shared between DHA and THCA (Fig. 2A).

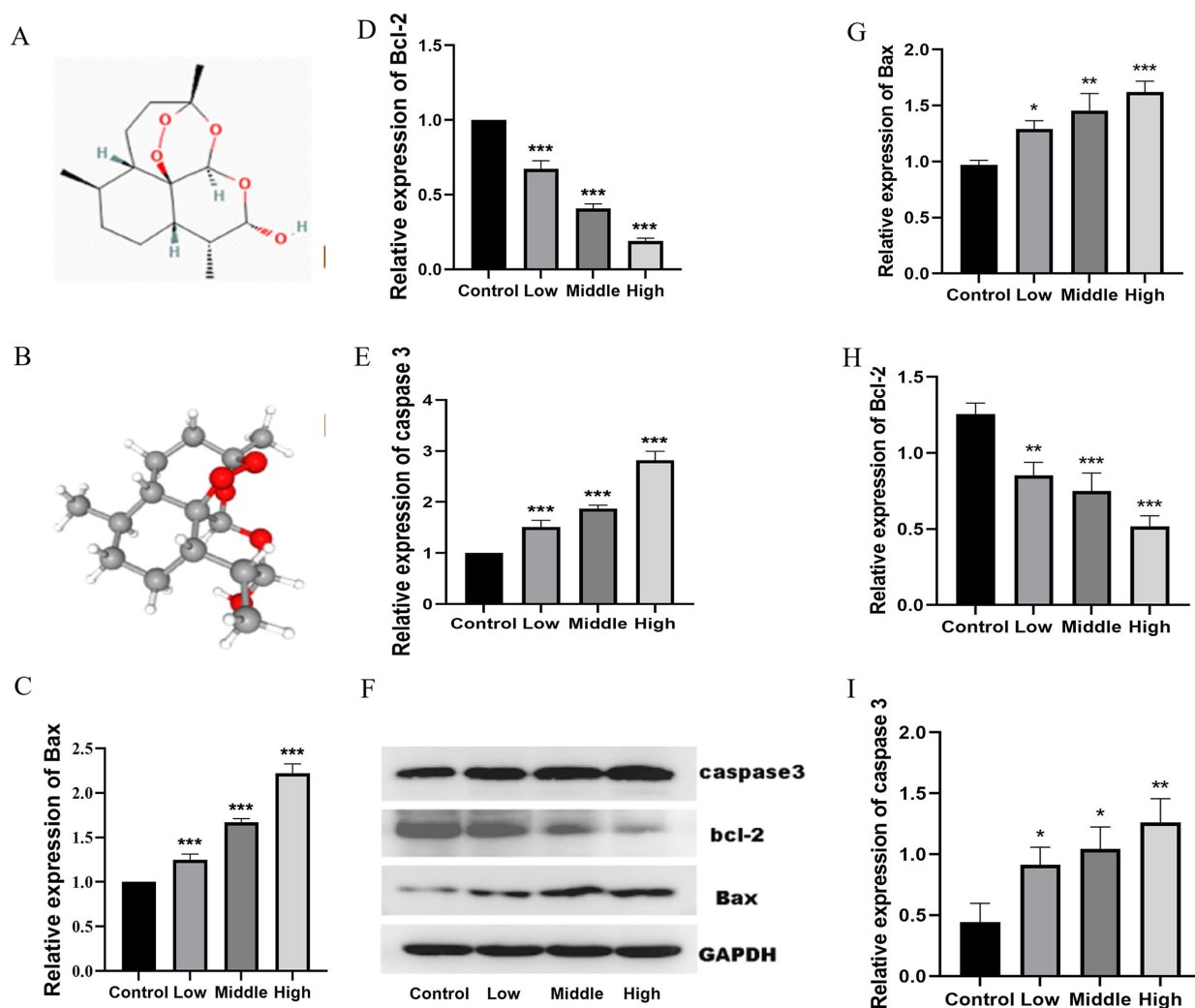


Fig. 1. Dihydroartemisinin-induced apoptosis of TPC-1 cells. (A) 2D structure of dihydroartemisinin (PubChem CID: 3000518, Molecular Formula: C₁₅H₂₄O₅, Molecular Weight: 284.35 g/mol). (B) 3D structure of dihydroartemisinin. (C–E) qRT-PCR detection of mRNA expression of Bax, Bcl-2 and Caspase-3 in TPC-1 cells after treatment with DHA (0, 6.25, 25, 100 μ M) for 24 h. (F) Representative western blots of Bax, Bcl-2 and Caspase-3. (G–I) Quantification of protein levels normalized to GAPDH. Data are mean $\pm$ SD (n = 3 independent experiments). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control. DHA, dihydroartemisinin.

3.3 PPI Network of Intersection Targets

The PPI network was generated based on STRING database analysis and subsequently visualized using Cytoscape software. Fig. 2B shows that the nodes are connected by interaction edges, and 168 genes were found to participate in PPI. Nodes highlighted in red exhibited higher degree values, indicating greater connectivity within the network. Among those, top 10 core targets: STAT3 (Signal transducer and activator of transcription 3), PIK3R1 (phosphoinositide-3-kinase regulatory subunit 1), PIK3CA (phosphatidylinositol 3-kinase catalytic subunit), PIK3CD (the p110 δ catalytic subunit of the phosphatidylinositol-3-OH kinase), MAPK1 (mitogen-activated protein kinase 1), MAPK3 (mitogen-activated protein kinase 3), EGFR (Epidermal Growth Factor Receptor), HSP90AA1 (Heat shock protein HSP 90- α), ESR1 (Estrogen Receptor 1),

PTPN11 (protein tyrosine phosphatase, non-receptor type 11) were identified (Fig. 2C). These findings suggest that these genes might be vital genes in THCA development.

3.4 GO Enrichment Analysis

To further explore the biological relevance of the 230 predicted genes, Gene Ontology (GO) enrichment analysis was carried out, covering BP, CC, and MF classifications. The results showed that these intersection target genes were evidently enriched in 2214 BPs, 118 CCs and 220 MFs by using p < 0.05. GO enrichment analysis revealed the top 10 BP terms (Fig. 3A), including response to peptide (GO:1901652), peptide hormone response (GO:0043434), calcium ion homeostasis (GO:0055074), cellular calcium ion homeostasis (GO:0006874), and other processes related to external stimulus response and cytosolic calcium

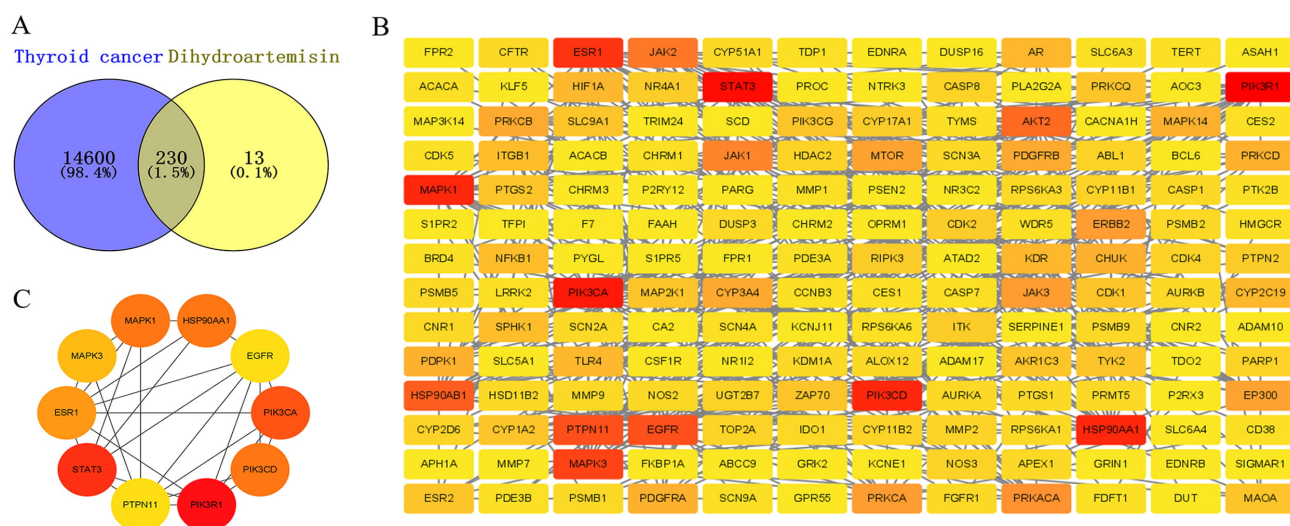


Fig. 2. PPI network of dihydroartemisinin in the treatment of thyroid cancer. (A) Venn diagram for the overlap analysis of dihydroartemisinin-targeted genes and thyroid cancer-related genes. (B) The PPI network was constructed using Cytoscape software. Node color from yellow to red indicates increasing degree (number of interactions). The top 10 hub genes are highlighted with red borders. (C) Top 10 hub genes in the PPI network.

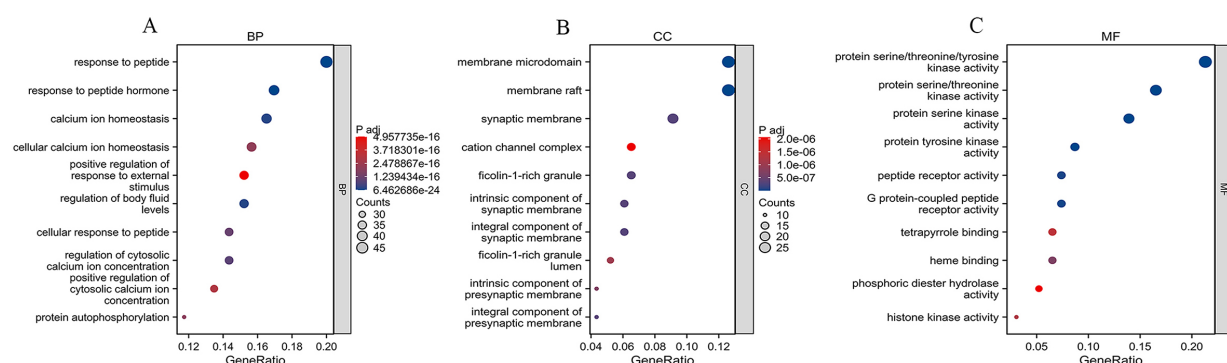


Fig. 3. GO analysis of the core targets of dihydroartemisinin-regulated thyroid cancer by bubble diagrams. (A) The top 10 terms of biological processes. (B) The top 10 terms of cellular components. (C) The top 10 terms of molecular functions.

regulation. For the CC (Fig. 3B), enriched terms were mainly associated with membrane-related structures, such as membrane microdomains, lipid rafts, synaptic membranes, cation channel complexes, and granule-associated components. MF analysis (Fig. 3C) indicated that kinase activities (serine/threonine, tyrosine), peptide receptor activity, G protein-coupled peptide receptor activity, heme/tetrapyrrole binding, phosphoric diester hydrolase activity, and histone kinase activity were significantly enriched.

3.5 KEGG Pathway Enrichment Analysis

KEGG enrichment analysis revealed 175 pathways potentially involved in DHA-mediated anti-THCA effects. Among them, the top enriched pathways included cancer-related signaling pathways such as proteoglycan interactions, HIF-1 signaling, PD-1/PD-L1 immune checkpoint regulation, as well as calcium signaling and drug resistance-

related pathways (Fig. 4). Among these pathways, those containing the largest numbers of enriched genes included chemical carcinogenesis - receptor activation (31 genes), calcium signaling pathway (29 genes), proteoglycans in cancer (28 genes), Hepatitis B (24 genes), HIF-1 signaling pathway (21 genes). The anti-THCA effect of DHA was closely linked to chemical carcinogenesis - receptor activation, PD-L1 expression and PD-1 checkpoint, HIF-1 signaling pathway and calcium signaling pathway. Based on the above results, the chemical carcinogenesis-receptor activation pathway (Fig. 5) that contained the most abundant number of enriched genes may play a greater role in THCA treatment.

3.6 The Expressions of Top 10 Genes in THCA Patients

By GEPIA database analysis, it was found that there were 512 THCA tumor samples and 337 normal samples. Compared with the normal group, *MAPK1*, *HSP90AA1* and

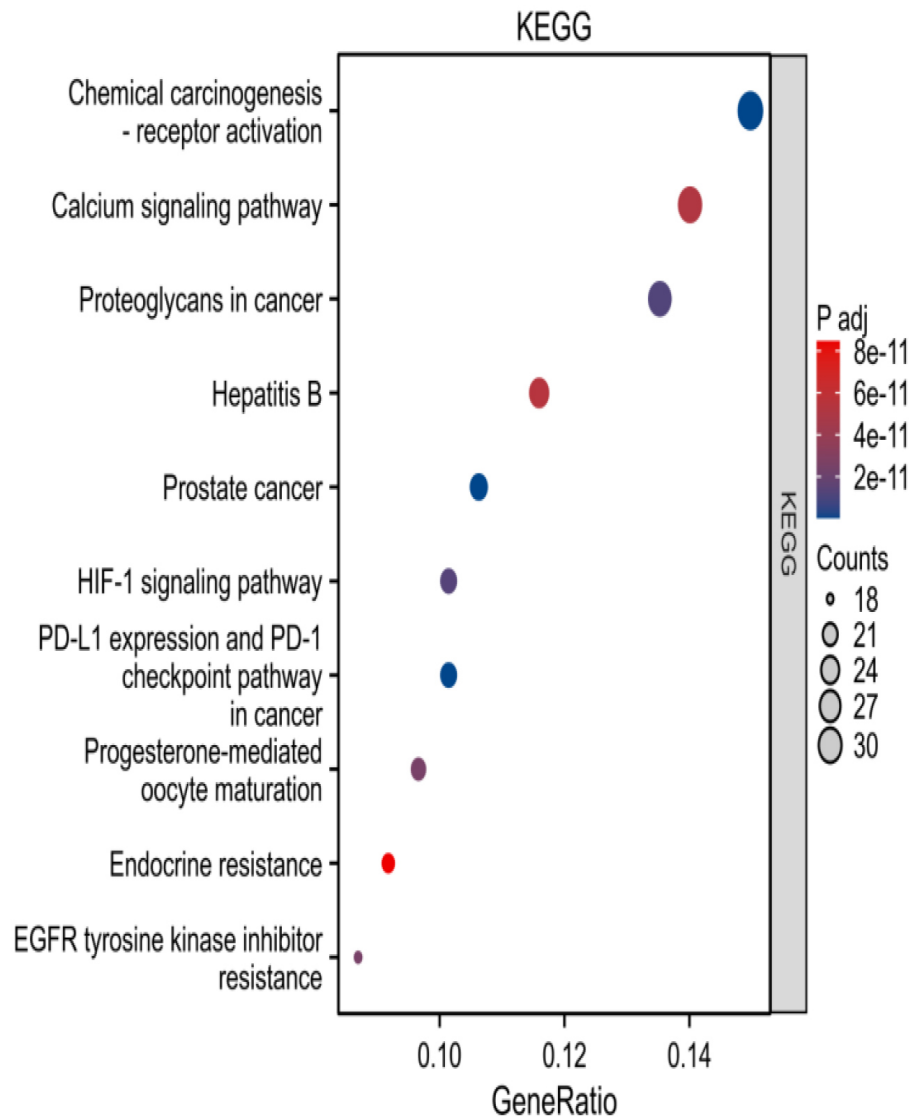


Fig. 4. The enrichment analysis of KEGG biological pathways.

PTPN11 were significantly overexpressed, but the levels of *STAT3*, *PIK3R1*, *PIK3CA*, *PIK3CD*, *MAPK3*, *EGFR* and *ESR1* were evidently decreased in the tumor group ($p < 0.01$) in Fig. 6.

4. Discussion

As a genetically controlled form of programmed cell death, apoptosis is critically involved in the early detection, prevention, and therapeutic management of THCA. For instance, the apoptosis-related gene CD44 expression is linked with tumor invasion and immune cell infiltration in THCA tissues. Also, autophagy correlated with apoptosis will promote the vitality and metastasis of THCA cells via maintaining cancer stem cells, supporting epithelial-mesenchymal transition (EMT) processes [16]. Anoikis is a specific type of apoptosis that is involved in cellular detachment from the matrix. CDKN2A, which is linked to anoikis-mediated apoptosis, has been suggested

to predict THCA prognosis and immune response, and may be involved in the regulation of tumor proliferation and metastasis-related processes [17]. Therefore, it could provide new strategies and methods for individualized treatment of THCA through deeply investigating apoptosis-related genes and mechanisms.

Numerous studies have demonstrated that DHA possesses significant anticancer activity with relatively low toxicity. This study showed that DHA increases apoptosis in THCA cells in a dose-dependent fashion, which is consistent with findings from previous research. Previous studies have shown that DHA induced caspase-dependent apoptosis in a time- and dose-dependent manner in primary effusion lymphoma [18]. DHA treatment could trigger apoptosis in U937 and KG-1 acute myeloid leukemia cell lines [19]. It has been demonstrated that DHA promotes apoptotic cell death in colon cancer cells through inhibition of the JAK2/STAT3 signaling pathway [20]. Consis-

[illegible]

tent with these findings, recent studies have further demonstrated the broad-spectrum anticancer potential of DHA. A comprehensive review also summarized that DHA exerts anticancer effects via multiple pathways, including induction of oxidative stress, programmed cell death, cell cycle arrest, and enhancement of antitumor immunity [21]. These observations across different cancer types support

the notion that DHA may have conserved antitumor mechanisms that are also relevant to thyroid cancer. However, the molecular mechanisms underlying DHA-induced apoptosis in THCA remain largely unexplored. We observed that there were 230 intersected targets within DHA and THCA by target prediction, STAT3, PIK3R1, PIK3CA, PIK3CD, MAPK1, MAPK3, EGFR, HSP90AA1, ESR1.

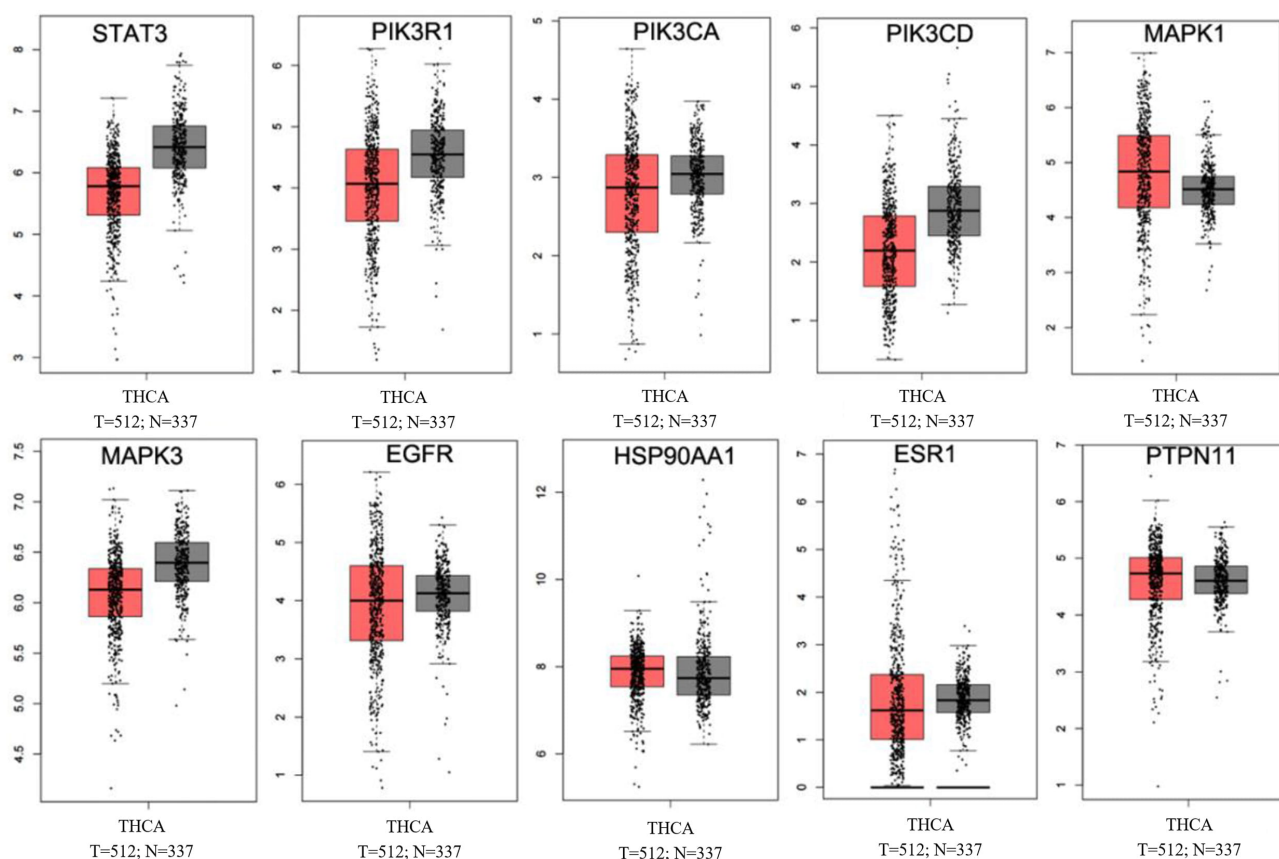


Fig. 6. The expressions of top 10 hub targets between thyroid cancer samples and normal samples were illustrated in the GEPIA database ($p < 0.01$). Red and gray bars indicate tumor and normal samples, respectively.

and PTPN11 were ranked among the top 10 by PPI network (Fig. 2) and their expressions were significantly different between THCA samples and normal samples (Fig. 6), suggesting that these top 10 core targets may be key genes of DHA acting on THCA. In THCA, STAT3 has been reported to show altered expression in certain tumor subtypes, which correlates with decreased malignancy. Its activation is regulated through multiple oncogenic kinase pathways, such as IL-6/gp130/JAK and HER2/CDK5 signaling axes [22]. Alterations in PIK3CA, such as mutations and genomic amplification of the PI3K p110 catalytic subunit, have been reported in thyroid cancers, and these findings have prompted clinical trials of pan-PI3K inhibitors like buparlisib [23,24]. Enrichment analysis of KEGG pathways (Fig. 4) identified multiple key signaling pathways, among which chemical carcinogenesis–receptor activation, PD-1/PD-L1 checkpoint regulation, and HIF-1 signaling were predominant. The highest gene count was observed in the chemical carcinogenesis–receptor activation pathway, suggesting its potential dominance in THCA-related therapeutic processes. Multiple downstream signaling pathways, such as PI3K/AKT/mTOR, JAK/STAT, and Wnt/ β -catenin, are involved in the chemical carcinogenesis–receptor activation pathway (hsa05207). These pathways are frequently hyperactivated in thyroid cancer due to mutations in BRAF,

RAS, or PIK3CA. DHA's predicted targeting of STAT3, PIK3R1, and PIK3CA suggests that DHA may interfere with these signaling axes, thereby suppressing cell proliferation and inducing apoptosis. Moreover, the enrichment of this pathway—with 31 genes—indicates that DHA may act on multiple nodes within the carcinogen-induced receptor activation network, offering a mechanistic basis for its anti-THCA activity [25,26,27,28].

However, there are some limitations in the current research. On the one hand, it is necessary to conduct molecular docking to choose the most potential ligands for further experimental verification or drug development. On the other hand, experimental validation using *in vitro* and *in vivo* models is required to better understand the mechanistic basis of THCA development.

5. Limitations

This study has several limitations. First, although network pharmacology predicted STAT3, PIK3R1, PIK3CA, etc. as core targets, molecular docking is needed to identify the most potent ligands for further experimental validation. Second, the apoptosis conclusion was primarily based on Bax/Bcl-2/Caspase-3 expression changes without direct apoptosis assays (e.g., flow cytometry with Annexin V/PI staining). Third, only one thyroid cancer cell line (TPC-1)

was used; results should be confirmed in additional THCA cell lines and *in vivo* models. These limitations may affect the generalizability of the findings, but they do not invalidate the observed correlations between DHA treatment and apoptosis-related markers. Dihydroartemisinin is known to exert its antimalarial effect through iron-dependent production of reactive oxygen species (ROS). In cancer cells, elevated intracellular iron levels may similarly sensitize cells to DHA-induced ferroptosis or apoptosis. Our network pharmacology results point to receptor-mediated pathways, but the ROS-mediated mechanism is not mutually exclusive. Future studies should assess ROS levels and iron chelator effects to delineate the relative contribution of oxidative stress in DHA-treated THCA cells.

6. Conclusion

In summary, network pharmacology analysis identified STAT3, PIK3R1, PIK3CA, PIK3CD, MAPK1, MAPK3, EGFR, HSP90AA1, ESR1, and PTPN11 as potential targets of dihydroartemisinin in thyroid cancer. In TPC-1 cells, DHA treatment led to changes in the expression of major apoptosis regulators, including Bax, Bcl-2, and Caspase-3. The effects may be mediated through pathways such as chemical carcinogenesis-receptor activation, PD-L1/PD-1, and HIF-1 signaling. However, these conclusions are primarily based on *in silico* prediction and preliminary *in vitro* experiments. Direct apoptosis assays, molecular docking, and *in vivo* studies are required to confirm the proposed mechanisms. The findings provide a basis for future research into DHA as a candidate for THCA therapy.

Availability of Data and Materials

All data are available from the corresponding author upon request.

Author Contributions

The integrity of the entire study and statistical analysis was guaranteed by JS. The study concepts, study design, definition of intellectual content, experimental studies, and data acquisition were performed by XX, JS, JD, BZ, LZ, and LN. The literature research and data analysis were conducted by JM. The manuscript was prepared by JD and BZ, edited by LZ, reviewed by LN and JS. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not Applicable.

Acknowledgment

Not Applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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