

Mechanism of 6-Gingerol against Cutaneous Squamous Cell Carcinoma Based on Network Pharmacology, Molecular Docking, and In Vitro Validation

Yang Xinyue¹, Li Chenlu¹, Zeng Yueqin^{2,3,*}, Chen Liang^{2,3,*}

¹Kunming Medical University, Kunming 650500, Yunnan, China

²Institute of Biomedical Engineering, Kunming Medical University, Kunming 650500, Yunnan, China

³Yunnan Key Laboratory of Precision Medicine for Breast Cancer, Kunming 650500, Yunnan, China

Received July 30, 2026
Revise August 20, 2026
Accepted August 31, 2026

***Corresponding author:**

Zeng Yueqin and Chen Liang, Institute of Biomedical Engineering, Kunming Medical University, Kunming 650500, Yunnan, China

E-mail:

z_yueqin@foxmail.com
chenliang@kmmu.edu.cn

©2026, Yang, X. et al.

This is an Open Access article published under a Creative Commons (CC BY-NC-ND 0.4) license.



ABSTRACT

Introduction: Cutaneous squamous cell carcinoma (cSCC) is a prevalent non-melanoma skin cancer with increasing global morbidity. Conventional therapies are limited by high recurrence rates and adverse reactions, creating an urgent demand for innovative therapeutic agents. 6-Gingerol, the primary bioactive constituent of ginger, exhibits broad antitumor activity across multiple malignancies, yet its functional mechanism against cSCC remains poorly defined. The study aimed to predict potential molecular mechanisms underlying the anti-cSCC activity of 6-gingerol via network pharmacology, molecular docking, and preliminary in-vitro morphological assays.

Methods: Compound targets of 6-gingerol and disease-related targets of cSCC were retrieved from multiple databases. Intersected targets were applied to construct a protein-protein interaction (PPI) network. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted to predict functional biological processes and key signaling cascades. Molecular docking was performed to assess binding affinity between 6-gingerol and hub targets. Hematoxylin-eosin (HE) staining was used to observe morphological alterations in A431 human cSCC cells.

Results: Sixty-one overlapping targets were screened. The PPI network consisted of 61 nodes and 1173 edges, with TP53, AKT1, BCL2, CASP3, and EGFR identified as core hub genes. A total of 522 enriched GO biological processes were obtained, while KEGG enrichment highlighted apoptosis, PI3K-Akt and MAPK signaling pathways. Molecular docking verified strong binding capacity between 6-gingerol and core targets (binding energy ≤ -7.0 kcal/mol). In vitro staining revealed that 6-gingerol induced morphological changes reminiscent of apoptosis-like phenotypes in A431 cells. Network pharmacology and molecular docking analyses predicted that 6-gingerol may exert anti-cSCC effects through the regulation of TP53, AKT1, BCL2, and the PI3K-Akt/MAPK pathways.

Conclusion: HE staining provided preliminary morphological evidence of apoptosis-like changes in A431 cells, but the predicted pathway interactions require further experimental validation at the protein and gene expression levels.

Keywords: Cutaneous squamous cell carcinoma; 6-Gingerol; Network pharmacology; Molecular docking

Introduction

Cutaneous squamous cell carcinoma (cSCC) is a common non-melanoma skin cancer whose incidence has increased markedly worldwide in recent years. cSCC is characterized by local invasion and regional lymph node metastasis. In advanced cases, patients frequently suffer from facial tissue defects and distant

organ metastasis, accompanied by substantially elevated risks of disability and mortality, which severely impairs patients' quality of life. Current clinical treatments mainly rely on surgical resection, radiotherapy, and chemotherapy; however, several unresolved challenges remain, including high recurrence rates, considerable toxic side effects, and unsatisfactory therapeutic responses in some patients [-8]. Therefore, exploring

safe and effective novel therapeutic strategies for cSCC bears great clinical significance.

Ginger (*Zingiber officinale*), a typical medicinal and edible plant, possesses a long history of medicinal value. Modern pharmacology has confirmed that ginger is rich in phenolic active substances, which can block ultraviolet-mediated skin carcinogenesis by scavenging oxidative free radicals and inhibiting chronic inflammatory microenvironments [9]. 6-Gingerol is one of the main active components of ginger, and its molecular structure contains phenolic hydroxyl and α -hydroxy ketone groups that confer potent antioxidant activity [10]. Recent studies have demonstrated that 6-gingerol exerts anti-proliferative effects in various tumor models, including gastric cancer and colorectal cancer, through mechanisms such as regulating the STAT3 signaling pathway, affecting cell cycle-related protein expression, and inducing apoptosis [11-15]. Although numerous studies have revealed the anticancer potential of 6-gingerol in multiple tumors [16-18], its role in cSCC remains insufficiently investigated, and whether it exerts anti-tumor effects through multi-target, multi-pathway synergistic regulation remains unclear. Therefore, in-depth investigation of the potential molecular mechanisms of 6-gingerol against cSCC from a systems biology perspective is of great research significance.

Network pharmacology integrates bioinformatics and systems biology, aligning with the "multi-component, multi-target, multi-pathway" characteristics of natural products. It can systematically predict the regulatory networks between small-molecule active ingredients and malignancies, compensating for the limitations of traditional single-target experiments. This approach has been widely applied in exploring the mechanisms of natural anti-tumor active substances [19, 20].

The present study combines network pharmacology, molecular docking and in vitro experiments to screen the core targets and signaling pathways of 6-gingerol in cSCC, predict molecular binding modes, and observe drug-induced morphological changes in A431 squamous carcinoma cells via HE staining. This work systematically elucidates the potential molecular mechanisms of 6-gingerol against cSCC, providing a theoretical foundation for subsequent in vivo animal experiments and candidate drug development.

Methods

Target screening and data acquisition

To systematically screen the potential targets of 6-gingerol and cSCC-related targets, data were obtained from multiple public databases.

Prediction of 6-gingerol potential targets: First, the chemical structure information of 6-gingerol, including its SMILES representation and 2D/3D molecular structure files, was obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>). Subsequently, with "*Homo sapiens*" as the species

restriction, target prediction was performed in the following databases: (i) TCMSP database for direct retrieval of known targets of 6-gingerol; (ii) SwissTargetPrediction database, where the SMILES of 6-gingerol was input and targets with prediction probability > 0 were retained; (iii) PharmMapper database, where the 3D structure (SDF file) of 6-gingerol was uploaded with species limited to "Human", and targets with Normalized Fit Score ≥ 0.6 were retained. The prediction results from the three databases were merged, duplicate targets removed, and all target protein names were standardized through the UniProt database, uniformly converted to human official gene symbols (Gene Symbol), with non-human and non-protein-coding gene entries excluded.

Acquisition of cSCC disease-related targets: Using "cutaneous squamous cell carcinoma" as the search term: (i) GeneCards and DisGeNET databases were searched for all associated targets and deduplicated; (ii) The OMIM database was searched for disease targets with relevance score ≥ 60 . Targets from the three databases were pooled, deduplicated again, and standardized through UniProt for subsequent use. **Overlapping target screening:** The Venny online tool was used to perform intersection analysis between drug targets and disease targets to obtain potential common targets of 6-gingerol acting on cSCC for subsequent analysis (<https://bioinfo.gp.cnb.csic.es/tools/venny>).

Protein-protein interaction (PPI) network construction and analysis

The overlapping targets of 6-gingerol and cSCC were imported into the STRING database (<https://string-db.org>) for protein-protein interaction (PPI) analysis. The species was restricted to "*Homo sapiens*," and the minimum required interaction confidence score was set to 0.400 to construct the PPI network, with isolated nodes removed to improve analytical reliability.

The interaction file (TSV format) of the PPI network was exported from STRING and imported into Cytoscape software (version 3.9.0) for visualization. The Network Analyzer tool built into Cytoscape was used to calculate node degree, betweenness centrality, and closeness centrality. Node degree was used as the primary criterion for identifying core targets, with higher degree values indicating more critical roles of the gene in the regulatory network.

Targets were sorted in descending order by node degree, and the top-ranked key targets were selected for subsequent molecular docking and mechanistic analysis.

2.3 GO and KEGG Enrichment Analysis. The overlapping targets of 6-gingerol and cSCC were imported into the DAVID database (<https://david.ncifcrf.gov>, latest version) for gene functional annotation and pathway enrichment analysis. The species was restricted to "*Homo sapiens*," and the

gene identifier type was set to "OFFICIAL GENE SYMBOL."

Gene Ontology (GO) functional enrichment analysis was performed, covering three dimensions: biological process (BP), cellular component (CC) and molecular function (MF). Simultaneously, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted to predict the signaling pathways potentially regulated by 6-gingerol.

FDR < 0.05 was used as the significance threshold for screening and sorting the enrichment results. The top-ranked GO terms and KEGG pathways were selected for visualization. Bubble charts were generated using the Bioinformatics Platform (<http://www.bioinformatics.com.cn>) to visually present the enrichment significance, gene counts, and pathway involvement of key GO terms and KEGG pathways.

Molecular Docking Analysis

To verify the binding ability between 6-gingerol and core targets, molecular docking was performed to analyze the binding modes between key target proteins and 6-gingerol. Ligand preparation: The three-dimensional structure file (SDF format) of 6-gingerol was obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>), and energy minimization optimization was performed on its structure for use as the docking ligand. Receptor preparation: Three-dimensional structures of core target proteins were obtained from the RCSB Protein Data Bank (PDB, <https://www.rcsb.org>). Selection criteria included: species "Homo sapiens," resolved by X-ray diffraction with resolution ≤ 2.5 Å, without large fragment deletions or mutations. Protein structure preprocessing included: (1) removal of crystal water molecules and unrelated ligand small molecules; (2) addition of polar hydrogen atoms; (3) completion of missing amino acid residues and side chains; (4) assignment of atomic charges and force field parameters. Molecular docking: The prepared ligands and receptors were imported into the CB-Dock2 online platform [31] (<http://clab.labshare.cn/cb-dock2>). This platform automatically identifies potential binding pockets on the protein surface based on a blind docking strategy and calculates the binding energy and optimal binding conformation between the ligand and receptor. Result evaluation and visualization: Binding energy was used as the evaluation metric. Binding energy ≤ -5.0 kcal/mol indicated binding activity between the small molecule and protein; binding energy ≤ -7.0 kcal/mol represented strong binding affinity and stable complex structure. Ligand-target combinations with lower binding energies were selected based on docking results, and PyMOL software (version 2.5 or higher) was used for visualization analysis of the optimal docking conformations, displaying the interaction modes (such as hydrogen bonds, hydrophobic interactions, π - π stacking, etc.) between the ligand and surrounding key amino acid residues.

Validation of docking reliability: CB-Dock2 performs blind docking without requiring predefined binding sites. Since most of the selected target proteins lack co-crystallized ligands, conventional redocking RMSD calculations were not applicable. To ensure reliability, we used the default parameters of CB-Dock2, which have been validated in previous studies, and the binding energies obtained were consistent across multiple iterations, supporting the robustness of the docking results.

In vitro experiments

Cell culture and treatment

The human cutaneous squamous cell carcinoma cell line A431 was purchased from a standard cell bank (e.g., ATCC or a domestically certified cell bank). Cells were cultured in high-glucose DMEM medium containing 10.0% fetal bovine serum (FBS) and 1.0% penicillin-streptomycin (100 U/mL penicillin, 100 µg/mL streptomycin) in a 37°C, 5.0% CO₂, saturated humidity incubator. 6-Gingerol was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution (e.g., 50 mM), aliquoted, and stored at -20°C protected from light. Before use, it was diluted with complete medium to working concentrations (10, 15, 20, 40 µM), ensuring the final DMSO concentration did not exceed 0.1% (v/v) to exclude solvent interference with cell activity. The experiment included different concentrations of 6-gingerol treatment groups and a solvent control group (0.1% DMSO). Cells were used for subsequent assays after 48 hours of drug treatment.

Cell morphological observation

To observe the effect of 6-gingerol on A431 cell morphology, cells were seeded in 6-well plates at 2×10^5 cells/well. After overnight routine culture, different concentrations (0, 20, 40 µM) of 6-gingerol were added and treated for 48 hours. After treatment, the culture medium was aspirated, and cells were gently washed twice with pre-chilled phosphate-buffered saline (PBS). One milliliter of 4.0% paraformaldehyde was added to each well and fixed at room temperature for 15 minutes. After fixation, the fixative was discarded, cells were washed twice with PBS, and routine hematoxylin-eosin (HE) staining was performed: fixation with 4% paraformaldehyde followed by PBS washing; hematoxylin staining for 5 minutes, tap water washing for blueing; 1% hydrochloric acid ethanol differentiation for 30 seconds; eosin staining for 2 minutes; dehydration through 70.0%–100.0% gradient ethanol, clearing with xylene, and mounting with neutral resin. An inverted microscope was used for observation and photography to record cell morphological changes. For semi-quantitative observation, a fixed-size counting frame (approximately 1.0 cm × 1.0 cm on the printed image) was applied to five randomly selected fields per group at ×40 magnification. Cell nuclei with intact morphology within the frame were manually counted,

and the representative cell counts were recorded for each group. HE staining provides morphological evidence of apoptosis-like changes but does not quantitatively measure cell viability or confirm specific apoptotic pathways or target regulation.

Results

Screening of potential targets of 6-Gingerol and cSCC

By integrating the TCMSP, SwissTargetPrediction, and PharmMapper databases, a total of 256 potential targets of 6-gingerol were obtained. Using "cutaneous squamous cell carcinoma" as the keyword, the

GeneCards, DisGeNET, and OMIM databases were searched, yielding 841 cSCC-related targets after deduplication. Intersection of drug targets and disease targets yielded 61 common targets, which were identified as key targets through which 6-gingerol may act on cSCC. The Venn diagram visually displays the above target screening results (Figure 1): 6-gingerol-specific targets accounted for 195 (18.8% of total targets), cSCC disease-specific targets accounted for 780 (75.3%), and the intersection of the two comprised 61 common targets (5.9%).

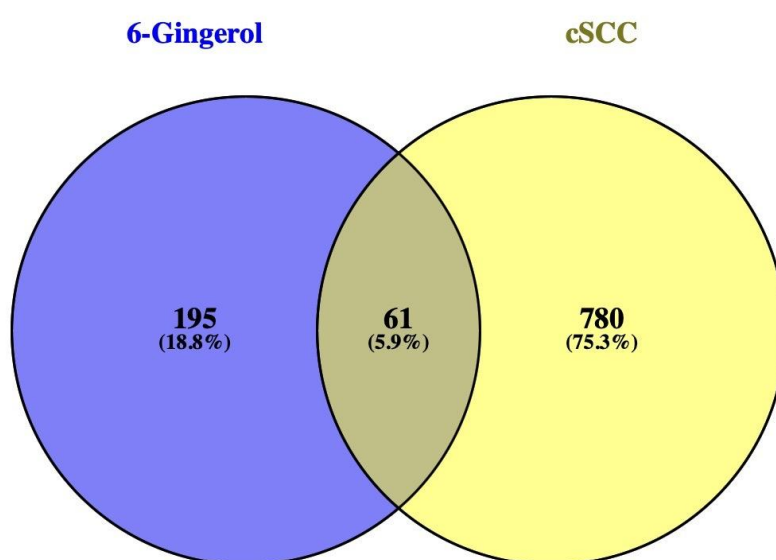


Figure 1 Venn diagram of common targets between 6-Gingerol and cutaneous squamous cell carcinoma.

Protein-protein interaction (PPI) network analysis

The 61 intersecting targets were imported into the STRING database to construct a PPI network and visualized (Figure 2). The network consisted of 61 nodes and 1,173 interaction edges, suggesting extensive interactions among the above targets. Topological analysis was performed using Cytoscape software, and targets were ranked by node degree. The results showed that targets with higher degree values included *CASP3*, *JUN*, *BCL2*, *HIF1A*, *CTNNB1*, *AKT1*, *CCND1*, *STAT3*, *TP53* and *EGFR* (Figure 2, Table 1), suggesting that these core targets may play key regulatory roles in the intervention of 6-gingerol in cSCC.

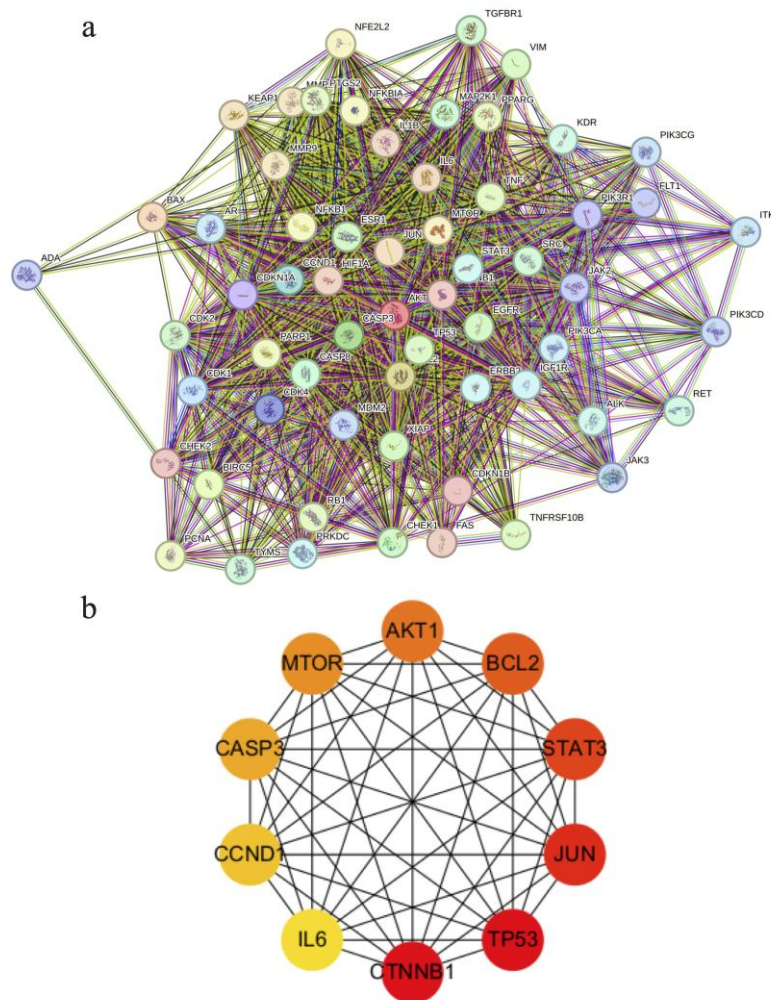


Figure 2 Protein-protein interaction network of overlapping targets between 6-gingerol and cSCC. (a) The whole interaction network of all overlapping targets; (b) Subnetwork of the top 10 core targets ranked by degree value.

Table 1 Topological parameters of the top 10 core targets ranked by degree in the PPI network.

Rank	Gene Symbol	Degree	Closeness centrality	Betweenness centrality
1	<i>TP53</i>	58	0.9677	69.6379
2	<i>EGFR</i>	56	0.9375	63.3634
3	<i>STAT3</i>	56	0.9375	57.2480
4	<i>BCL2</i>	56	0.9375	52.3782
5	<i>CTNNB1</i>	56	0.9375	50.5786
6	<i>JUN</i>	55	0.9231	50.9928
7	<i>CASP3</i>	55	0.9231	46.6883
8	<i>AKT1</i>	54	0.9091	45.6010
9	<i>CCND1</i>	53	0.8955	45.1082
10	<i>HIF1A</i>	53	0.8955	40.4415

GO and KEGG enrichment analysis results

GO functional enrichment analysis was performed on the 61 intersecting targets, yielding 711 significantly enriched entries (FDR < 0.05), including 522 biological processes (BP), 129 molecular functions (MF), and 60 cellular components (CC). The top 20 enriched entries were selected for visualization.

GO analysis results showed that in terms of biological processes, targets were mainly involved in apoptosis regulation, cell proliferation, PI3K/Akt signaling pathway and MAPK signaling pathway; in terms of molecular function, they were mainly involved in DNA binding, transcription factor activity and protein binding; in terms of cellular components, targets were mainly distributed in the nucleus, cytoplasm and protein complexes (Figure 3a, b, c).

KEGG pathway enrichment analysis yielded 152 significantly enriched pathways (FDR < 0.05), with the top 20 pathways visualized (Figure 3d). The results showed that related targets were mainly enriched in cancer pathways, apoptosis pathways, and classical signaling pathways such as PI3K-Akt and MAPK.

These results suggest that 6-gingerol may exert multi-target synergistic effects in cSCC by regulating multiple tumor-related signaling pathways.

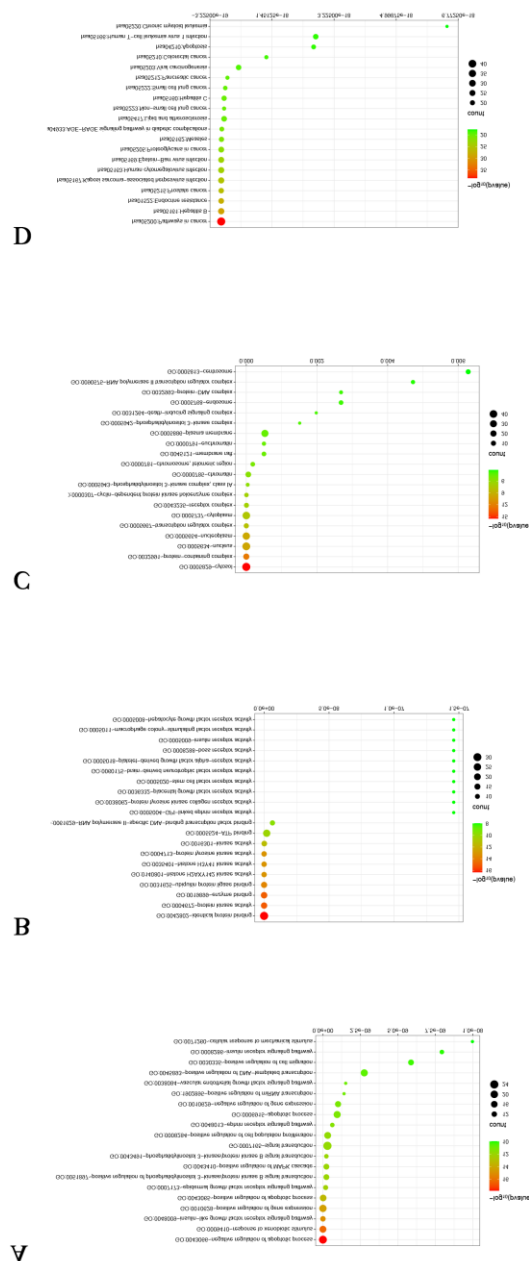


Figure 3 Bubble diagram of GO and KEGG enrichment for overlapping targets. (a) Biological Process (BP); (b) Molecular Function (MF); (c) Cellular Component (CC); (d) KEGG signaling pathways. The horizontal axis represents the ratio of enriched genes to total targets; the size of dots indicates the number of enriched genes, and the color gradient corresponds to adjusted FDR values (darker color means higher enrichment significance). Only the top 20 terms sorted by FDR are displayed in each module.

Molecular Docking Analysis

To verify the binding ability between 6-gingerol and key targets, molecular docking analysis was performed on the selected core targets. The results showed that most targets exhibited good binding activity with 6-gingerol, with binding energies lower than -6.0 kcal/mol (Table 2). Among them, *TP53*, *JUN*, *EGFR*, *CCND1*, *AKT1* and *BCL2* showed stronger binding ability, with binding energies lower than -7.0 kcal/mol, suggesting computationally-predicted high binding potential between 6-gingerol and the above targets.

Further binding mode analysis was performed on the four targets with the lowest binding energies (Figure 4). The analysis showed that 6-gingerol could form stable binding with key amino acid residues at the active sites of target proteins through hydrogen bonds and hydrophobic interactions.

These docking-derived results computationally suggest that 6-gingerol may exert its anti-cSCC effects via predicted binding to multiple key targets.

Table 2 Molecular docking binding energies between 6-gingerol and core targets

Core Target	Uniprot ID	Binding energy(kcal · mol ⁻¹)	Binding activity
<i>TP53</i>	P04637	-9.0	Strong
<i>JUN</i>	P05412	-7.9	Strong
<i>EGFR</i>	P00533	-7.5	Strong
<i>CCND1</i>	P24385	-7.3	Strong
<i>AKT1</i>	P31749	-7.1	Strong
<i>BCL2</i>	P10415	-7.1	Strong
<i>CTNNB1</i>	P35222	-7.1	Strong
<i>CASP3</i>	P42574	-7.0	Strong
<i>HIF1A</i>	Q16665	-6.9	Moderate
<i>STAT3</i>	P40763	-6.5	Moderate

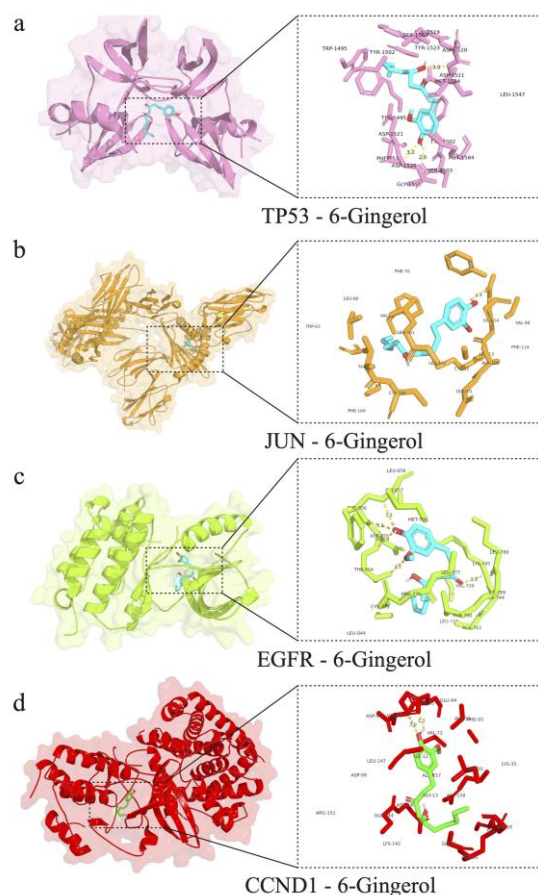


Figure 4 The optimal docking conformations of 6-Gingerol with four core targets (*TP53*, *JUN*, *EGFR*, *CCND1*). Hydrogen bonds and hydrophobic interactions between the ligand and key amino acid residues of proteins are displayed.

Effect of 6-Gingerol on A431 Cell Morphology

Cell morphological changes were observed by HE staining. As shown in Figure 5, control group cells exhibited irregular elongated or spindle shapes, adhered to the wall, and had good morphology. After 48 hours of treatment with 20 $\mu\text{mol/L}$ and 40 $\mu\text{mol/L}$ 6-gingerol, cell numbers decreased, and cells exhibited morphological changes including cell shrinkage and irregular morphology, which are morphological phenotypes reminiscent of apoptotic changes.

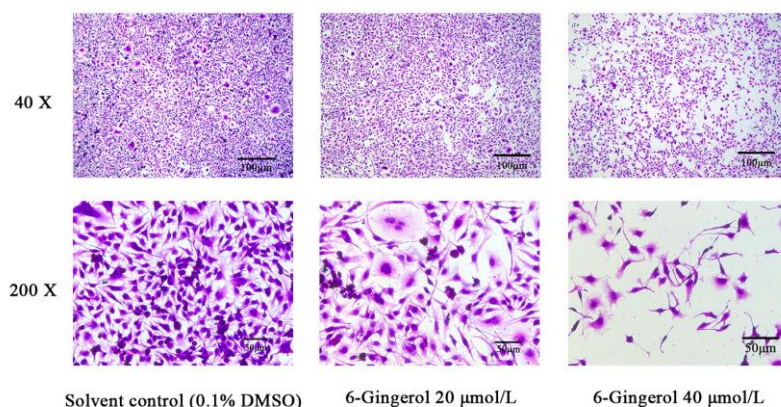


Figure 5 Representative HE staining images of A431 cells treated with 0 (control), 20, and 40 $\mu\text{mol/L}$ 6-gingerol for 48 h. Scale bars: 250 μm (40 $\times$) and 50 μm (200 $\times$). Cells with apoptotic-like morphological features, including cell shrinkage and irregular nuclear outlines, were observed in 6-gingerol-treated groups. Manual counting of five random fields per group at $\times 40$ magnification revealed that 6-gingerol at 40 $\mu\text{mol/L}$ reduced the average cell number per field from approximately 153 (control) to 80, representing a reduction of about 48%. The 20 $\mu\text{mol/L}$ group showed a less pronounced reduction (approximately 145 cells per field). These results suggest a concentration-dependent decrease in cell density following 6-gingerol treatment in this representative experiment.

Discussion

This study, through the integration of network pharmacology, molecular docking and in vitro experiments, preliminarily revealed the potential targets and related mechanisms of 6-gingerol against cSCC. A total of 61 common targets of 6-gingerol and cSCC were identified, among which *TP53*, *AKT1*, *BCL2*, *CASP3* and *EGFR* were key core targets. Enrichment analysis indicated that these targets were mainly enriched in classical signaling pathways such as PI3K-Akt and MAPK. Molecular docking results showed that 6-gingerol exhibited strong binding affinity to core targets (binding energy ≤ -7.0 kcal/mol). Morphological HE staining demonstrated that 6-gingerol could interfere with cell morphology, inducing cell shrinkage and irregular morphology, exerting an intervention effect on cSCC.

In the PPI network, *TP53* had the highest degree value (degree = 58), suggesting it may be one of the key targets through which 6-gingerol exerts its anti-cSCC effects. *TP53* is a classic tumor suppressor gene that plays a key role in cell cycle regulation and apoptosis. Studies have shown that activation of *TP53* can induce cell cycle arrest, promote DNA repair, and drive apoptosis. Furthermore, *TP53* is also involved in the regulation of tumor metabolism, ferroptosis, tumor microenvironment signaling pathways, and stem cell self-renewal and differentiation, collectively constituting a tumor suppressor barrier [21]. Previous

studies have reported that 6-gingerol can inhibit breast cancer cell proliferation through *TP53*-dependent mitochondrial apoptosis pathways [17]. This finding is consistent with the PPI network prediction results of this study, further supporting *TP53* as a key target of 6-gingerol against cSCC.

AKT1 is a core molecule of the PI3K-Akt signaling pathway and may also be a key factor mediating the anti-cSCC effects of 6-gingerol. Our network analysis showed that *AKT1* had a degree value of 54, and molecular docking results further indicated that the binding energy between 6-gingerol and *AKT1* reached -7.1 kcal/mol, suggesting strong binding affinity between 6-gingerol and *AKT1*. Abnormal activation of *AKT1* can promote tumor cell survival and proliferation and inhibit apoptosis [22]. Previous studies have shown that 6-gingerol can induce G1 phase arrest in renal carcinoma cells through the AKT-GSK3 β -cyclin D1 pathway [18]. In summary, it can be inferred that 6-gingerol may target *AKT1* to intervene in the PI3K-Akt signaling pathway, thereby inhibiting cSCC cell proliferation.

In addition, targets such as *BCL2*, *CASP3* and *CCND1* are also worthy of attention. *BCL2* and *CASP3* are involved in the regulation and execution of the mitochondrial apoptosis pathway, respectively. GO enrichment analysis showed that the screened targets were significantly enriched in "regulation of apoptotic process." Combined with the apoptosis-like morphological changes observed in cell morphology,

such as cell shrinkage and irregular morphology [23], this suggests that 6-gingerol may induce cSCC cell death through *BCL2/CASP3*-related pathways [24]. Of note, the morphological changes observed in the present study are consistent with those reported by Nigam et al. [24], who described similar apoptosis-like features in A431 cells upon 6-gingerol treatment. However, while Nigam et al [24] focused on the ROS-mediated mitochondrial pathway, our study additionally predicts the involvement of a broader regulatory network including PI3K-Akt and MAPK pathways based on network pharmacology analysis. *CCND1* is a key protein regulating the G1/S phase transition of the cell cycle. In this study, *CCND1* had a degree value of 53 and a molecular docking binding energy of -7.3 kcal/mol, suggesting that 6-gingerol may interact with *CCND1* to affect cell cycle progression, thereby inhibiting cSCC cell proliferation. This speculation is consistent with previous reports that 6-gingerol induces cell cycle arrest through the AKT-GSK3 β -cyclin D1 pathway [18].

GO functional enrichment analysis showed that the potential targets of 6-gingerol were mainly enriched in biological processes such as "signal transduction" and "regulation of cell proliferation." KEGG enrichment analysis further revealed that the potential targets of 6-gingerol were significantly enriched in the PI3K-Akt and MAPK signaling pathways. Previous studies have shown that the above two pathways are often abnormally activated in cSCC, promoting tumor cell proliferation [25, 26]. Based on this, it is speculated that 6-gingerol may inhibit cSCC cell proliferation by intervening in the PI3K-Akt and MAPK signaling pathways, but this speculation still requires further experimental verification.

Molecular docking results supported the binding ability of 6-gingerol to core targets at the computational level. In this study, the four targets with the lowest binding energies (*TP53*, *JUN*, *EGFR*, *CCND1*) were selected for binding mode analysis. The binding energies were all lower than -7.0 kcal/mol, with *TP53* having the lowest binding energy at -9.0 kcal/mol, suggesting that 6-gingerol may have strong interactions with these targets. Binding mode analysis showed that 6-gingerol could form stable binding with key amino acid residues at the active sites of target proteins through hydrogen bonds and hydrophobic interactions.

These computational predictions were preliminarily validated by in vitro experiments. HE staining results showed that 6-gingerol-treated A431 cells exhibited changes including reduced cell numbers, cell shrinkage and irregular morphology, which are consistent with the characteristics of apoptotic cells. Although direct verification at the protein level (such as Western blot) was not performed, the cell morphological changes were consistent with the trend of molecular docking predictions, preliminarily supporting that 6-gingerol may exert anti-cSCC effects by targeting key proteins such as *TP53* and *AKT1*.

Integrating network pharmacology analysis, molecular docking results and in vitro experimental results, this study proposes a possible mode of action: 6-gingerol may interact with key targets such as *TP53*, *BCL2* and *AKT1* to regulate signaling networks related to cell proliferation and apoptosis, thereby exerting anti-cSCC effects at the multi-target and multi-pathway level.

Several limitations of this study should be acknowledged. First, our in-vitro experimental evidence is preliminary and restricted to morphological observation using HE staining. We did not perform quantitative cell-viability assays (e.g., MTT, CCK-8), apoptosis-detection assays (e.g., Annexin V/PI flow cytometry), nor target-related protein or gene expression measurements (e.g., Western blot, RT-qPCR for *TP53*, *AKT1* and *BCL2*). Although HE staining can show apoptosis-like morphological alterations, it cannot quantitatively evaluate cell viability or confirm specific apoptotic pathways and target-gene regulation. Unlike Nigam et al [24], which reported direct molecular evidence for single-pathway mechanisms, our present findings are mainly computationally generated hypotheses; the predicted pathway crosstalk and target-regulatory relationships still require further wet-lab validation.

Second, the cell-density assessment was only carried out on a single batch of HE-stained samples, without independent biological replicates. Manual visual counting was applied, and no formal statistical tests were performed. Neither cell-viability quantification nor apoptotic-index calculation was implemented in this preliminary investigation. Accordingly, these counting results should only be regarded as descriptive observations, rather than definitive quantitative conclusions.

Third, molecular docking is purely an in-silico simulation. Satisfactory binding-energy values cannot be treated as direct proof of real-world physical interactions between small-molecule 6-gingerol and target proteins. Biophysical experiments including SPR or MST are necessary to verify direct binding events. In addition, target prediction relying on public databases may introduce false-positive hits; all targets and signaling pathways screened via network-pharmacology analysis remain computational hypotheses.

Finally, only the A431 epidermoid carcinoma cell line was adopted in this work. This cell model cannot fully recapitulate the pathological characteristics of primary cutaneous squamous cell carcinoma, which limits the generalizability and extrapolation of our observations. All above-mentioned limitations will be addressed in our follow-up research.

Conclusions

In summary, this study integrated network pharmacology, molecular docking simulation and in vitro cell morphology experiments to systematically screen 61 intersecting targets of 6-gingerol in cSCC, identifying *TP53*, *AKT1*, *BCL2*, *CASP3* and *EGFR* as

core targets. Enrichment analysis suggested that the drug may primarily regulate the biological processes of apoptosis and proliferation, potentially acting on the classical tumor signaling pathways PI3K-Akt and MAPK. Molecular docking indicated that 6-gingerol has strong affinity for the above core targets and can stably bind to the active regions of target proteins through hydrogen bonds and hydrophobic interactions. HE staining morphological observation preliminarily revealed that 6-gingerol can induce apoptosis-like morphological changes in A431 squamous carcinoma cells. These findings provide preliminary evidence and theoretical support for 6-gingerol as a candidate anti-cSCC agent; however, the mechanisms predicted in this study remain to be verified through further molecular biology and in vivo experiments.

Declarations

Ethics approval and consent to participate

Not applicable. This study only adopted the commercially purchased A431 human cutaneous squamous cell carcinoma cell line, and no human tissue samples or live animal models were used in all experimental procedures, thus ethical approval and participant consent were not required.

Consent for publication

Not applicable. No individual participant data, clinical images or personal information were involved in this manuscript.

Conflicts of interest

The authors declare that there are no potential conflicts of interest regarding the research, authorship, and publication of this article.

Funding

This work did not receive any financial support from public, commercial or non-profit funding agencies.

References

- [1] Brantsch KD, Meisner C, Schönfish B, Trilling B, Wehner-Caroli J, Röcken M, et al. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. *Lancet Oncol*. 2008;9(8):713-20. doi:10.1016/S1470-2045(08)70178-5.
- [2] Burton KA, Ashack KA, Khachemoune A. Cutaneous squamous cell carcinoma: a review of high-risk and metastatic disease. *Am J Clin Dermatol*. 2016;17(5):491-508. doi:10.1007/s40257-016-0207-3.
- [3] Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. *J Am Acad Dermatol*. 2013;68(6):957-66. doi:10.1016/j.jaad.2012.11.037.
- [4] Karia PS, Jambusaria-Pahlajani A, Harrington DP, Murphy GF, Qureshi AA, Schmults CD. Evaluation of American Joint Committee on Cancer, International Union Against Cancer, and Brigham and Women's Hospital tumor staging for cutaneous squamous cell carcinoma. *J Clin Oncol*. 2014;32(4):327-34. doi:10.1200/JCO.2012.48.5326.
- [5] Kim JYS, Kozlow JH, Mittal B, Moyer J, Olenecki T, Rodgers P. Guidelines of care for the management of cutaneous squamous cell carcinoma. *J Am Acad Dermatol*. 2018;78(3):560-78. doi:10.1016/j.jaad.2017.10.007.
- [6] Que SKT, Zwald FO, Schmults CD. Cutaneous squamous cell carcinoma: incidence, risk factors, diagnosis, and staging. *J Am Acad Dermatol*. 2018;78(2):237-47. doi:10.1016/j.jaad.2017.08.059.
- [7] Schmults CD, Karia PS, Carter JB, Han J, Qureshi AA. Factors predictive of recurrence and death from cutaneous squamous cell carcinoma: a 10-year, single-institution cohort study. *JAMA Dermatol*. 2013;149(5):541-7. doi:10.1001/jamadermatol.2013.2139.
- [8] Stratigos A, Garbe C, Lebbe C, Malvehy J, del Marmol V, Pehamberger H, et al. Diagnosis and treatment of invasive squamous cell carcinoma of the skin: European consensus-based interdisciplinary guideline. *Eur J Cancer*. 2015;51(14):1989-2007. doi:10.1016/j.ejca.2015.06.110.
- [9] Alhanshali L, Khachemoune A. Clinical dermatologic applications of ginger: a focused review. *Int J Dermatol*. 2024;63(4):438-42. doi:10.1111/ijd.16963.
- [10] Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, et al. Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). *Foods*. 2019;8(6):185. doi:10.3390/foods8060185.
- [11] Kapoor V, Aggarwal S, Das SN. 6-Gingerol mediates its anti tumor activities in human oral and cervical cancer cell lines through apoptosis and cell cycle arrest. *Phytother Res*. 2016;30(4):588-95. doi:10.1002/ptr.5561.
- [12] Lee SH, Cekanova M, Baek SJ. Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells. *Mol Carcinog*. 2008;47(3):197-208. doi:10.1002/mc.20374.
- [13] Lin CB, Lin CC, Tsay GJ. 6-Gingerol inhibits growth of colon cancer cell LoVo via induction of G2/M arrest. *Evid Based Complement Alternat Med*. 2012;2012:326096. doi:10.1155/2012/326096.
- [14] Prasad S, Tyagi AK. Ginger and its constituents: role in prevention and treatment of gastrointestinal cancer. *Gastroenterol Res Pract*. 2015;2015:142979. doi:10.1155/2015/142979.

- [15] Shukla Y, Singh M. Cancer preventive properties of ginger: a brief review. *Food Chem Toxicol*. 2007;45(5):683-90. doi:10.1016/j.fct.2006.11.002.
- [16] Lv L, Chen H, Soroka D, Chen X, Leung T, Sang S. 6-Gingerdiols as the major metabolites of 6-gingerol in cancer cells and in mice and their cytotoxic effects on human cancer cells. *J Agric Food Chem*. 2012;60(45):11372-7. doi:10.1021/jf303879b.
- [17] Sp N, Kang DY, Lee JM, Bae SW, Jang KJ. Potential antitumor effects of 6-gingerol in p53-dependent mitochondrial apoptosis and inhibition of tumor sphere formation in breast cancer cells. *Int J Mol Sci*. 2021;22(9):4660. doi:10.3390/ijms22094660.
- [18] Xu S, Zhang H, Liu T, Yang W, Lv W, He D, et al. 6-Gingerol induces cell-cycle G1-phase arrest through AKT-GSK 3 β -cyclin D1 pathway in renal-cell carcinoma. *Cancer Chemother Pharmacol*. 2020;85(2):379-90. doi:10.1007/s00280-019-03999-9.
- [19] Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol*. 2008;4(11):682-90. doi:10.1038/nchembio.118.
- [20] Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Med*. 2013;11(2):110-20. doi:10.1016/S1875-5364(13)60037-0.
- [21] Wang H, Guo M, Wei H, Chen Y. Targeting p53 pathways: mechanisms, structures, and advances in therapy. *Signal Transduct Target Ther*. 2023;8(1):92. doi:10.1038/s41392-023-01347-1.
- [22] Hua H, Zhang H, Chen J, Wang J, Liu J, Jiang Y. Targeting Akt in cancer for precision therapy. *J Hematol Oncol*. 2021;14(1):128. doi:10.1186/s13045-021-01137-8.
- [23] Belicza M. [Evaluation of morphologically determined apoptotic index]. *Acta Med Croatica*. 2009;63 Suppl 2:3-12.
- [24] Nigam N, Bhui K, Prasad S, George J, Shukla Y. [6]-Gingerol induces reactive oxygen species regulated mitochondrial cell death pathway in human epidermoid carcinoma A431 cells. *Chem Biol Interact*. 2009;181(1):77-84. doi:10.1016/j.cbi.2009.05.012.
- [25] Zhou K, Wu C, Cheng W, Zhang B, Wei R, Cheng D, et al. Transglutaminase 3 regulates cutaneous squamous carcinoma differentiation and inhibits progression via PI3K-AKT signaling pathway-mediated Keratin 14 degradation. *Cell Death Dis*. 2024;15(4):252. doi:10.1038/s41419-024-06626-5.
- [26] Centeno PP, Chester C, Kanellos G, Ford CA, Cammareri P, Inman GJ, et al. SOX2 confers tumour permissiveness in a specific skin progenitor population. *Nat Commun*. 2026;17(1):304. doi:10.1038/s41467-025-66251-4.