Original Article

Exploring the molecular targets and mechanisms of [10]-Gingerol for treating triple-negative breast cancer using bioinformatics approaches, molecular docking, and *in vivo* experiments

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Background: Triple-negative breast cancer (TNBC) is the most aggressive among breast cancer subtypes with the worst prognosis. Ginger is widely used in pharmaceuticals and as food. Its anticancer properties are known, but the mechanism is still unclear. [10]-Gingerol is one of the main phenolic compounds isolated from ginger. Studying the biological effects of [10]-Gingerol is of great significance to understand the efficacy of ginger.

Methods: In this study, the therapeutic effects of [10]-Gingerol on TNBC cells were studied using network pharmacology, molecular docking, and in vitro experiments, and the target and mechanism of action were explained.

Results: A total of 48 targets of ginger for the treatment of TNBC were found. These targets might interfere with the growth of TNBC by participating in many pathways, such as endocrine resistance, progesterone-mediated oocyte maturation, estrogen signaling pathway, and cellular senescence. Prognostic analyses indicated that the *JUN*, *FASN*, *ADRB2*, *ADRA2A*, and *PGR* were the hub genes, while molecular docking predicted the stable binding of ADRB2 protein with drug compounds. Additionally, [10]-Gingerol could induce apoptosis by regulating the caspase activation.

Conclusions: [10]-Gingerol affects the growth of TNBC through multiple action targets and participating in multiple action pathways. ADRB2 and apoptosis pathways might be important target pathways for [10]-Gingerol in the treatment of TNBC.

Keywords: Triple-negative breast cancer (TNBC); [10]-Gingerol; natural product; network pharmacology; molecular docking; apoptosis

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Introduction

Breast cancer is the most frequent cancer in women worldwide and has caused the highest cancer deaths in 2020 (1). In developing countries, it has the highest morbidity and mortality in women (2). Triple-negative breast cancer (TNBC) accounts for 20–30% of breast cancer cases and is characterized by the lack of estrogen (ER), progesterone (PR) and human epidermal growth factor receptor-2 (HER2) receptor expression, and its rapid progression to metastasis (2-4). Due to the lack of receptors' expression for estrogen, progesterone, or HER2, the patients with TNBC do not respond to endocrine or HER2-targeted therapies, leaving chemotherapy as the only option for the primary treatment of TNBC. TNBC is initially very sensitive to chemotherapy; relapse is common with the emergence of chemotherapy-resistant metastases, inevitably leading to death (5-7). Therefore, the long-term efficacy of chemotherapy for advanced TNBC is limited and the TNBC patients and their normal tissue toxicity remain a major concern. Therefore, the search for natural drugs with fewer side effects is needed as an alternative or complementary chemotherapy for advanced breast cancer.

Ginger (*Zingiber officinale Roscoe*, *Zingiberaceae*) is a natural dietary rhizome of the plant and is widely used as a traditional herb and flavoring agent (8). Ginger contains a variety of biologically active ingredients, such as gingerol, shogaols, polyphenols, and gingerone, indicating its role in mediating the anti-inflammatory and anti-tumor activities (9-12). Among the bioactive components of ginger, [6]-Gingerol and [8]-Gingerol have been widely reported to exhibit antitumor activity in multiple tumors by inhibiting the proliferation and migration of cells and inducing the apoptosis of tumor cells (13-16). [10]-Gingerol is one of the main phenolic compounds isolated from ginger and can inhibit the proliferation of tumor cells and induce the apoptosis of ovarian, colon, and prostate cancer cells (17-19). Although [10]-Gingerol has been reported to exhibit anti-breast cancer cell activity, its molecular mechanism is poorly understood (20). In this study, first, apoptosis and proliferation assays were used to identify the anti-cancer ability and pro-apoptotic pathways for [10]-Gingerol in TNBC. Then, the network pharmacology and molecular biology approaches were used to investigate the bioactive compounds and underlying mechanisms of ginger and [10]-Gingerol for the treatment of TNBC. This study demonstrated potential mechanisms that might underlie the therapeutic effects of ginger's potent constituent, [10]-Gingerol, against TNBC and provided evidence to support its clinical use. We present the following article in accordance with the MDAR reporting checklist (Available at https://dx.doi.org/10.21037/tcr-21-1138).

Methods

Identification of [10]-Gingerol as the key component of ginger

The bioactive compounds of ginger were obtained from the Traditional Chinese Medicine System Pharmacology database and analysis platform (TCMSP, http://lsp.nwu.edu.cn/). The main bioactive compounds of ginger were screened using criteria OB ≥15% and DL ≥0.15, among which [10]-Gingerol was one of the important components of ginger (*Table 1*).

Therapeutic targets for ginger

In order to obtain the putative targets of ginger, PubChem (https://pubchem.ncbi.nlm.nih.gov/) and TCMSP databases were used. The canonical SMILES strings for all the chemical compounds of ginger were obtained from PubChem. These canonical SMILES were submitted into the TCMSP database to obtain the main targets of ginger. The duplications in compounds were deleted to obtain the final target of ginger.

Potential target genes of TNBC

The mRNA expression data of TNBC and adjacent tissues were downloaded from the Cancer Genome Atlas (TCGA) website (https://portal.gdc.cancer.) and the datasets GSE76124 and GSE112825 were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds/). Then, the Omicshare (https://www.omicshare.com/tools/index.php/) online system was used to normalize the included datasets.

R 'limma' package (version 3.32.10) (21) was used to assess differentially expressed genes (DEGs). DEGs were screened according to $P<0.05$ and $|\log2\ FC| > 1.0$. The screening of DEGs resulted in obtaining the TNBC-associated genes.

Protein-protein interaction (PPI) network

The DEGs, obtained from GEO and TCGA databases,

Table 1 The main active component of Ginger

Target name	Compound name	BC	CC	DG	OB, %	DL
MOL002459	10-gingerol	0.029	0.398	20	19.14	0.28
MOL000449	Stigmasterol	0.029	0.482	11	43.83	0.76
MOL000358	beta-sitosterol	0.002	0.385	8	36.91	0.75
MOL002467	6-gingerol	0.002	0.376	5	35.64	0.16
MOL003358	Euxanthone	0.009	0.418	5	92.98	0.16

BC, betweenness centrality; CC, closeness centrality; DG, degree; OB, oral bioavailability; DL: drug-likeness.

and ginger targets were imported to Venny (https://bioinfogp.cnb.csic.es/tools/venny/) online software to obtain the intersection of three datasets. The overlapped genes were potential targets for the ginger intervention in TNBC. In order to obtain a PPI network for the potential targets of ginger intervention for TNBC, these overlapped targets were imported into STRING (https://string-db.org/) database; the human was set as target species and the confidence score of 0.400 was selected as a threshold value. Finally, Cytoscape software version 3.8.1 (22) was used to visualize the PPI network.

Construction of components-targets network and screening of core targets

The corresponding targets of ginger's bioactive compounds and the PPI network of potential targets of ginger intervention for TNBC were imported into Cytoscape software version 3.8.1 (22) to build a "components-targets" network. Then, the "Network Analyzer", a plug-in that comes with Cytoscape, was used to analyze the topology properties of the "components-targets" network. The core targets were selected with the node >2-fold Degree (DG), >1-fold Betweenness Centrality (BC), and >1-fold Closeness Centrality (CC).

GO and KEGG pathway enrichment analysis

DAVID (https://david.ncifcrf.gov/) (23) was used to conduct the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the related coding genes. The GO analyses included biological process (BP), cellular component (CC), and molecular function (MF). Significant pathways and biological functions were screened according to $P<0.05$.

Survival analysis

The data obtained from the TCGA dataset were used to validate the survival effects. In this study, R 'survminer' package (https://CRAN.R-project.org/package=survminer) was used to perform the overall survival analysis of the above core targets.

Molecular docking

The 3D structures of the core targets obtained in the previous step were downloaded from the Protein Data Bank database (PDB; https://www.rcsb.org/) and the MOL2 structure file for each key compound was obtained from the TCMSP database. Ligand-target separation was performed on each target using Discovery Studio software for obtaining the protein and ligand structures of each target. The proteins were prepared for docking by dehydrating, hydrogenating, calculating the charge, and assigning atomic type. The ligands were prepared by hydrogenating, adding charge, removing non-polar hydrogen, deleting lone pair electrons, and establishing special rotatable bonds. Then, AutoDock software was used to set binding pockets for molecular docking using the original ligands of each target as a reference. Finally, the AutoDock Vina was used to conduct the molecular docking of each target protein with ligand and the binding energy data were obtained for analysis.

Cell culture and the preparation of [10]-Gingerol

Human TNBC cell lines MDA-MB-231 and non-tumor breast cell line MCF-10A were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cell lines were cultured in Dulbecco's Modified

Eagle Medium (DMEM), which was supplemented with 10% fetal bovine serum (FBS), and incubated in an incubator (WCI-180, Wiggens, Germany) with a humid atmosphere at 37 ℃ and 5% CO_2. All the cell culture reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). [10]-Gingerol was purchased from Chengdu Ruifensi Biotechnology Co., Ltd. (Chengdu, China).

Cell proliferation assay

The viability of [10]-Gingerol in TNBC cells was analyzed using a CCK-8 assay following the manufacturer's instructions. The MDA-MB-231 cells were inoculated into 96-well plates with a cell density of 4×10^3 per well for 24 hours. After 24 hours, the different concentrations of [10]-Gingerol compounds (0, 50, 75, 100, 125, and 150 μmol/L) were used to treat the cells with 3 holes in each group. After 24 hours of incubation, 10 μL of CCK-8 solution was added to the cells for 2 hours at 37 ℃. The OD value was measured using the enzyme labeling instrument at 450 nm. Each experiment was carried out in triplicate; the half inhibitory concentration (IC_{50}) was calculated using Prism 7 (GraphPad software).

Cell apoptosis assay by flow cytometry

The breast cancer cells, at the logarithmic growth phase, were inoculated on a 6-well plate at the density of 1×10^5 cells per well; after adhering to the wall, the cells were treated with the indicated doses of [10]-Gingerol. After 48 hours, the cells were collected. According to Annexin-V-FITC/PI staining instructions (KeyGEN Biotech, Jiangsu, China), 5-μL FITC Annexin V and 5-μL PI were added to each tube and incubated in dark for 15 min. Then, 200-μL Binding Buffer was added. Apoptosis was detected using Cytomic FC500 flow cytometry (Beckman Coulter, Inc., Brea, CA). The data were analyzed using NovoExpress software.

Western blot (WB)

After flow cytometry analysis, the cells were collected for lysis. Protein concentration was determined using Keygen BCA Protein Assay Kit (KEYGEN, China). An equivalent amount of 20 μg protein was disintegrated using 10% SDS-PAGE and then transferred to the PVDF membrane. The membrane was blocked with 5% blocking buffer (5% skimmed milk powder + TBST) for 60 min at room temperature. Subsequently, the membrane was incubated with primary antibodies, including Caspase-3 antibody (Cell Signaling Technology, CST#9662S; 1:1,000), Caspase-7 antibody (CST#12827S; 1:1,000), and Caspase-8 antibody (CST#4790S; 1:1,000), overnight at 4 °C. The secondary antibodies were then added on the second day and incubated for 60 min. After the membranes were washed, the bands were visualized using ChemiDoc TMXRS+ (Bio-RAD, USA). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

All the measurements were taken in triplicates and the data were expressed as mean ± The Student's *t*-test was used to compare the statistical differences the between two groups. The comparison of multiple groups was carried out using analysis of variance (ANOVA) followed by the Tukey range test to analyze the differences between conditions. In each case, the value of $P<0.05$ was considered statistically significant.

Results

Identification of potential targets of ginger and TNBC

After differential analysis, a total of 3,228 DEGs (1,250 up-regulated and 1,977 down-regulated) were identified in the TCGA database, while 3,674 DEGs (2,745 up-regulated and 929 down-regulated) were identified in the GEO database and are presented as volcano plots (*Figure 1A*,*1B*). The "up-regulated" or "down-regulated" polarity of genes in this article is described as tumor *vs.* normal.

A total of 265 compounds of ginger were obtained from the TCMSP database (Available online: https://cdn.amegroups.cn/static/public/tcr-21-1138-1.xlsx). According to the results of PubChem and TCMSP databases, a total of 402 therapeutic targets were identified for all the compounds of ginger. Meanwhile, Venn Diagram online software was used to determine the intersection of DEGs from TCGA, GEO, and ginger targets (*Figure 1C*). A total of 48 genes were identified as potential targets in the three data sets, which suggested that ginger had 48 therapeutic targets for TNBC (*Figure 1D*). Moreover, these 48 therapeutic targets corresponded to 117 compounds of ginger (Available online: https://cdn.amegroups.cn/static/

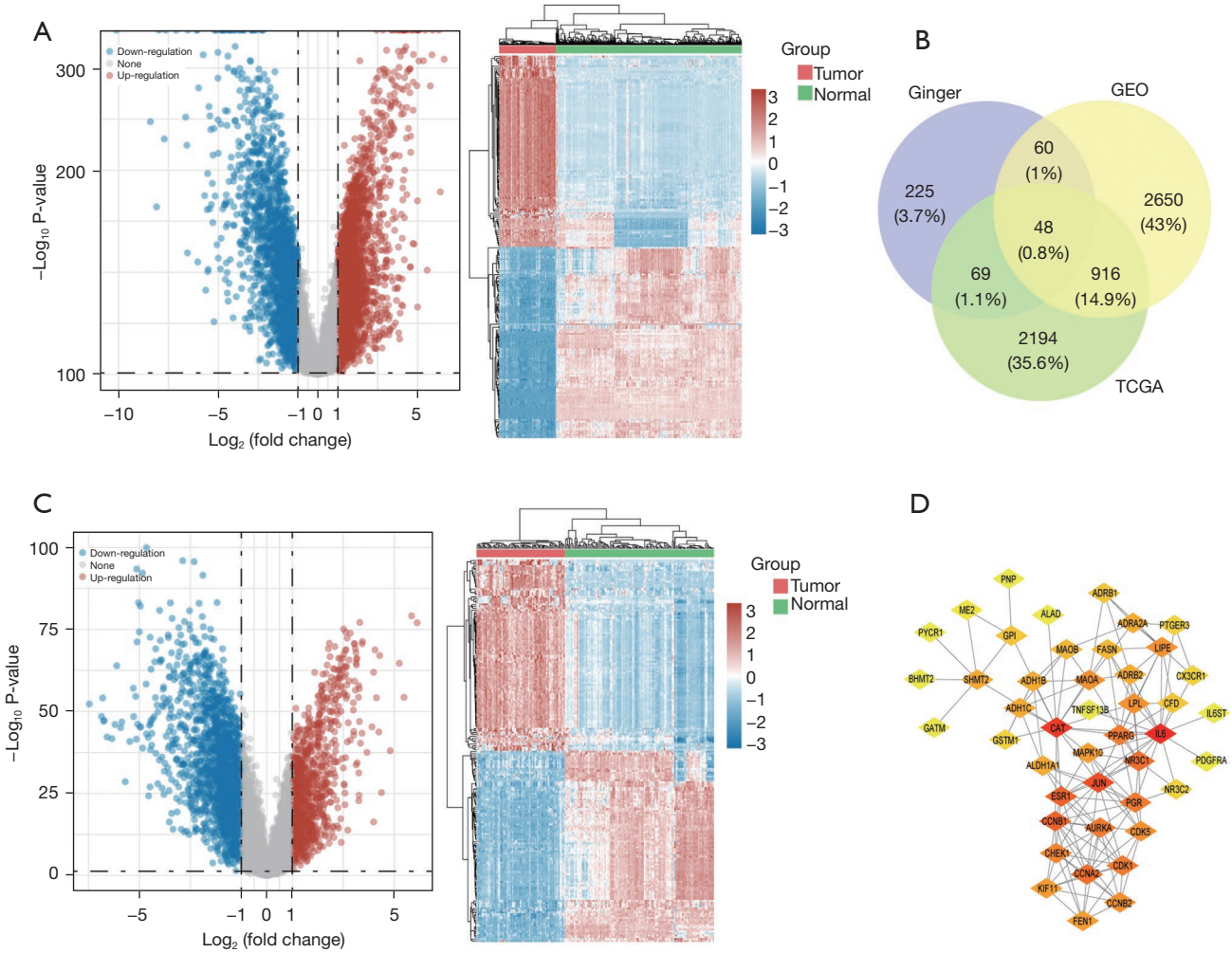

Figure 1 Identification of the potential targets of Ginger-TNBC. (A) Identification of the potential targets in the TCGA cohort; (B) identification of the potential targets in the GEO cohort; (C) Venn diagram to identify TNBC expressed genes between TCGA, GEO and Ginger; (D) the PPI network of 108 potential targets of Ginger-TNBC. TNBC, triple-negative breast cancer; TCGA, The Cancer Genome Atlas; GEO, gene expression omnibus; PPI, protein-protein interactions.

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PPI network analysis and screening of core targets

A total of 108 potential targets were submitted to the STRING database to build a PPI network (*Figure 2A*). The network was analyzed with Network Analyzer. The core targets were selected with the node >2-fold DG, >1-fold BC, and >1-fold CC. Among them, a total of 29 therapeutic targets were identified, including ESR1, ADH1C, MAOB, ADRB2, PPARG, NR3C1, ADH1B, CCNA2, PGR, IL6, ADRB1, JUN, CAT, MAOA, CHEK1, ADRA2A, CCNB1, CDK1, AURKA, LPL, MAPK10, LIPE, CDK5, SHMT2, ALDH1A1, NR3C2, GPI, FASN, and PTGER3, which indicated that these targets might be the core therapeutic targets for TNBC by ginger (*Table 2*, *Figure 2B*).

Functional enrichment analysis of core targets

KEGG and GO analyses were performed for all the 29 core targets. For KEGG pathway analysis, the hub genes were mainly enriched in endocrine resistance, progesterone-mediated oocyte maturation, estrogen signaling pathway, breast cancer, and cellular senescence (*Figure 3A*).

Table 2 The topological parameters of core candidate targets

Target name	Betweenness centrality	Closeness centrality	Degree
ESR1	0.370	0.526	74
ADH1C	0.234	0.473	45
MAOB	0.176	0.449	34
ADRB2	0.070	0.425	31
PPARG	0.061	0.442	30
NR3C1	0.072	0.457	30
ADH1B	0.082	0.451	29
CCNA2	0.014	0.415	23
PGR	0.016	0.446	22
IL6	0.055	0.458	20
ADRB1	0.021	0.384	18
JUN	0.023	0.448	18
CAT	0.124	0.513	17
MAOA	0.039	0.454	16
CHEK1	0.017	0.385	16
ADRA2A	0.023	0.389	15
CCNB1	0.011	0.430	13
CDK1	0.003	0.374	12
AURKA	0.006	0.404	11
LPL	0.007	0.402	10
MAPK10	0.006	0.433	9
LIPE	0.004	0.366	9
CDK5	0.001	0.355	9
SHMT2	0.009	0.356	7
ALDH1A1	0.033	0.453	7
NR3C2	0.000	0.369	6
GPI	0.007	0.355	6
FASN	0.013	0.378	6
PTGER3	0.006	0.374	4

GO analysis included biological process (BP), cellular component (CC), and molecular function (MF). The top five BP terms for GO analysis included a response to DNA-templated transcription, initiation, rhythmic process, transcription initiation from RNA polymerase II promoter, muscle cell proliferation, and regulation of developmental growth (*Figure 3B*). The top five CC terms for GO analysis included spindle, transferase complex, transferring phosphorus-containing groups, serine/threonine-protein kinase complex, protein kinase complex, and perikaryon. The top five MF terms for GO analysis included nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, steroid hormone receptor activity, nuclear hormone receptor binding, and RNA polymerase II transcription factor binding.

Overall survival analysis of core targets

In order to assess whether the mRNA levels of core targets harbored prognostic implications for TNBC, the RNA-seq data of TNBC patients obtained from the TCGA database was analyzed and found that the low expressions of *JUN* (HR =0.88, log-rank P=0.031), *ADRB2* (HR =0.64, log-rank P<0.001), *ADRA2A* (HR =0.6, log-rank P<0.001), *PGR* (HR =0.54, log-rank P<0.001), and *FASN* (HR =0.79, log-rank P<0.001) were significantly associated with lower overall survival (*Figure 4*).

Docking results of [10]-Gingerol hub genes

The [10]-Gingerol was docked with 5 hub targets that harbored prognostic implications, including ADRA2A, ADRB2, PGR, JUN, and ALDOA. The binding energies are listed in *Table 3*. As compared to the reference ligand, the binding energies of [10]-Gingerol with ADRB2, ADRA2A, and ALDOA were generally ideal. Of these, the affinity by ADRB2 performed best and the docking patterns of [10]-Gingerol with ADRB2 are presented in *Figure 5*.

[10]-Gingerol effectively inhibits the growth of TNBC cells

The effect of [10]-Gingerol on the cellular viability of MDA-MB-231 and MCF-10A was detected using the CCK-8 assay. Cells were exposed to the various concentrations (50–150 μM) of [10]-Gingerol for 24 hours and their viabilities were determined. As shown in *Figure 6*, [10]-Gingerol significantly inhibited the proliferation of MDA-MB-231 cells in a dose-dependent manner. After 24 hours of exposure, the IC_{50} value of [10]-Gingerol was 122.450 μM (*Figure 7A*). In comparison, the lowest inhibition was observed in the [10]-Gingerol-treated MCF-10A cells, where the IC_{50} value was 581.546 μM (*Figure 7B*). Therefore, [10]-Gingerol exhibited strong inhibitory effects on the growth of TNBC cells, while had low inhibition on

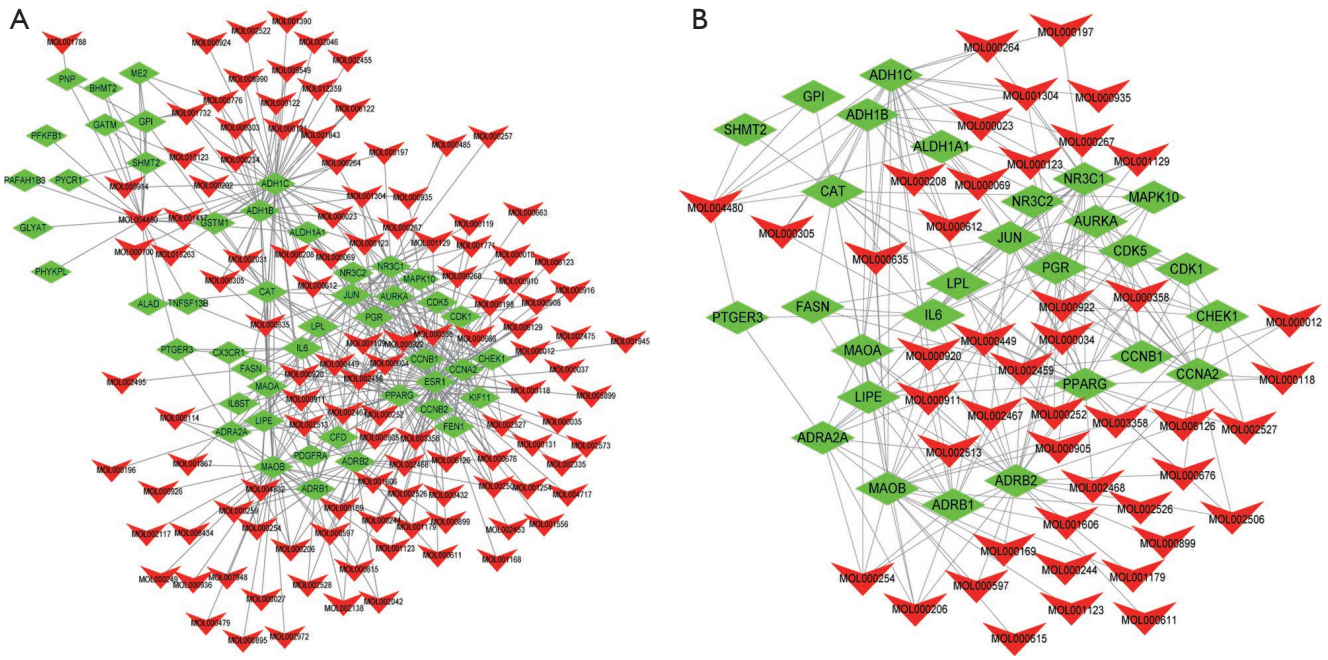

Figure 2 The Ginger component-therapeutic target network. (A) The PPI network of 48 potential targets; (B) the PPI network of core candidate targets. PPI, protein-protein interactions.

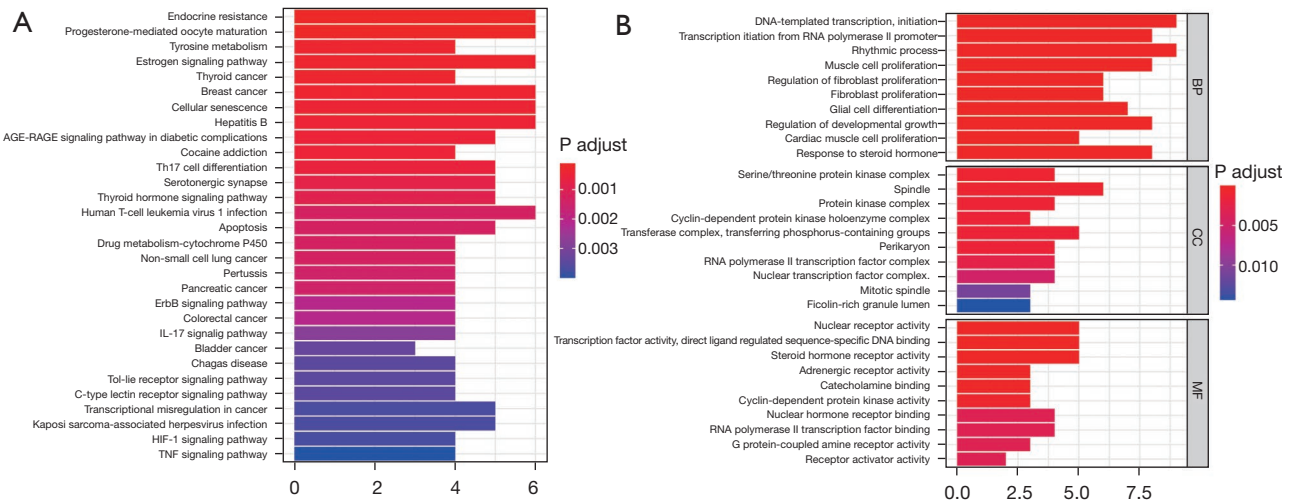

Figure 3 Functional enrichment analysis of 30 core targets. (A) KEGG analysis; (B) functional enrichment analysis including cellular component, molecular function, biological process. KEGG, Kyoto Encyclopedia of Genes and Genomes.

healthy breast epithelial cells.

[10]-Gingerol specifically induces apoptosis in TNBC cells

The role of [10]-Gingerol in the induction of apoptosis in TNBC cells was analyzed. Flow cytometry analysis validated that the treatment of cells with [10]-Gingerol significantly increased the percentage of apoptotic cells as compared to the untreated control cells. The total apoptosis rates in the MDA-MB-231 human TNBC cells

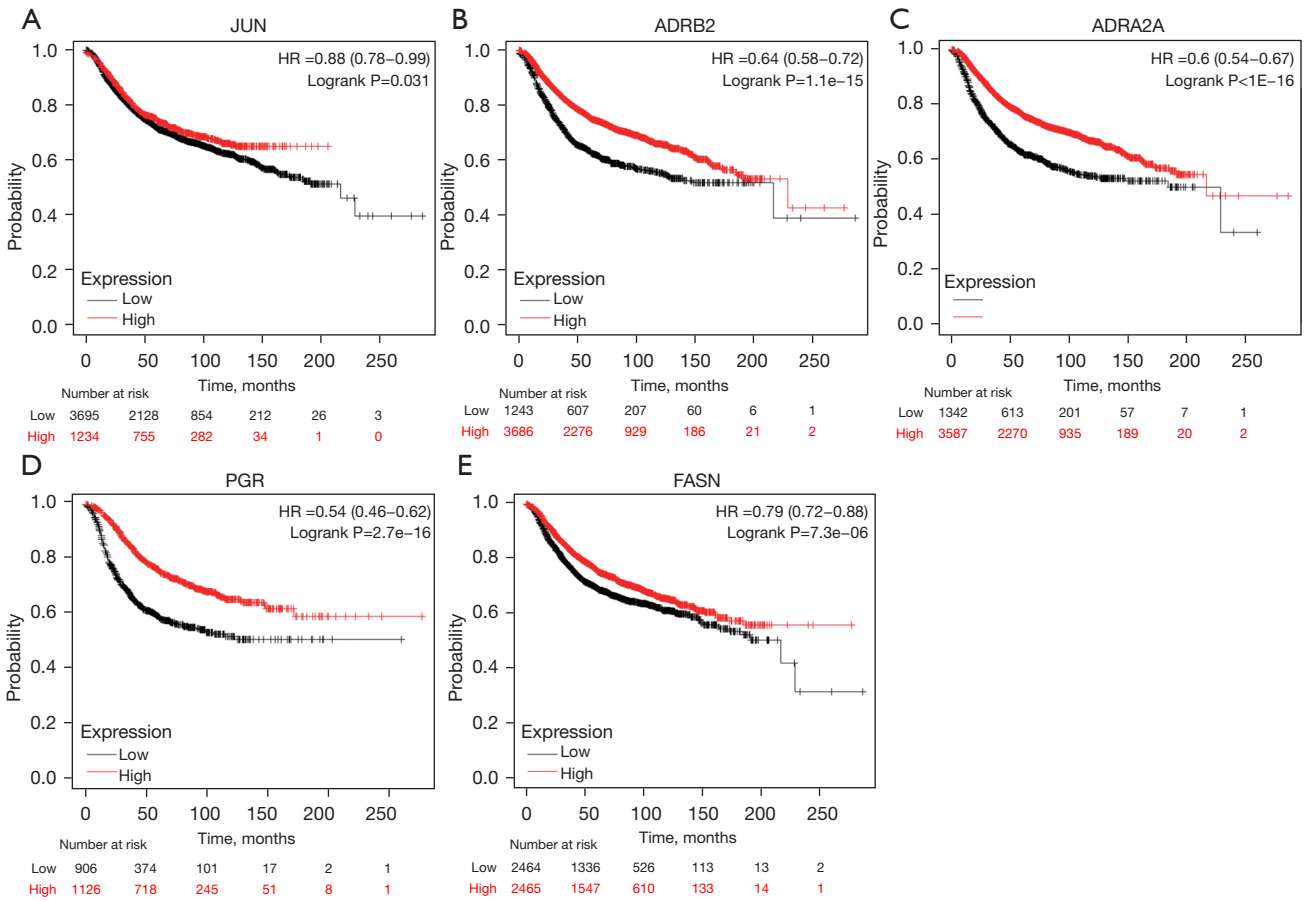

Figure 4 Kaplan-Meier analysis of the association between hub genes and the OS times of patients with TNBC. OS, overall survival; TNBC, triple-negative breast cancer.

Table 3 The affinity of 5 hub targets and [10]-Gingerol

Ligands targets	Affinity (kcal/mol)				
	ADRA2A (6k42)	ADRB2 (3ny8)	FASN (2cg5)	JUN (4hmy)	PGR (1a28)
Reference ligands					
CZX	−7				
JRZ		−7.9			
COA			−6.3		
GTP				−6.6	
STR					−11
[10]-Gingerol	−7.1	−8	−7.8	−6	−7

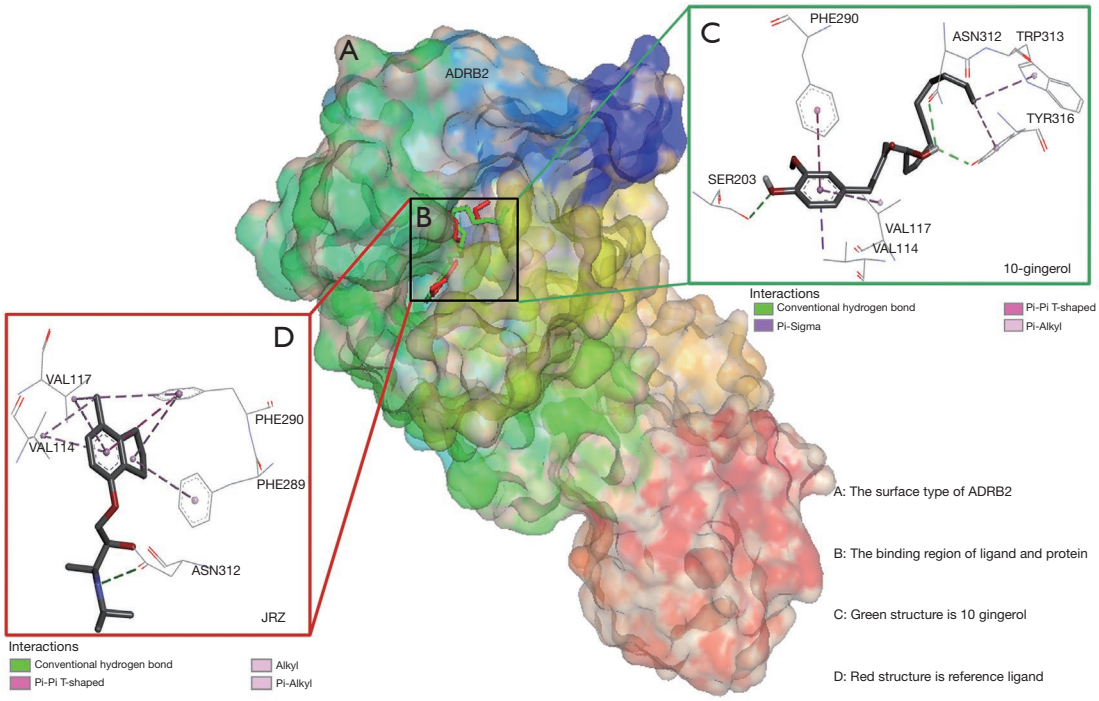

Figure 5 The visual annotation of [10]-Gingerol combined with ADRB2. TNBC, triple-negative breast cancer.

were 7.3%, 13.2%, and 21.9%, when the cells were treated with [10]-Gingerol at the concentrations of 0, 50, and 100 μM, respectively (*Figure 6A*,*6B*). The apoptosis rate in the MCF-10A cells was 6.5%, 6.3%, and 6.8%, when the cells were treated with [10]-Gingerol at the concentrations of 0, 50, and 100 μM, respectively (*Figure 6A*,*6C*). The results suggested that the apoptotic rate in TNBC cells induced by [10]-Gingerol increased with increasing the dose. In contrast, [10]-Gingerol had lesser pronounced pro-apoptotic effects on normal breast epithelial MCF-10A cells.

In order to study the apoptotic effects of [10]-Gingerol on TNBC cells, the expression levels of several apoptosis-related proteins, including Caspase-3, Caspase-7, and Caspase-8, were examined. The western blotting analysis demonstrated that, as compared to the DMSO group, after [10]-Gingerol treatment, the expression of Caspase-3, Caspase-7, and Caspase-8 increased (*Figure 6D*,*6E*). Notably, the up-regulatory effects of [10]-Gingerol on Caspase-7 and Caspase-8 increased with increasing the dose, suggesting a dose-dependent effect on their expression levels in TNBC cells. The result above indicated that [10]-Gingerol could inhibit the growth of TNBC by inducing apoptosis.

Discussion

The possible anticancer potential of ginger is receiving a great deal of attention. Information on the potential mechanism of ginger's bioactive compounds, such as [10]-Gingerol, is important for understanding the biological effects of ginger. This study indicated that [10]-Gingerol could inhibit TNBC through the induction of apoptosis by activating the caspase-family protein. Moreover, combined with the network pharmacology and molecular docking analysis, this study demonstrated for the first time that [10]-Gingerol could inhibit the proliferation of TNBC cells and induce their apoptosis through the ADRB2 pathway.

Fresh ginger is rich in [6]-Gingerol, [8]-Gingerol, and [10]-Gingerol but most of the studies, reporting the anti-tumor ability of gingerols, are focused on [6]-Gingerol. However, some studies have illustrated that [10]-Gingerol has more significant anti-tumor activity than that of [6]-Gingerol (24). In this study, the anti-tumor activities of [10]-Gingerol were investigated against TNBC cells *in vitro* and demonstrated that [10]-Gingerol could inhibit the proliferation of MDA-MB-231 cells with an IC_{50} value of 122.450±0.5 μM. Moreover, this concentration had no obvious toxicity to non-tumor breast cell line (MCF-10A).

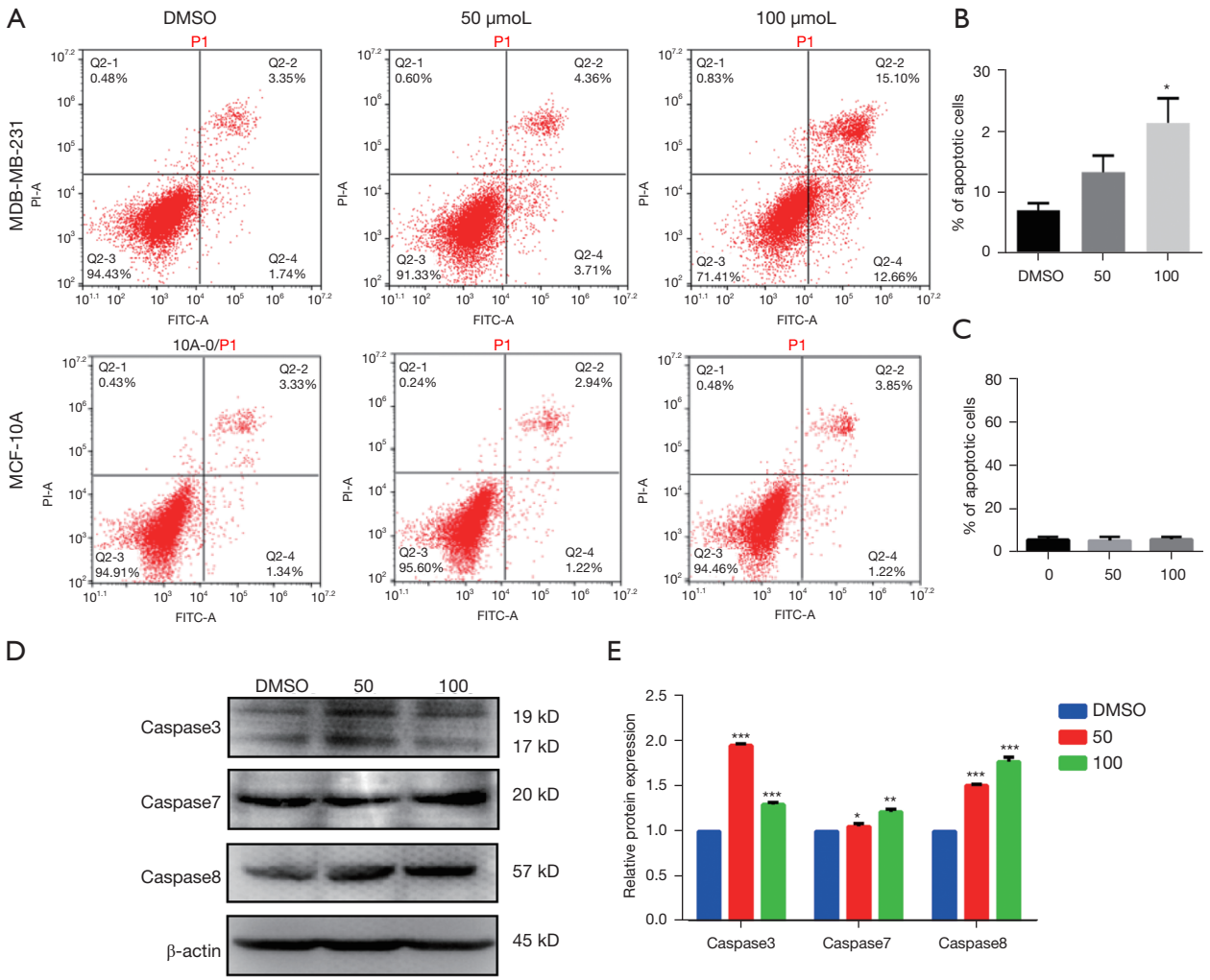

Figure 6 Induction of apoptosis by [10]-Gingerol in MDA-MB-231 human TNBC cells and MCF-10A normal breast epithelial cells. (A) Flow cytometric analysis of MDA-MB-231 and MCF-10A cells incubated with [10]-Gingerol for 24 h; (B) statistical analysis of apoptosis in MDA-MB-231; (C) statistical analysis of apoptosis in MCF-10A; (D) Western blotting analysis of apoptosis-related proteins in MDAMB-231; (E) Statistical analysis of apoptosis-related proteins. *P<0.05, **P<0.01, ***P<0.001, significantly different from control cells.

In this study, multiple *in vitro* experiments were conducted on the effect of [10]-Ginger on TNBC cells and compared to the non-tumor MCF-10A breast cells. The IC_{50} value for non-tumor cytotoxicity was 581.546±2.5 μM, with the selectivity index (SI) of 4.78 (IC_{50} of non-tumor cells/IC_{50} of tumor cells), which is a high value as compared to the standard chemotherapeutics used in the clinic. These results confirmed that [10]-Gingerol had better tumor suppressor ability in TNBC.

Notably, recent studies have demonstrated that [10]-Gingerol can inhibit the growth of TNBC cells by affecting its cell cycle, but the results from these studies are contradictory. A study reported that [10]-Gingerol could inhibit the growth of TNBC cells at the concentration of 10 μM *in vitro* (25), while another study showed that [10]-Gingerol could inhibit the growth of TNBC cells at the concentration of >50 μM *in vitro* (26). Furthermore, another study reported that [10]-Gingerol could inhibit the growth of TNBC cells at the concentration of 666.2 μM *in vitro* (27). The IC_{50}, which was obtained in this study, was different from the other studies, which might be due to differences in the production process of [10]-Gingerol in different companies, leading to different purities of [10]-Gingerol.

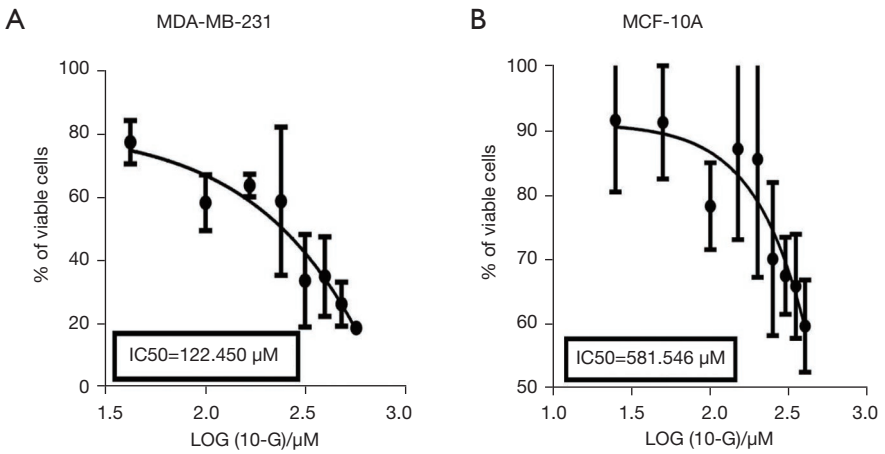

Figure 7 Cell viability at the indicated concentrations of [10]-Gingerol in MDA-MB-231 human TNBC cells and MCF-10A normal breast epithelial cells at 24 h was assessed by CCK-8 assay. (A) Determination of IC50 of [10]-Gingerol on MDA-MB-231 cells; (B) determination of IC50 of [10]-Gingerol on MCF-10A cells.

Moreover, [10]-Gingerol has been reported to exhibit anti-tumor activity by inducing apoptosis through the MAPK pathway in HCT116 human colon cancer cells (18). Gingerol has been subsequently shown to induce apoptosis in a variety of cancer cell types (17,28-30). In this study, [10]-Gingerol induced apoptosis by activating the caspase-family proteins (Caspase-3, Caspase-7, and Caspase-8).

Furthermore, the information from literature and publicly available databases were integrated to find the potential therapeutic protein targets for the TNBC treatment by ginger. A total of 265 bioactive compounds of ginger were found from previous studies, including [10]-Gingerol and others, among which 29 core targets of ginger for the treatment of TNBC were screened out. Enrichment analysis showed that endocrine resistance, progesterone-mediated oocyte maturation, estrogen signaling pathway, and cellular senescence were the potential target pathways for ginger's bioactive compounds to inhibit the growth of TNBC cells.

Five pivotal proteins (ADRA2A, ADRB2, PGR, JUN, and FASN) not only had high BC and DG values in the PPI network but also identified as prognostic risk factors, suggesting that they might be the hub regulatory factors in the response of TNBC cells to ginger. In previous studies, these pivotal proteins have already been shown to participate in the pathophysiology and treatment of cancer. ADRA2A and ADRB2 belong to adrenoceptors and can regulate stress hormones. The high expression of ADRA2A and ADRB2 were related to the increased distant metastasis-free survival (DMFS); ADRA2A was related to the inhibition of tumor cell proliferation, while ADRB2 was involved in anti-tumor immunity (31). However, studies have demonstrated that dexmedetomidine, an ADRA2A agonist, could promote metastasis in breast cancer via triggering the prolactin signaling pathway (32,33). Moreover, the blockage of ADRB2 was reported to improve the apoptosis in pancreatic cancer cells, suggesting that [10]-Gingerol could improve the TNBC apoptosis via regulating ADRB2 (34). PGR is a ligand-activated nuclear transcription factor that mediates progesterone activity; it is the most important prognostic and predictive immunoinflammatory marker in breast cancer (35). The variants of PGR were found to be associated with increased breast cancer susceptibility (36). FASN is involved in the *de novo* lipogenesis and regulation of ERα signaling (37). FASN is overexpressed in breast cancer cells and its silencing could result in the suppression of cell growth, but it also enhanced the cell invasion (38,39). JUN is an oncogenic transcription factor, which is an important regulator for a wide range of biological processes, such as the proliferation, differentiation, invasion, migration, and apoptosis of cells (40). JUN is highly expressed in various tumors, providing feedback on environmental stimuli, such as DNA damage (41).

This evidence supports that ginger might work against TNBC by regulating the expression of these hub genes. The current study aided in understanding the pathophysiology and roles of these genes in TNBC.

A molecular docking approach was also used to predict specific interactions between [10]-Gingerol and these five pivotal target proteins for ginger. The results showed

that [10]-Gingerol could exert therapeutic effects against TNBC by targeting ADRB2, ADRA2A, and FASN. Among them, ADRB2 exhibited the highest affinity for [10]-Gingerol. The current results suggested that ginger and compound [10]-Gingerol might be the promising anti-cancer agent. Stress hormones or glycolysis might alternative effective approaches for these compounds to inhibit the growth of TNBC.

Conclusions

To sum up, [10]-Gingerol is a compound of ginger, which inhibited the proliferation of TNBC and induced apoptosis by activating the caspase-family protein. Moreover, the combination of network pharmacology method and molecular docking illuminated the molecular and pharmacological mechanisms of ginger and [10]-Gingerol against TNBC. ADRA2A, ADRB2, PGR, JUN, and FASN might play key roles in the effects of ginger against TNBC; among them, ADRA2A, ADRB2, and FASN might be the main targets for [10]-Gingerol in TNBC treatment. The current study might inspire and guide further work to verify the molecular targets of ginger and [10]-Gingerol against TNBC and its development in clinical use.

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