

Original Article

Exploring the molecular mechanism of cellular senescence induced by *Picrasma quassioides* in HepG2 based on RNA sequencing

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Abstract: Objective: This study investigates the impact of *Picrasma quassioides* (PQ) on senescence and proliferation in the human hepatoma cell line HepG2, alongside its potential molecular mechanisms, using RNA sequencing (RNA-seq) in conjunction with *in vitro* experiments. The study aims to provide a novel theoretical foundation for PQ-based hepatocellular carcinoma (HCC) treatments. Methods: The analysis of chemical components in PQ-containing serum was conducted using UHPLC-Q-Orbitrap HRMS. To assess cell viability, proliferation, and senescence, CCK-8 assays, EdU staining assays, and SA- β -Gal staining assays were performed. Additionally, core differential genes were screened through transcriptome sequencing and network pharmacology. The molecular mechanisms underlying PQ-induced senescence in HepG2 cells were thoroughly elucidated via functional enrichment analysis, construction of protein-protein interaction networks, and molecular docking techniques. Results: Using UHPLC-Q-Orbitrap HRMS technology, 33 chemical components were identified in PQ. The CCK8 assay demonstrated that PQ exhibited a cytotoxic effect on HepG2 cells, while EdU and SA- β -Gal assays confirmed that PQ could induce cellular senescence and inhibit excessive proliferation. RNA sequencing, in conjunction with network pharmacology, identified 146 key genes, and enrichment analysis revealed that these genes were significantly enriched in the MAPK and PI3K-Akt pathways. Among these, five core targets were identified: MMP2, TGFB1, CXCL8, JUN, and FOS. Molecular docking studies suggested that these core genes could interact with PQ-like compounds. Conclusions: PQ exerts anti-cancer effects on HCC by inducing cellular senescence, potentially through the MAPK and PI3K-Akt signaling pathways. MMP2, TGFB1, CXCL8, JUN, and FOS may represent key targets of PQ-induced senescence.

Keywords: RNA sequencing, *Picrasma quassioides*, hepatocellular carcinoma, cellular senescence, molecular mechanisms

Introduction

Globally, liver cancer ranks as one of the most common cancers. Histopathologically, it is primarily classified into three types: hepatocellular carcinoma (HCC), cholangiocarcinoma, and mixed liver cancer [1]. Among these, HCC stands out as the predominant type, representing about 90% of all liver cancer instances [2]. HCC poses a significant health threat due to its high incidence, aggressive invasiveness and metastasis, and poor prognosis. Early-stage HCC often presents without specific symptoms, and by the time it is diagnosed, the majority of patients are in the middle or late phases, miss-

ing the optimal window for surgical intervention. Furthermore, current clinical treatments, such as interventional therapies, immunotherapy, and targeted drugs, still fail to achieve ideal therapeutic outcomes and are associated with notable adverse reactions and side effects [3]. Therefore, the development of novel therapeutic drugs for HCC remains a critical challenge.

Picrasma quassioides (PQ), a traditional Chinese medicine, has often been used to treat various ailments such as anemopyretic cold, eczema, and venomous snake bites. PQ contains a range of chemical components, including alkaloids and quassinoids, along with vola-

tile oils, triterpenoids, saponins, and other bio-active compounds. Modern pharmacological studies have demonstrated that PQ extracts possess significant anti-inflammatory, anti-tumor, and antihypertensive properties [4]. Gong et al. [5] showed that PQ extract activates the p38 MAPK signaling pathway by increasing reactive oxygen species (ROS) accumulation in cells and mitochondria, promoting apoptosis in cervical cancer cells. Moreover, Picrasidine I, an active component derived from PQ, has been found to induce apoptosis in melanoma cells by activating the ERK and JNK pathways while inhibiting the Akt signaling pathway [6]. However, the precise anti-cancer mechanisms of PQ within the realm of HCC are still not completely elucidated.

Cellular senescence is a state of persistent, irreversible cell cycle arrest, characterized by stable growth inhibition, morphological changes, and functional decline or loss [7]. Various stressors, such as telomere shortening, DNA damage, mitochondrial dysfunction, and mutations in oncogenes, can trigger cellular senescence [8]. Furthermore, recent research [9] highlights the critical role of cellular senescence in tumor immunity. By inhibiting the excessive proliferation of tumor cells, senescence serves as an effective mechanism to prevent malignant growth and metastasis. This study employs RNA sequencing (RNA-seq) and *in vitro* experiments to examine the effects of PQ on cellular senescence and proliferation in HepG2 cells, as well as its underlying molecular mechanisms, providing a new theoretical foundation for PQ-based therapies in HCC.

Materials and methods

Extract preparation

PQ dried stems (serial No. 051310101) were purchased from Anhui Weiwu Traditional Chinese Medicine Co., Ltd. (Bozhou, China). The stems were boiled in water at a 1:10 (w/v) ratio for 1 hour, followed by filtration. The residue was boiled again at a 1:8 (w/v) ratio for 0.5 hours and filtered once more. The two filtrates were merged and concentrated using a rotary evaporator (Shanghai, China) to a final stock solution concentration of 0.63 g/mL, which was then stored at below 4°C for subsequent use in experiments.

Experimental animals

Twenty 8-week-old male Sprague-Dawley rats (190 ± 10 g) were purchased from the Experimental Animal Center of Anhui Medical University. The rats were housed in the specialized animal facility of the Fourth Affiliated Hospital of Anhui Medical University, with a controlled environment including constant temperature (22 ± 2°C), constant humidity (50% ± 10%), and a 12-hour light-dark cycle. The animals were acclimatized to the experimental environment for one week prior to subsequent experiments. The use of all animals was approved by the local medical ethics committee (Approval No. KYXM-202403-012).

Preparation of PQ medicated serum

Rats were assigned at random to either the PQ group or the control group, with 10 rats in each group. Rats in the PQ group were administered 6.3 g/kg by gavage once daily for 7 days, while the control group received an equivalent volume of physiological saline. The mice were euthanized under deep anesthesia via cervical dislocation after 2 h, and care was taken to reduce animal suffering. After collecting blood samples from the abdominal aorta, the serum was separated by centrifugation at 3,000 rpm for 20 minutes, inactivated at 56°C for 30 minutes, and filtered using a 0.22 µm microporous membrane. The serum was stored at -80°C for future use.

Identification of components in PQ medicated serum

One hundred µL of PQ sample was put into a centrifuge tube, 300 µL of methanol was added, and vortexed for 1 min. Next, it was centrifuged at 4°C and 12,000 rpm for 10 min. Then 100 µL of the supernatant was taken and added to 100 µL of ultrapure water, mixed well, and injected for analysis.

One hundred µL of serum was placed into a centrifuge tube, mixed with 300 µL of methanol, and vortexed for 10 min. Next it was centrifuged at 4°C and 12,000 rpm for 10 min. Then 270 µL of the supernatant was vacuum concentrated for 4 h, and then 90 µL of 50% methanol solution was added and vortexed for 1 min. The resulting mix was centrifuged under

Table 1. Elution gradient

Time (min)	Mobile phase	
	A (v%)	B (v%)
0	98	2
1.0	98	2
14.0	70	30
25.0	0	100
28.0	0	100
28.1	98	2
30.0	98	2

the same conditions for 10 min, and 80 μ L of the supernatant was used for analysis.

Chromatographic separation was performed using the Vanquish Flex UHPLC (Thermo Fisher Scientific Inc., Waltham, MA, USA). The mobile phase consisted of water (0.1% formic acid, phase A) and acetonitrile (phase B) with a flow rate of 0.3 mL/min and the column temperature was 40°C. The elution gradient is shown in **Table 1** and the injection volume was 6.0 μ L.

The MS data was collected by a hybrid quadrupole orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific, Waltham, MA, USA). The data was acquired in “Full scan/dd-MS2” mode.

The MS data was processed by Progenesis QI 3.0 (Waters Corp., Milford, MA, USA). Subsequently, the reference substance database (TCM Pro 2.0, Beijing Hexin Technology Co., Ltd.) and the theoretical database (constructed through literature, public databases, etc.) were searched. Through a comprehensive multi-dimensional analysis of reference substance retention time error, isotope distribution, and peak intensity, the final identification results were obtained.

Cell culture

The human liver cancer cell line HepG2 (CL-0103) was obtained from Procell Life Science & Technology Co., Ltd. (Wuhan, China). The cells were cultured in DMEM medium (Servicebio, Wuhan, China), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated in a 5% CO₂ atmosphere at 37°C. After 24 hours of treatment, HepG2 cells were collected for further experiments.

CCK-8 assay

Cell viability was assessed using the CCK-8 assay. HepG2 cell suspensions were seeded into a 96-well plate at a density of 1.0×10^5 cells per well and incubated overnight in a 5% CO₂ atmosphere at 37°C. Following treatment with PQ medicated serum at various concentrations for 24, 48, or 72 hours; 10 μ L of CCK-8 reagent (Biosharp, Shanghai, China) was added to each well and incubated for 1 hour. Absorbance at OD450 nm was measured using an enzyme-linked immunosorbent assay (ELISA) reader.

EdU assay

EdU assay was conducted to assess cell proliferation. Following the manufacturer's instructions, EdU solution (Elabscience, Wuhan, China) was added to each well, and cells were incubated for 2 hours. After incubation, the EdU solution was removed, and the cells were fixed with 1 mL of 4% paraformaldehyde in PBS for 15 minutes at room temperature. The cells were then permeabilized with 1 mL of PBS containing 0.3% Triton X-100 for 20 minutes. After washing three times with PBS, each well received 500 μ L of Click reaction solution, which was allowed to incubate at room temperature for 30 minutes. Following three PBS washes, 500 μ L of DAPI staining solution was added to stain the cells for 10 minutes at room temperature. After another PBS wash, fluorescence staining was observed using an inverted microscope (OLYMPUS, Japan).

Senescence associated β -gal staining (SA- β -gal staining)

To evaluate SA- β -gal activity, cells were fixed as per the instructions, washed three times with PBS, and then incubated overnight at 37°C with an appropriate amount of staining solution (Beyotime, Shanghai, China). After PBS washes, images were captured using an inverted microscope.

Western blot (WB)

Cellular proteins were extracted using RIPA lysis buffer (Biosharp, Shanghai, China) supplemented with 1 mM phenylmethanesulfonyl fluoride (PMSF). Equal amounts of proteins were separated by 10% SDS-PAGE and transferred

to a polyvinylidene fluoride (PVDF) membrane. The membranes were blocked with 5% skim milk powder at room temperature for 2 hours, followed by overnight incubation at 4°C with primary antibodies, including Ki67, P21, TGFβ1, CXCL8, JUN, and FOS (1:1000, Affinity, USA), SMP30 (1:1000, Zenbio, Sichuan, China), MMP2 (1:1000, Beyotime, Shanghai, China). The membranes were then incubated with horseradish peroxidase (HRP)-labeled secondary antibody (1:10,000, Zs-BIO, Beijing, China), and signals were detected using the ECL Hypersensitive Luminescence Kit (Biosharp, Shanghai, China). Finally, Image J software was utilized for densitometric analysis.

RNA-seq and data processing

Total RNA was extracted using the TRIzol reagent (Invitrogen, USA). The quantification of RNA was performed with the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), while its integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Libraries were constructed with the VAHTS Universal V6 RNA-seq Library Prep Kit, following the manufacturer's instructions. The sequencing process was carried out using the Illumina Novaseq 6000 platform, resulting in the generation of 150 bp paired-end reads. Raw reads were processed using Fastp (version 0.20.1), where low-quality reads were filtered to produce clean reads. Clean reads were aligned to the human reference genome GRCH38 with HISAT2 (version 2.1.0). Differential expression analysis was conducted using the DESeq2 package in R. Genes with a *P*-value of less than 0.05 and an absolute fold change ($|\log_2FC|$) greater than 0.585 were identified as significantly differentially expressed genes. To facilitate the visualization of results, gene expression levels were normalized to fragments per kilobase of transcript per million mapped reads (FPKM). Based on the normalized FPKM values, the Pearson correlation coefficient was calculated to evaluate sample reproducibility, and the correlation matrix was visualized as a heatmap using the ggcorrplot package in R.

Screening of aging-related differentially expressed genes (AgDEGs)

Differential expression analysis was performed utilizing DESeq2 (version 1.22.2) [10], with a

fold change ≥ 1.5 and $P < 0.05$ as the thresholds for identifying significantly differentially expressed genes (DEGs). A hierarchical clustering analysis of DEGs was conducted in R (version 3.2.0) to visualize gene expression patterns across groups and samples. Aging-related genes (AGs) were sourced from the GeneCards [11] database (<https://www.genecards.org>) and filtered based on correlation scores above the average threshold. Intersection of DEGs and AGs identified aging-related differentially expressed genes (AgDEGs). A radar chart was constructed in R using the ggradar package to display the top 10 upregulated and downregulated AgDEGs.

Enrichment analysis

The enrichment analyses of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were conducted using the DAVID [12] database (<https://david.ncifcrf.gov>) to investigate the biological relevance of AgDEGs. The results of enrichment analysis were visualized using the Microbiography platform (<https://www.bioinformatics.com.cn/login/>).

Identification of hub AgDEGs

A protein-protein interaction (PPI) network was constructed using the STRING [13] database (<https://string-db.org/>), applying a high-confidence interaction score (≥ 0.7) to enhance reliability. The interaction data were downloaded and imported into Cytoscape (version 3.9.1) [14] for network visualization. Hub AgDEGs were identified using five algorithms from the CytoHubba plugin: MCC, MNC, Degree, Closeness, and Betweenness.

Molecular docking

The structures of small molecule compounds and hub AgDEGs were retrieved from the PubChem [15] database (<https://pubchem.ncbi.nlm.nih.gov/>) and the PDB [16] database (<https://www.rcsb.org/>), respectively. AutoDock Vina was employed for docking simulations between active ingredients and protein receptors, selecting the conformation with the lowest binding energy for further analysis. Non-covalent interactions between molecules were analyzed using PLIP [17] (<https://plip-tool.bio->

tec.tu-dresden.de/plip-web/plip/index), and PyMOL [18] was used for visualization of these interactions.

Statistical analysis

Data analysis was performed using GraphPad Prism (version 8.0.2). All experiments were conducted in triplicate, with results expressed as mean $\pm$ standard deviation (SD). Statistical significance between two groups was determined using the t-test. Statistical significance was set at * $P < 0.05$ and ** $P < 0.01$.

Results

Identification of components in PQ medicated serum

The BPI chromatogram is constructed by continuously plotting the ion features with the highest abundance at each time point. **Figure 1** displays the BPI of PQ water extract (A) and medicated serum (B) in both positive and negative ion modes. We isolated 33 compounds from medicated serum and selected the top 10 compounds with the highest peak areas as the main compounds of PQ medicated serum (**Table 2; Figure 2**).

Screening the intervention concentration and time of PQ medicated serum

Cells were pretreated with medicated serum at varying concentrations for 24, 48, or 72 hours. The results demonstrated a concentration- and time-dependent increase in the inhibition rate of PQ on HepG2 cells (**Figure 3**). Specifically, as the PQ concentration and treatment duration increased, the inhibitory effect on HepG2 cells became more pronounced. At a 10% PQ medicated serum concentration with a 72-hour intervention, the cell inhibition rate reached 50% (IC₅₀). Consequently, 10% PQ medicated serum and a 72-hour treatment duration were selected for subsequent experiments. Cells in the zero-concentration treatment group were treated with serum from rats in the blank control group. All experiments were performed with 6 independent biological replicates ($n = 6$).

PQ induces cellular senescence in HepG2 and inhibits excessive cell proliferation

The EdU assay was performed to assess the impact of PQ on HepG2 cell proliferation. As

shown in **Figure 4A**, an increase in the concentration of medicated serum led to a corresponding decrease in cell proliferation, confirming the anti-proliferative effects of PQ. SA- β -Gal staining revealed a dose-dependent increase in the positive cell rate with rising medicated serum concentration (**Figure 4B**). Additionally, WB was conducted to measure the protein expression of the proliferation marker Ki67, as well as the senescence markers P21 and SMP30. As illustrated in **Figure 4C**, PQ treatment resulted in a decrease in Ki67 expression, while the expression of P21 and SMP30 was upregulated. These results collectively suggest that PQ induces cellular senescence in HepG2 cells, inhibiting excessive cell proliferation.

PQ inhibits excessive cell proliferation by inducing cellular senescence in HepG2

To examine whether PQ inhibits excessive proliferation by inducing cellular senescence, a recovery experiment was performed using the cell senescence inhibitor rapamycin (RAPA). The results indicated that RAPA partially reversed the effects of PQ on cellular senescence and proliferation in HepG2 cells (**Figure 5A, 5B**). WB further confirmed the findings from the EdU and SA- β -Gal assays (**Figure 5C**). These data suggest that PQ inhibits excessive cell proliferation through the induction of cellular senescence in HepG2 cells.

Quality control of RNA-seq data

To elucidate the mechanisms underlying PQ-induced cellular senescence, RNA-seq analysis was conducted to identify differential gene expression between the HepG2 and HepG2+PQ groups. PCA showed a trend toward separation between the two groups along PC1. (**Figure 6A**), and the correlation heatmap visually highlighted the consistency within groups and the differences between them (**Figure 6B**). These quality control assessments confirmed the suitability of the RNA-seq data for subsequent analysis.

Screening of AgDEGs

Compared to the HepG2 group, PQ treatment led to the identification of 1,170 DEGs, with 504 genes upregulated and 666 downregulated (**Figure 7A**). A heatmap illustrating the DEGs

Picrasma quassioides-induced senescence in HepG2

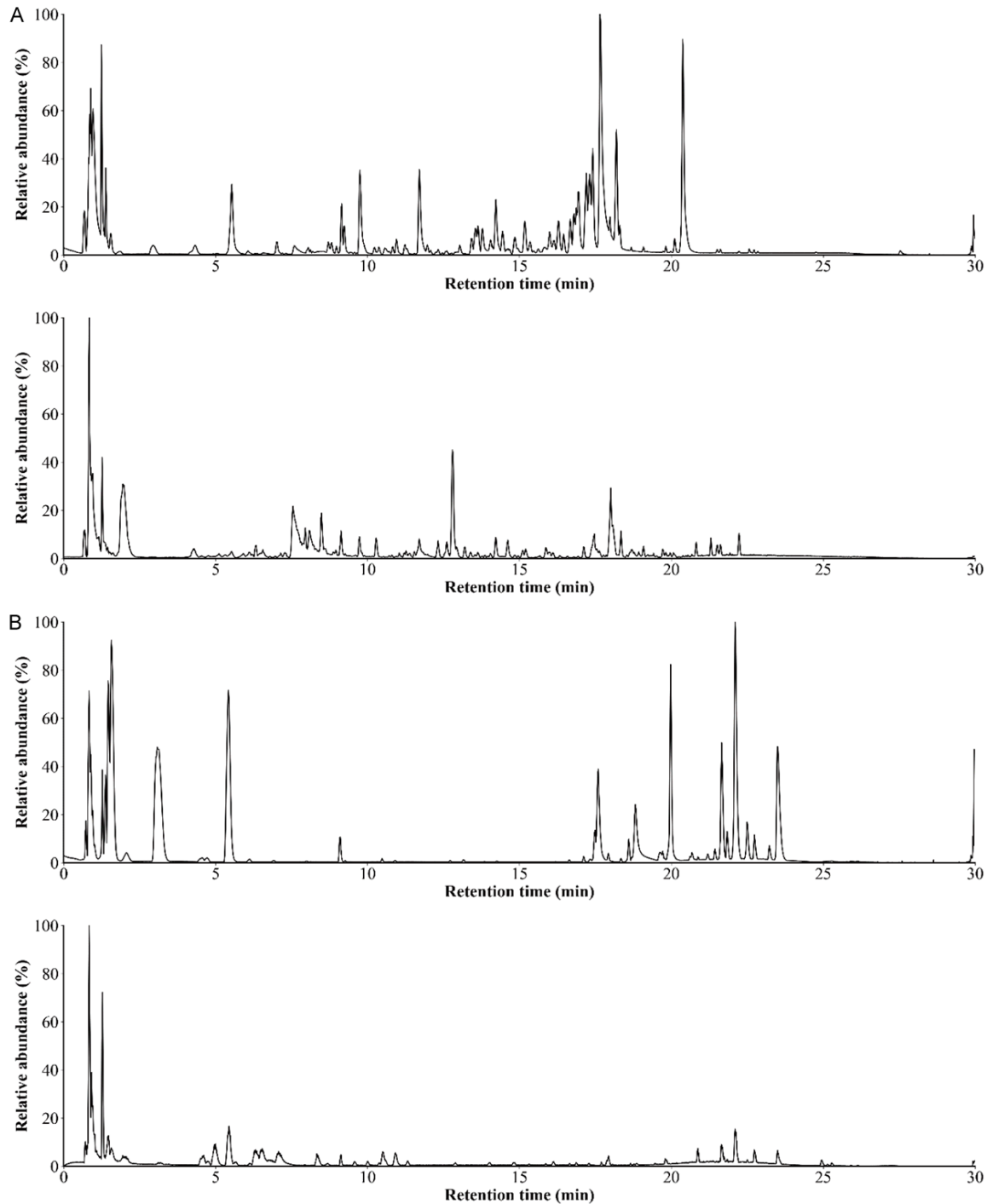


Figure 1. Base peak ions (BPI). A. BPI chromatograms of *Picrasma quassioides* (PQ) detected in positive and negative modes. B. BPI chromatograms of medicated serum detected in positive and negative modes.

between the HepG2 and HepG2+PQ groups is shown in **Figure 7B**. The intersection of DEGs and AGs revealed 146 AgDEGs (**Figure 7C**). The radar chart in **Figure 7D** displays the top 10 upregulated and downregulated AgDEGs (**Table 3**). Despite the high correlation observed

between the HepG2 and HepG2+PQ groups in the sample correlation analysis (**Figure 6B**), significant differences were evident between them. Thus, the underlying mechanisms of PQ-induced cellular senescence in HepG2 may be linked to these AgDEGs.

Table 2. Main components of medicated serum

Components	Experimental m/z	Formula	Retention time (min)	Peak area	Type
Hancogenin B	393.1902	C ₂₁ H ₂₈ O ₇	12.71	421951	Prototype
Glaucogenin A	377.1954	C ₂₁ H ₂₈ O ₆	14.26	333213	Prototype
Picrasin D	391.1747	C ₂₁ H ₃₀ O ₆	13.86	61969	Metabolite
Picrasinoside C	558.294	C ₂₇ H ₄₀ O ₁₁	15.01	46635	Metabolite
Nigakilactone F	409.2219	C ₂₂ H ₃₂ O ₇	14.36	45793	Prototype
Nigakihemiacetal D	475.23	C ₂₄ H ₃₆ O ₈	15.75	38192	Prototype
Nigakilactone N	395.2061	C ₂₁ H ₃₀ O ₇	12.69	26492	Prototype
1-Ethoxycarbonyl-beta-carboline	417.129	C ₂₀ H ₂₀ N ₂ O ₈	9.58	17170	Metabolite
Nigakihemiacetal B	435.1668	C ₂₁ H ₃₀ O ₆	13.87	14659	Metabolite
Prostratin	409.2218	C ₂₂ H ₃₂ O ₇	14.06	12797	Metabolite

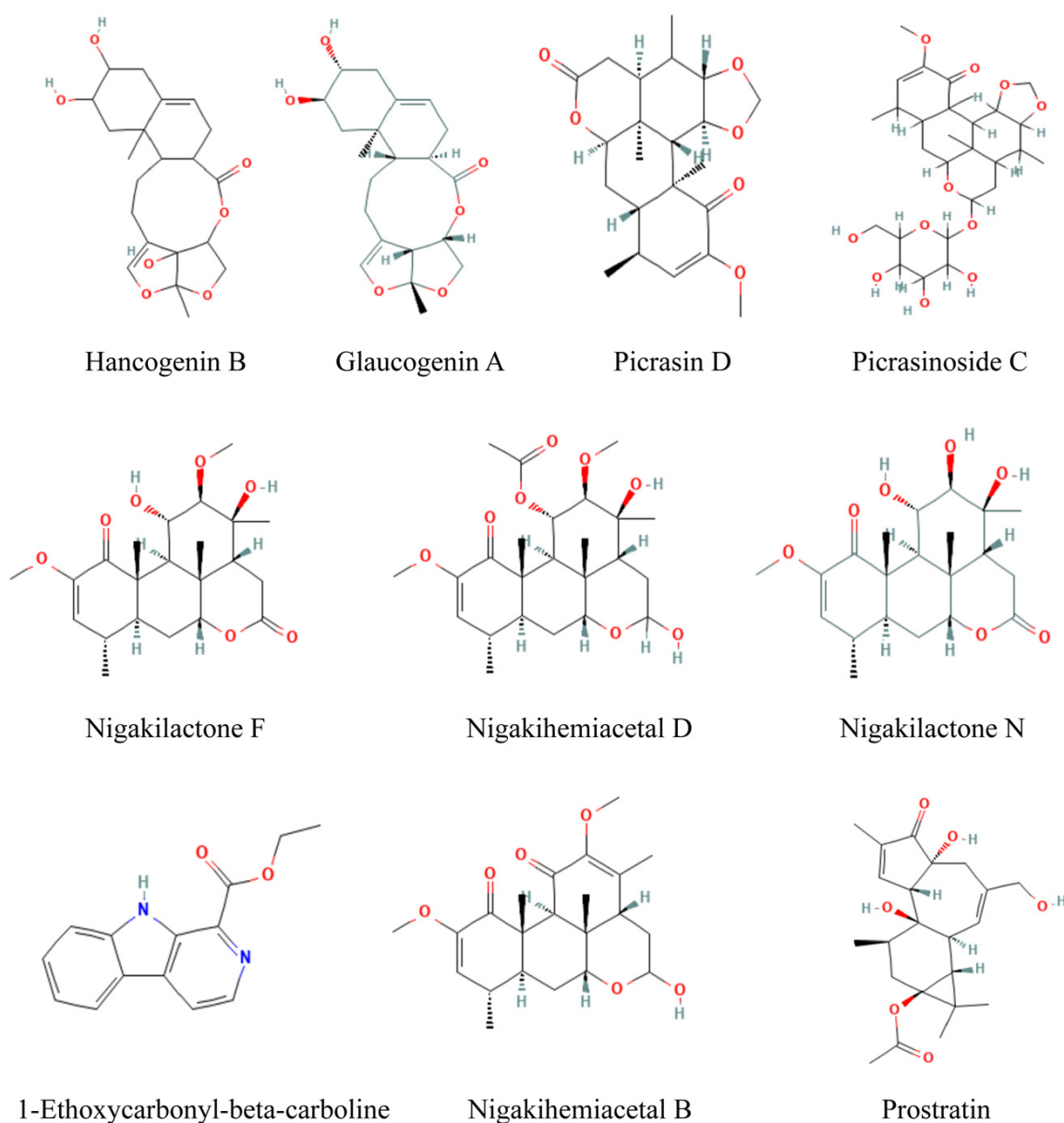


Figure 2. Compound structural formula.

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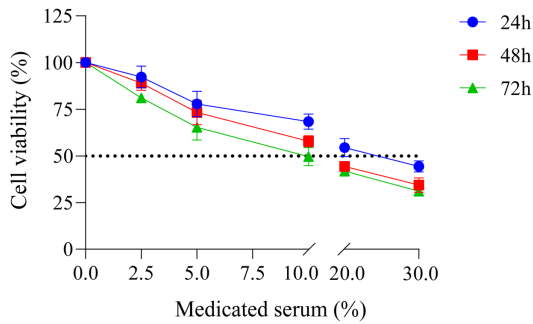


Figure 3. Screening the intervention concentration and time of PQ medicated serum. n = 6.

Enrichment analysis of AgDEGs

To investigate the biological functions and associated pathways of AgDEGs, GO and KEGG pathway enrichment analyses were conducted. **Figure 8** presents the top 10 GO terms, categorized into biological process (BP), cellular component (CC), and molecular function (MF), along with the KEGG pathways, ranked by *P*-value. GO analysis revealed 316 significant entries ($P < 0.05$), with 240, 35, and 41 entries significantly enriched in BP, CC, and MF categories, respectively. **Figure 8A** highlights the top 10 terms in each category. In the BP category, the top three terms included “response to xenobiotic stimulus”, “positive regulation of cell population proliferation”, and “positive regulation of cell migration”. In the CC category, the leading terms were “extracellular space”, “extracellular region”, and “collagen-containing extracellular matrix”. In the MF category, the top terms were “protein binding”, “identical protein binding”, and “growth factor activity”.

KEGG pathway analysis identified 76 signaling pathways significantly enriched in AgDEGs ($P < 0.05$). The top 10 enriched KEGG pathways included the AGE-RAGE signaling pathway in diabetic complications, pathways in cancer, focal adhesion, fluid shear stress and atherosclerosis, cellular senescence, MAPK signaling pathway, osteoclast differentiation, rheumatoid arthritis, PI3K-Akt signaling pathway, and Yersinia infection (**Figure 8B**). These pathways were categorized into four groups: environmental information processing, cellular processes, organismal systems, and human diseases (**Figure 8C**). The Sankey diagram in **Figure 8D** illustrates the distribution of AgDEGs across the top 10 KEGG pathways. These

results suggest that PQ may induce cellular senescence by modulating the MAPK and PI3K-Akt signaling pathways.

Identification of Hub AgDEGs

To further identify hub AgDEGs, the AgDEGs were uploaded to the STRING database, and a PPI network containing 145 nodes and 234 edges was constructed (**Figure 9A**). Using the Cytoscape plugin CytoHubba, hub AgDEGs were identified from the PPI network. To ensure reliability, five algorithms were applied, and the top 10 genes ranked by each algorithm were selected (**Table 4**). By intersecting the results from all five algorithms, five hub AgDEGs were identified: MMP2, TGFB1, CXCL8, JUN, and FOS (**Figure 9B**). Detailed information on these hub AgDEGs is provided in **Table 5**.

Molecular docking

Molecular docking was performed between the five hub AgDEGs and PQ compounds. The docking results were listed in **Table 6**. Lower negative binding energy correlates with stronger affinity. **Figure 10** illustrates the binding affinities between the five hub AgDEGs (MMP2 (**Figure 10A**), TGFB1 (**Figure 10B**), CXCL8 (**Figure 10C**), JUN (**Figure 10D**), and FOS (**Figure 10E**)) and PQ compounds. Among these, MMP2 demonstrated the strongest affinity with 1-Ethoxycarbonyl-beta-carboline (affinity value = -8.8) and had the lowest average binding energy among the hub AgDEGs, suggesting it may serve as a key target for PQ-induced cellular senescence.

Validation of hub AgDEGs

WB was conducted to validate the expression of hub AgDEGs. As shown in **Figure 11**, PQ treatment led to reduced expression of MMP2, TGFB1, CXCL8, JUN, and FOS compared to the HepG2 group, consistent with the RNA-seq results. These results confirm that PQ regulates the expression of the five hub AgDEGs.

Discussion

In recent years, increasing evidence has confirmed that inducing cellular senescence is an effective anti-tumor strategy. For instance, a study on breast cancer demonstrated that YAP1 knockout activated YPEL3 through the

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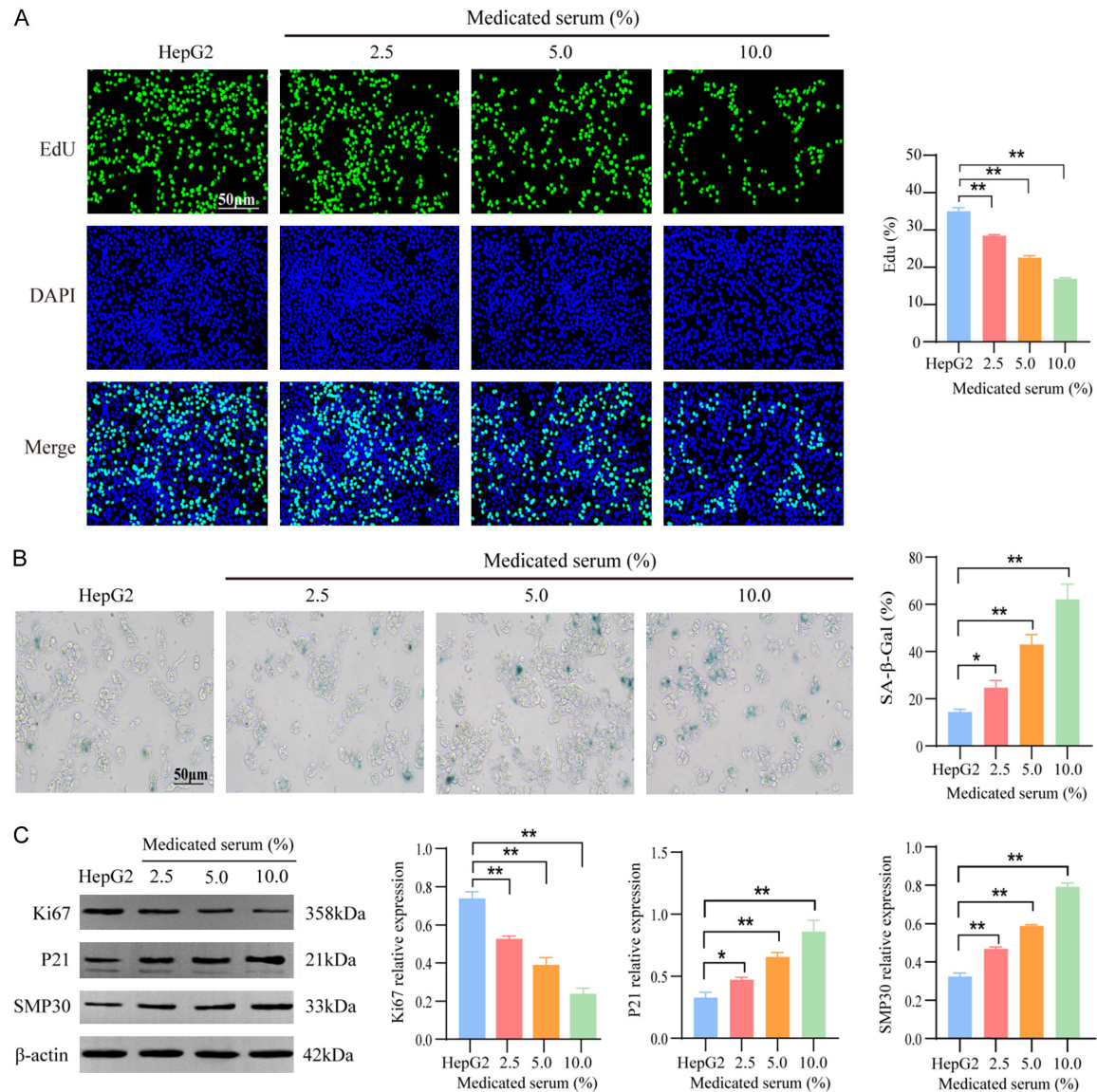


Figure 4. PQ induces cellular senescence in HepG2 and inhibits excessive cell proliferation. A. The effect of PQ on the proliferation of HepG2 was assessed by the EdU assay. B. SA- β -Gal staining was used to evaluate the effect of PQ on cellular senescence in HepG2. Senescent cells are blue-green. C. WB was performed to detect the protein expression levels of Ki67, P21, and SMP30. The results are presented as mean $\pm$ SD, n = 3. * P < 0.05, ** P < 0.01.

Hippo pathway, leading to elevated mitochondrial oxygen consumption and oxidative stress, which promoted senescence and apoptosis in breast cancer cells [19]. In hepatocellular carcinoma, knockdown of RRP15 induced senescence through activation of the p53-p21 signaling pathway, inhibiting the proliferation of p53 wild-type HepG2 cells. Interestingly, in p53-mutant PLC5 and p53-deleted Hep3B cells, RRP15 depletion regulated the glycolytic pentose phosphate pathway, enhanced oxidative-induced senescence, and promoted cell apop-

tosis both *in vitro* and *in vivo* [20]. In lung cancer, the mitochondrial deacetylase Sirt3 activated autophagy by inactivating the PI3K/AKT/mTOR pathway in A549 cells, which counteracted DOX-induced senescence and promoted cancer cell proliferation [21]. In gastric cancer, cystathionine- β -synthase (CBS) plays a critical role in AKT-induced senescence, with CBS deficiency reducing oxidative stress and alleviating cellular senescence through activation of the PI3K/AKT pathway, thereby promoting cancer cell growth [22]. Gas plasma therapy in head

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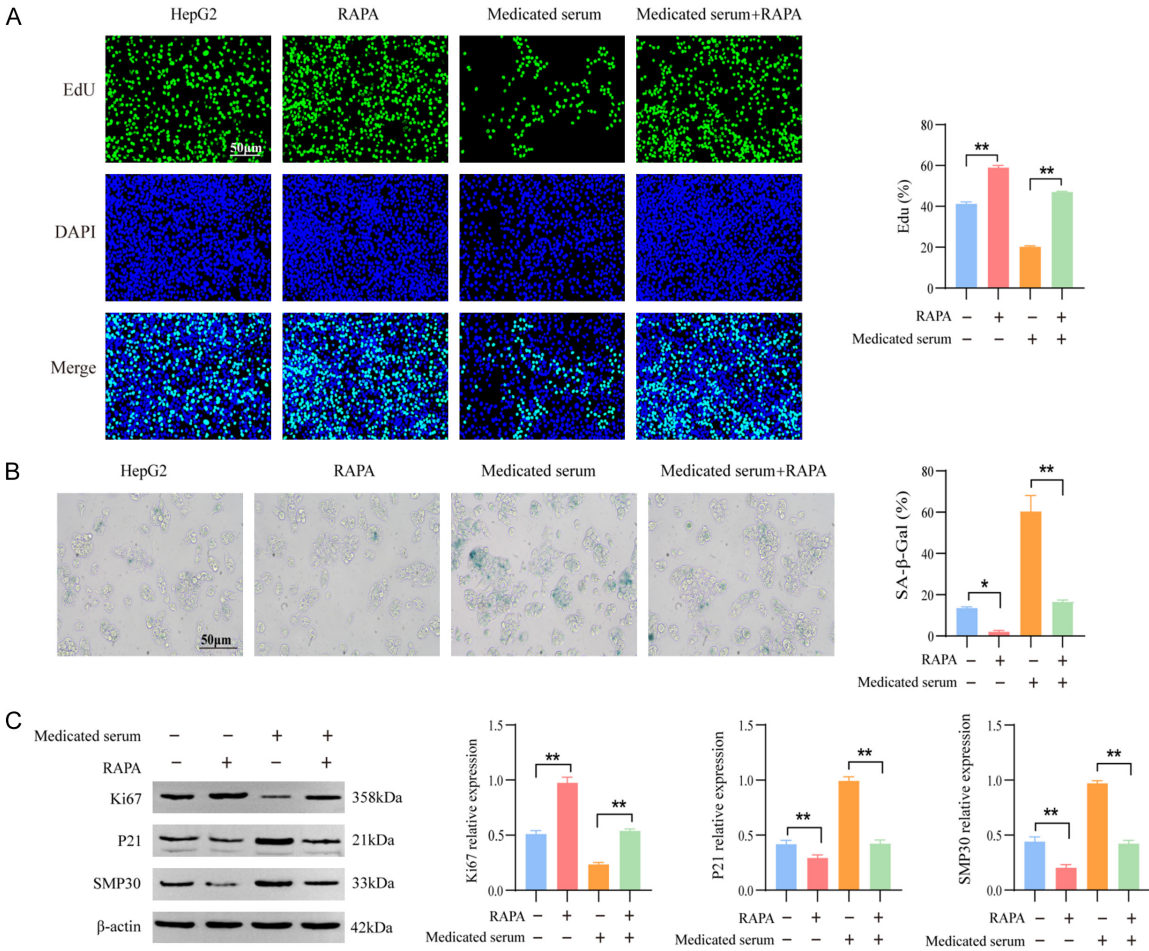


Figure 5. PQ inhibits excessive cell proliferation by inducing cellular senescence in HepG2. **A.** RAPA partially reversed the effects of PQ on HepG2 cell proliferation. **B.** RAPA partially reversed the effects of PQ on cellular senescence in HepG2. **C.** WB was employed to detect the protein expression levels of Ki67, P21, and SMP30. The results are presented as mean $\pm$ SD, $n = 3$. * $P < 0.05$, ** $P < 0.01$.

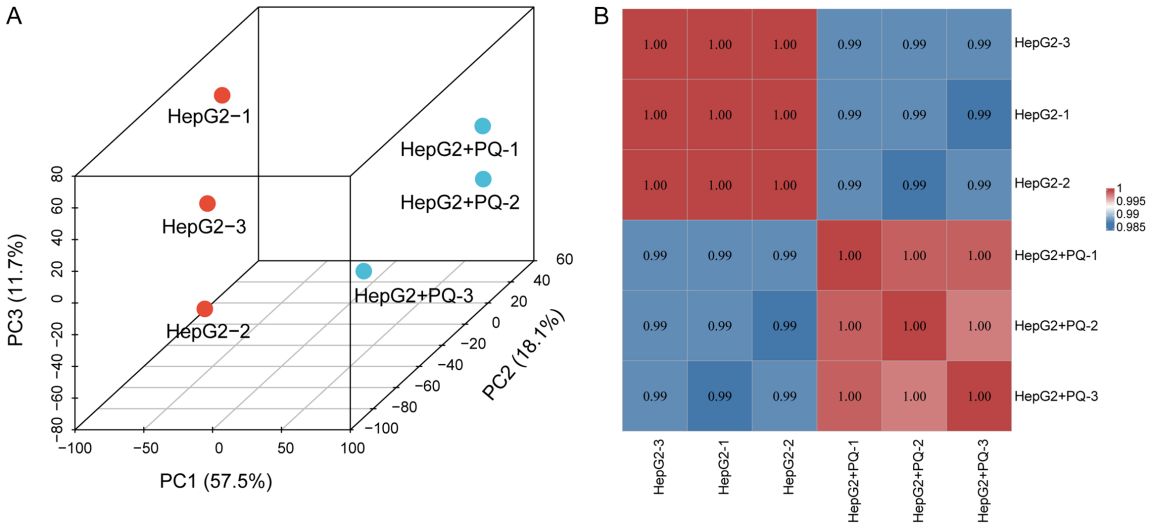


Figure 6. Quality control of RNA-seq data. **A.** Three-dimensional PCA plot of all RNA-seq samples. **B.** Correlation heatmap of all RNA-seq samples.

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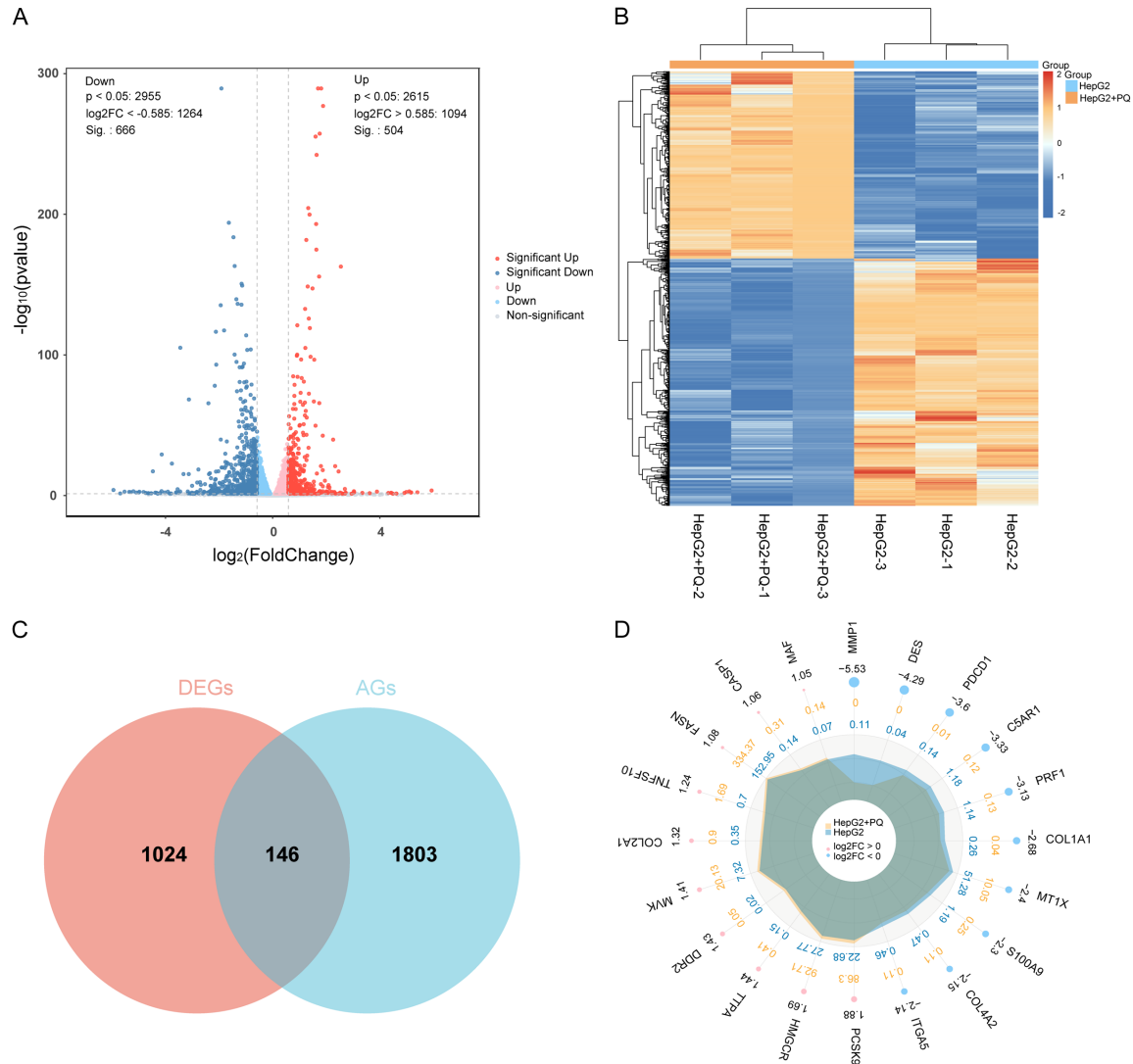


Figure 7. Screening of AgDEGs. A. The volcano plot shows the DEGs between the HepG2 and HepG2+PQ groups. B. The heatmap of DEGs between the HepG2 and HepG2+PQ groups. C. Venn diagram of DEGs and AGs. D. The radar chart displays the top 10 upregulated and downregulated AgDEGs. First circle: light blue indicates downregulated genes and light red indicates upregulated genes, with circle size varying according to the log₂FC value; second circle: outer circle data represents the average expression level in the HepG2+PQ group; inner circle data represents the average expression level in the HepG2 group; third circle: average expression levels of each gene in the HepG2 and HepG2+PQ groups.

and neck cancer has also been shown to induce cellular senescence through oxidative stress accumulation, reducing tumor proliferation *in vivo* [23]. Additionally, 1-Methyl-4-phenylpyridinium upregulated LincRNA-p21, which induces oxidative stress by downregulating the Wnt/ β -catenin pathway, thereby promoting senescence in neuroblastoma cells and reducing tumor cell proliferation and viability [24]. In the present study, PQ inhibited excessive cell proliferation by inducing cellular senescence in HepG2 cells, exerting an anti-HCC effect.

Therefore, inducing cellular senescence serves a vital function in tumor treatment, and targeting cellular senescence holds promise for controlling cancer initiation and progression.

RNA-seq analysis further explored the potential mechanisms underlying PQ-induced cellular senescence in HepG2. RNA-seq revealed that PQ treatment induced 1,170 DEGs, of which 504 were upregulated and 666 downregulated. These DEGs were intersected with AGs, yielding 146 AgDEGs. KEGG pathway enrich-

Table 3. Top 10 upregulated and downregulated AgDEGs

Gene	Log ₂ FC	P-value	Regulation
PCSK9	1.8829	0.0000	Up
HMGCR	1.6945	0.0000	Up
TTPA	1.4376	0.0022	Up
DDR2	1.4301	0.0235	Up
MVK	1.4150	0.0000	Up
COL2A1	1.3174	0.0000	Up
TNFSF10	1.2361	0.0000	Up
FASN	1.0829	0.0000	Up
CASP1	1.0606	0.0451	Up
MAF	1.0511	0.0000	Up
MMP1	-5.5255	0.0018	Down
DES	-4.2914	0.0423	Down
PDCD1	-3.6040	0.0061	Down
C5AR1	-3.3256	0.0000	Down
PRF1	-3.1291	0.0000	Down
COL1A1	-2.6774	0.0000	Down
MT1X	-2.3965	0.0000	Down
S100A9	-2.2997	0.0007	Down
COL4A2	-2.1477	0.0000	Down
ITGA5	-2.1448	0.0000	Down

ment analysis showed that the AgDEGs were significantly enriched in the MAPK and PI3K-Akt signaling pathways. Both the MAPK and PI3K-Akt pathways are known to play pivotal roles in various physiological processes, including cell proliferation, survival, and metastasis, with their dysregulation being implicated in numerous cancers [25, 26]. It has been reported that MAPKs regulate pro-inflammatory factors and key components of the p21/p53 and p16/RB pathways, which are critical for implementing senescence-associated traits, such as growth arrest, cell survival, and the senescence-associated secretory phenotype (SASP) [27]. Furthermore, Sun et al. [28] demonstrated that chronic unpredictable stress-induced downregulation of IDH1 expression inhibits granulosa cell proliferation and accelerates senescence, which is associated with ROS-mediated activation of the MAPK signaling pathway. Li et al. [29] demonstrated that genkwanin can reduce inflammation in nucleus pulposus cells and inhibit cellular senescence and apoptosis by regulating the ITGA2/PI3K/AKT, NF- κ B, and MAPK signaling pathways, thereby alleviating intervertebral disc degeneration. Another study found that MG-132 acti-

vates autophagy by inhibiting the PI3K/AKT/mTOR pathway, counteracting proliferation, migration, and oxidative stress in vascular smooth muscle cells induced by palmitate, and suppressing cellular senescence [30]. Additionally, RAS-activated PI3K/AKT signaling sustains senescence in psoriatic keratinocytes by upregulating p53/p21 expression [31]. These studies underscore the critical role of the MAPK and PI3K-Akt signaling pathways in cellular senescence. In the present study, PQ may induce cellular senescence by modulating these pathways.

Through the construction of the PPI network, five hub AgDEGs - MMP2, TGFB1, CXCL8, JUN, and FOS - were identified. Molecular docking results indicated that these hub AgDEGs are capable of binding with PQ compounds. To validate the RNA-seq findings, WB was performed on these hub AgDEGs. The results showed that, compared to the HepG2 group, MMP2, TGFB1, CXCL8, JUN, and FOS were significantly downregulated in the HepG2+PQ group. Research has shown that MMP2, as part of the SASP, participates in the degradation of the extracellular matrix (ECM) and cell invasion. Additionally, blocking the cellular senescence signaling pathway results in decreased expression and activity of MMP2 [32]. TGFB1, a member of the TGF- β family, regulates multiple aspects of aging, which may involve processes such as cell proliferation, cell cycle regulation, and DNA damage repair [33]. Liu et al. [34] reported that shikonin induces senescence and exerts anti-tumor effects in colon cancer cells by reducing the levels of CDKN2A and CXCL8. JUN has been shown to slow the progression of prostate cancer by mediating the transcription of senescence- and inflammation-related genes [35]. A recent investigation established that GADD45B collaborates with FOS to facilitate cellular senescence in human bronchial epithelial cells independently of p38 phosphorylation [36]. Based on our findings, it is hypothesized that MMP2, TGFB1, CXCL8, JUN, and FOS may serve as potential targets for PQ-induced cellular senescence.

This study highlights the critical role of PQ in regulating cellular senescence and excessive proliferation in HepG2 cells. However, this study has some limitations. Further in vivo evaluation and clinical trials are needed to verify the reliability of our results. In addition, the medicated

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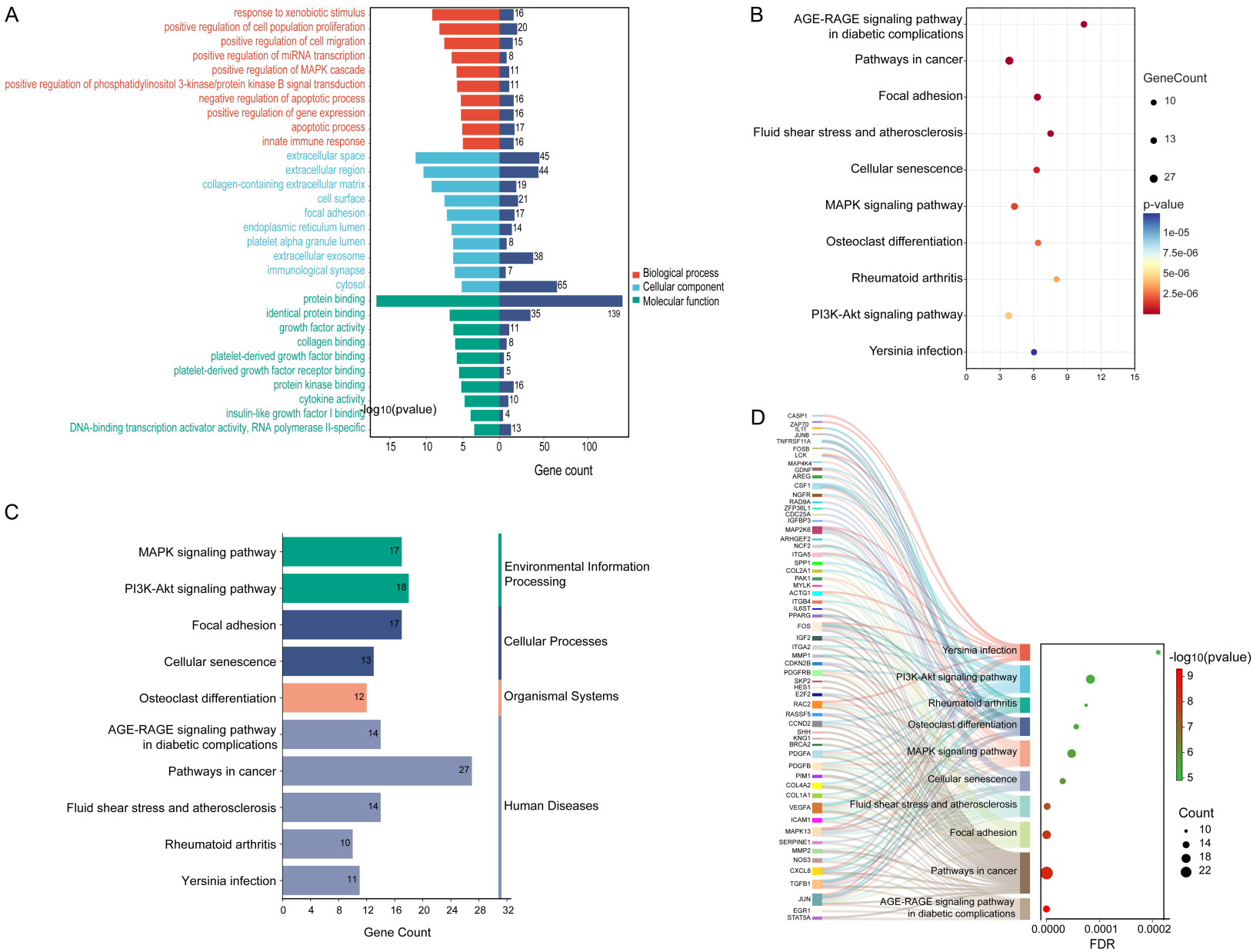


Figure 8. GO and KEGG analyses of AgDEGs. A. Top 10 enriched BP, CC, and MF terms of GO classification. B. Top 10 enriched KEGG pathways. C. Classification of KEGG pathways. D. Sankey diagram showing AgDEGs enriched in KEGG pathways.

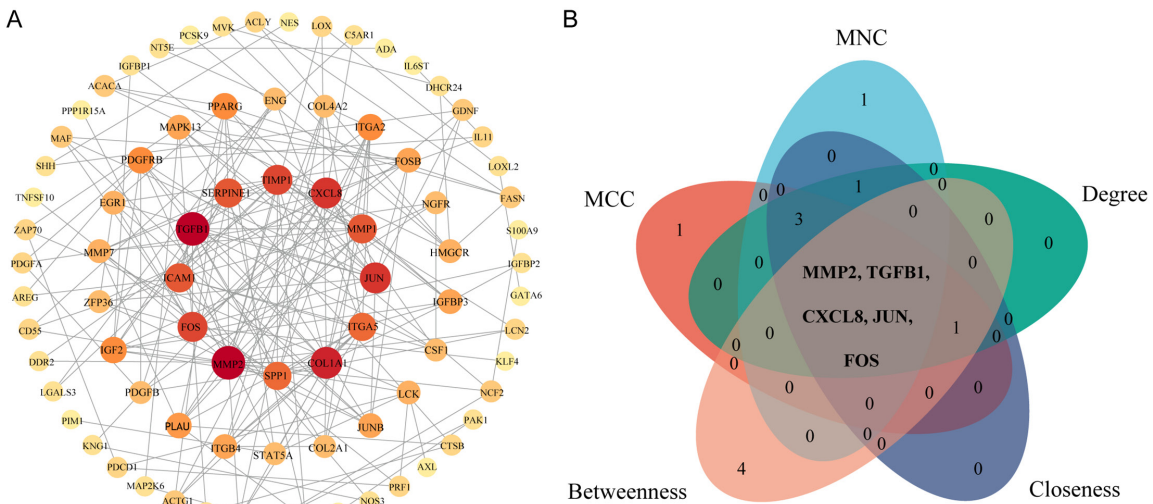


Figure 9. Identification on hub AgDEGs. A. PPI network of AgDEGs. The dots represent proteins, and the lines represent interactions between proteins. The dot size and color intensity are proportional to the score. B. Hub AgDEGs identified by five algorithms.

Table 4. Top 10 genes by five topological analysis methods

MCC	MNC	Degree	Closeness	Betweenness
MMP2	TGFB1	TGFB1	TGFB1	PPARG
TGFB1	MMP2	MMP2	MMP2	TGFB1
TIMP1	COL1A1	COL1A1	JUN	JUN
MMP1	JUN	JUN	CXCL8	MMP2
CXCL8	SERPINE1	CXCL8	TIMP1	FOS
SERPINE1	MMP1	TIMP1	MMP1	CXCL8
ICAM1	TIMP1	FOS	COL1A1	STAT5A
JUN	SPP1	SERPINE1	SERPINE1	ICAM1
FOS	CXCL8	MMP1	ICAM1	NCF2
JUNB	FOS	ICAM1	FOS	LCK

Abbreviations: MCC, Maximal Clique Centrality; MNC, Maximum Neighborhood Component; Degree, Degree Centrality; Closeness, Closeness Centrality; Betweenness, Betweenness Centrality.

Table 5. Basic information of hub AgDEGs

Gene	Log ₂ FC	P-value	Description
MMP2	-0.6851	0.0002	Matrix metalloproteinase 2
TGFB1	-1.0801	0.0000	Transforming growth factor beta 1
CXCL8	-0.6132	0.0000	C-X-C motif chemokine ligand 8
JUN	-0.6531	0.0000	Jun proto-oncogene AP-1 transcription factor subunit
FOS	-1.7664	0.0000	Fos proto-oncogene AP-1 transcription factor subunit

serum method has certain limitations, including complex serum components and potential interference from endogenous factors, which should be considered when interpreting the

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Table 6. Binding affinity between five hub AgDEGs and their bonded compound ligands

Gene	PDB	Compound	Affinity (kcal/mol)
MMP2	7XJO	1-Ethoxycarbonyl-beta-carboline	-8.8
		Picrasinoside C	-8.4
		Prostratin	-8.1
		Glaucogenin A	-8.0
		Hancogenin B	-7.8
		Nigakilactone N	-7.6
		Nigakihemiacetal B	-7.5
		Picrasin D	-7.4
		Nigakilactone F	-6.8
		Nigakihemiacetal D	-6.4
TGFB1	8UDZ	Picrasinoside C	-8.0
		1-Ethoxycarbonyl-beta-carboline	-7.4
		Picrasin D	-6.8
		Nigakihemiacetal B	-6.7
		Hancogenin B	-6.6
		Glaucogenin A	-6.6
		Nigakilactone N	-6.6
		Nigakihemiacetal D	-6.3
		Nigakilactone F	-6.2
		Prostratin	-6.0
CXCL8	6WZM	Glaucogenin A	-7.2
		Hancogenin B	-7
		Picrasinoside C	-6.6
		Picrasin D	-6.2
		Nigakilactone N	-6
		Nigakihemiacetal B	-5.9
		Nigakilactone F	-5.8
		Prostratin	-5.7
		1-Ethoxycarbonyl-beta-carboline	-5.6
		Nigakihemiacetal D	-5.3
JUN	8SOS	Hancogenin B	-8.2
		1-Ethoxycarbonyl-beta-carboline	-8.2
		Picrasinoside C	-8.1
		Picrasin D	-8.0
		Glaucogenin A	-7.9
		Nigakihemiacetal B	-7.7
		Nigakilactone N	-7.1
		Prostratin	-7.1
		Nigakilactone F	-7
		Nigakihemiacetal D	-6.7
FOS	1A02	Glaucogenin A	-6.0
		Picrasinoside C	-5.9
		Nigakihemiacetal B	-5.9
		Hancogenin B	-5.7
		Picrasin D	-5.7
		Nigakilactone N	-5.7
		Nigakilactone F	-5.3
		Nigakihemiacetal D	-5.0
		Prostratin	-5.0
		1-Ethoxycarbonyl-beta-carboline	-4.6

Abbreviation: PDB, Protein Data Bank.

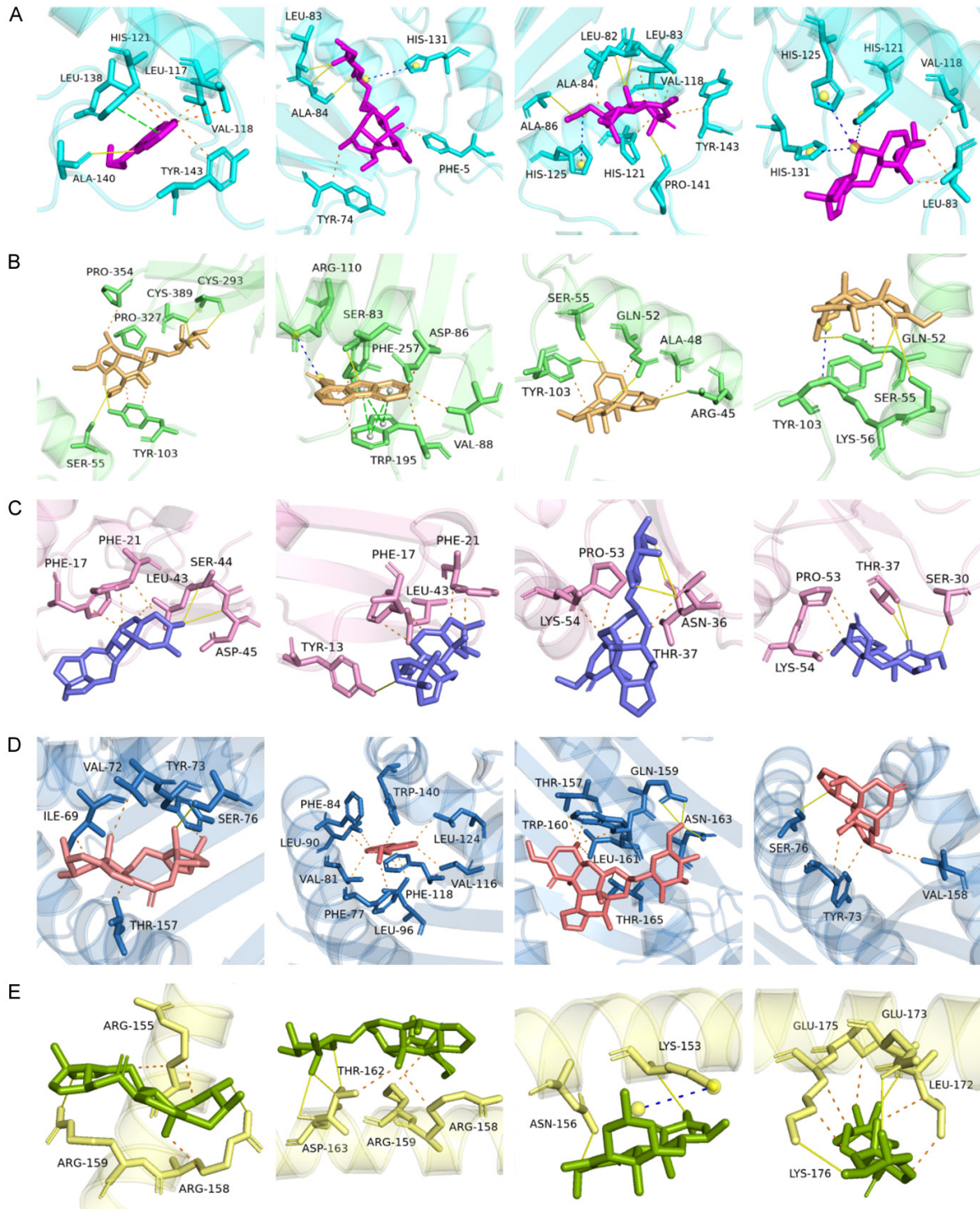


Figure 10. Molecular docking of five hub AgDEGs and PQ compounds. A. MMP2, with 1-Ethoxycarbonyl-beta-carboline, Picrasinoside C, Prostratin, Glaucogenin A; B. TGFβ1, with Picrasinoside C, 1-Ethoxycarbonyl-beta-carboline, Picrasin D, Nigakihemiacetal B; C. CXCL8, with Glaucogenin A, Hancogenin B, Picrasinoside C, Picrasin D; D. JUN, with Hancogenin B, 1-Ethoxycarbonyl-beta-carboline, Picrasinoside C, Picrasin D; E. FOS, with Glaucogenin A, Picrasinoside C, Nigakihemiacetal B, Hancogenin B. (Hydrogen bonds are denoted by yellow solid lines, hydrophobic interactions by orange dashed lines, π-stacking by green dashed lines, and salt bridges interactions by blue dashed lines).

data. Despite some limitations, the study offers several key insights. First, this is the first report

to demonstrate that PQ resists HCC by inducing cellular senescence, providing a new theoretic

Picrasma quassioides-induced senescence in HepG2

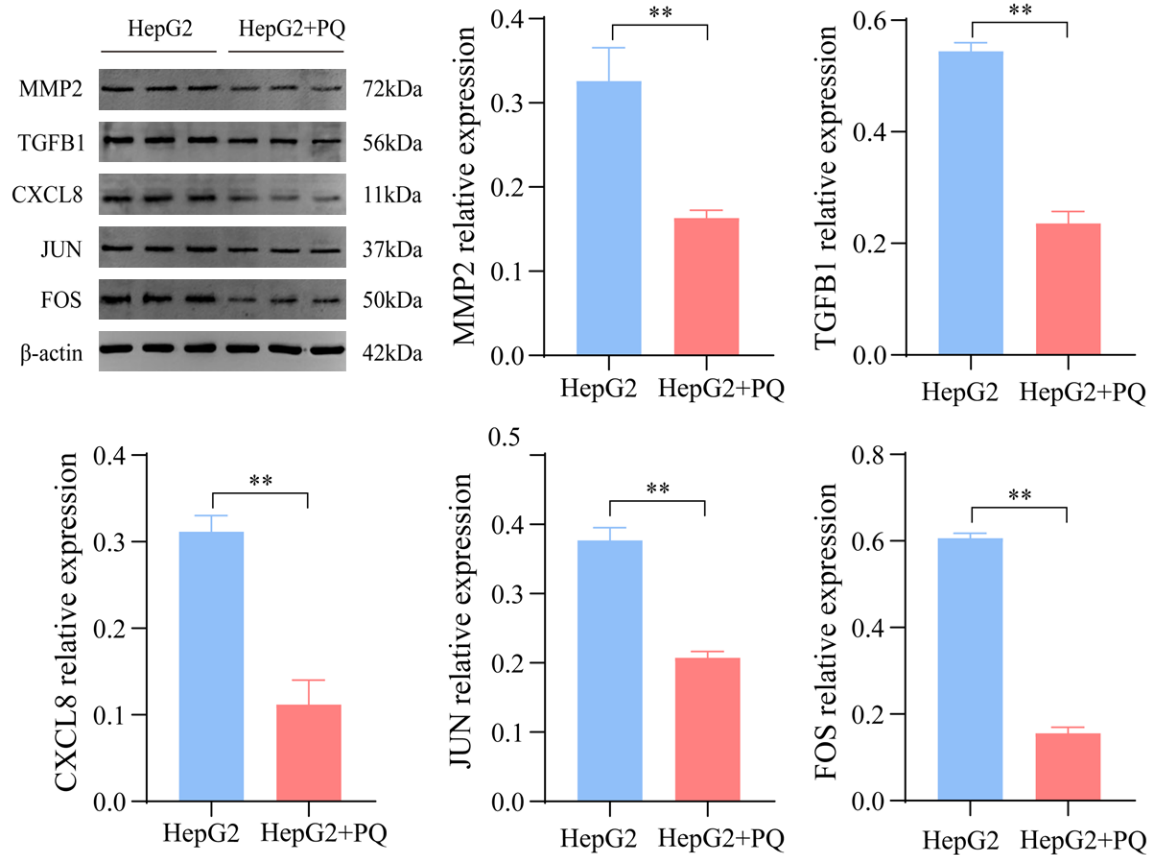


Figure 11. Validation of hub AgDEGs. Relative expression of MMP2, TGFB1, CXCL8, JUN, and FOS in the HepG2 and HepG2+PQ groups using WB. The results are presented as mean $\pm$ SD, $n = 3$. $**P < 0.01$.

cal basis for PQ in the treatment of HCC and offering a reference for future clinical development. Second, the use of RNA-seq analysis to explore the molecular mechanisms of PQ-induced cellular senescence in HepG2 represents a novel research approach. Lastly, targeting cellular senescence could provide a promising strategy for the treatment and intervention of HCC.

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Disclosure of conflict of interest

None.

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References

- [1] Zhou J, Sun H, Wang Z, Cong W, Zeng M, Zhou W, Bie P, Liu L, Wen T, Kuang M, Han G, Yan Z, Wang M, Liu R, Lu L, Ren Z, Zeng Z, Liang P, Liang C, Chen M, Yan F, Wang W, Hou J, Ji Y, Yun J, Bai X, Cai D, Chen W, Chen Y, Cheng W, Cheng S, Dai C, Guo W, Guo Y, Hua B, Huang X, Jia W, Li Q, Li T, Li X, Li Y, Li Y, Liang J, Ling C, Liu T, Liu X, Lu S, Lv G, Mao Y, Meng Z, Peng T, Ren W, Shi H, Shi G, Shi M, Song T, Tao K, Wang J, Wang K, Wang L, Wang W, Wang X, Wang Z, Xiang B, Xing B, Xu J, Yang J, Yang J, Yang Y, Yang Y, Ye S, Yin Z, Zeng Y, Zhang B, Zhang B, Zhang L, Zhang S, Zhang T, Zhang Y, Zhao M, Zhao Y, Zheng H, Zhou L, Zhu J, Zhu K, Liu R, Shi Y, Xiao Y, Zhang L, Yang C, Wu Z, Dai Z, Chen M, Cai J, Wang W, Cai X, Li Q, Shen F, Qin S, Teng G, Dong J and Fan J. Guidelines for the diagnosis and treatment of primary liver

- cancer (2022 Edition). Liver Cancer 2023; 12: 405-444.
- [2] Zhang Y, Wang J, Qu Y and Chen Y. 6-Shogaol suppresses the progression of liver cancer via the inactivation of Wnt/ β -catenin signaling by regulating TLR4. Am J Chin Med 2021; 49: 2033-2048.
- [3] Tian K, Ying Y, Huang J, Wu H, Wei C, Li L, Chen L and Wu L. The expression, immune infiltration, prognosis, and experimental validation of OSBPL family genes in liver cancer. BMC Cancer 2023; 23: 244.
- [4] Hsieh MC, Lo YS, Chuang YC, Lin CC, Ho HY, Hsieh MJ and Lin JT. Dehydrocrenatidine extracted from *Picrasma quassioides* induces the apoptosis of nasopharyngeal carcinoma cells through the JNK and ERK signaling pathways. Oncol Rep 2021; 46: 166.
- [5] Gong YX, Liu Y, Jin YH, Jin MH, Han YH, Li J, Shen GN, Xie DP, Ren CX, Yu LY, Lee DS, Kim JS, Jo YJ, Kwon J, Lee J, Park YH, Kwon T, Cui YD and Sun HN. *Picrasma quassioides* extract elevates the cervical cancer cell apoptosis through ROS-mitochondrial axis activated p38 MAPK signaling pathway. In Vivo 2020; 34: 1823-1833.
- [6] Shieu MK, Lin CC, Ho HY, Lo YS, Chuang YC and Hsieh MJ. Picrasidine I regulates apoptosis in melanoma cell lines by activating ERK and JNK pathways and suppressing AKT signaling. Environ Toxicol 2024; 39: 5309-5320.
- [7] Yang D, Guo Q, Liang Y, Zhao Y, Tian X, Ye Y, Tian J, Wu T and Lu N. Wogonin induces cellular senescence in breast cancer via suppressing TXNRD2 expression. Arch Toxicol 2020; 94: 3433-3447.
- [8] Kang D, Hwang HJ, Baek Y, Sung JY, Kim K, Park HJ, Ko YG, Kim YN and Lee JS. TRIM22 induces cellular senescence by targeting PHLPP2 in hepatocellular carcinoma. Cell Death Dis 2024; 15: 26.
- [9] Wada H, Otsuka R, Germeraad WTV, Murata T, Kondo T and Seino KI. Tumor cell-induced macrophage senescence plays a pivotal role in tumor initiation followed by stable growth in immunocompetent condition. J Immunother Cancer 2023; 11: e006677.
- [10] Love MI, Huber W and Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014; 15: 550.
- [11] Safran M, Dalah I, Alexander J, Rosen N, Iny Stein T, Shmoish M, Nativ N, Bahir I, Doniger T, Krug H, Sirota-Madi A, Olender T, Golan Y, Stelzer G, Harel A and Lancet D. GeneCards version 3: the human gene integrator. Database (Oxford) 2010; 2010: baq020.
- [12] Huang da W, Sherman BT and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.
- [13] Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R, Hu D, Peluso ME, Huang Q, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ and von Mering C. The STRING database in 2025: protein networks with directionality of regulation. Nucleic Acids Res 2025; 53: D730-D737.
- [14] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
- [15] Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J and Bolton EE. PubChem 2025 update. Nucleic Acids Res 2025; 53: D1516-D1525.
- [16] Vallat B, Rose Y, Piehl DW, Duarte JM, Bittrich S, Bi C, Segura J, Zalevsky A, Sekharan MR, Webb BM, Bhikadiya C, Voigt M, Henry J, Chao H, Pingale A, Raymond D, Sagendorf J, Peisach E, Feng Z, Guranovic V, Smith J, Rose AS, Young JY, Berman HM, Sali A and Burley SK. RCSB protein data bank: delivering integrative structures alongside experimental structures and computed structure models. Nucleic Acids Res 2026; 54: D489-D498.
- [17] Schake P, Bolz SN, Linnemann K and Schroeder M. PLIP 2025: introducing protein-protein interactions to the protein-ligand interaction profiler. Nucleic Acids Res 2025; 53: W463-W465.
- [18] Seeliger D and de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des 2010; 24: 417-422.
- [19] Kwon Y, Lee H, Park H, Lee B, Kwon TU, Kwon YJ and Chun YJ. YPEL3 expression induces cellular senescence via the Hippo signaling pathway in human breast cancer cells. Toxicol Res 2023; 39: 711-719.
- [20] Zhao D, Qian L, Zhuang D, Wang L, Cao Y, Zhou F, Zhang S, Liu Y, Liang Y, Zhang W, Kang W, Zhang M, Wang Y, Zhang F, Zhang W, Xiao J, Xu G, Lv Y, Zou X, Zhuge Y and Zhang B. Inhibition of ribosomal RNA processing 15 Homolog (RRP15), which is overexpressed in hepatocellular carcinoma, suppresses tumour growth via induction of senescence and apoptosis. Cancer Lett 2021; 519: 315-327.
- [21] Fan X, He Y, Wu G, Chen H, Cheng X, Zhan Y, An C, Chen T and Wang X. Sirt3 activates autophagy to prevent DOX-induced senescence by inactivating PI3K/AKT/mTOR pathway in A549 cells. Biochim Biophys Acta Mol Cell Res 2023; 1870: 119411.

- [22] Zhu H, Chan KT, Huang X, Cerra C, Blake S, Trigos AS, Anderson D, Creek DJ, De Souza DP, Wang X, Fu C, Jana M, Sanij E, Pearson RB and Kang J. Cystathionine- β -synthase is essential for AKT-induced senescence and suppresses the development of gastric cancers with PI3K/AKT activation. *ELife* 2022; 11: e71929.
- [23] Berner J, Miebach L, Kordt M, Seebauer C, Schmidt A, Lalk M, Vollmar B, Metelmann HR and Bekeschus S. Chronic oxidative stress adaptation in head and neck cancer cells generates slow-cyclers with decreased tumour growth in vivo. *Br J Cancer* 2023; 129: 869-883.
- [24] Zhu J and Chen L. LincRNA-p21 promotes cellular senescence by down-regulating theWnt/ β -catenin pathway in MPP+-treated SH-SY5Y cells. *Comb Chem High Throughput Screen* 2023; 26: 2476-2486.
- [25] Ci M, Zhao G, Li C, Liu R, Hu X, Pan J, Shen Y, Zhang G, Li Y, Zhang L, Liang P and Cui H. OTUD4 promotes the progression of glioblastoma by deubiquitinating CDK1 and activating MAPK signaling pathway. *Cell Death Dis* 2024; 15: 179.
- [26] Saisana M, Griffin SM and May FEB. Insulin and the insulin receptor collaborate to promote human gastric cancer. *Gastric Cancer* 2022; 25: 107-123.
- [27] Anerillas C, Abdelmohsen K and Gorospe M. Regulation of senescence traits by MAPKs. *GeroScience* 2020; 42: 397-408.
- [28] Sun J, Guo Y, Fan Y, Wang Q, Zhang Q and Lai D. Decreased expression of IDH1 by chronic unpredictable stress suppresses proliferation and accelerates senescence of granulosa cells through ROS activated MAPK signaling pathways. *Free Radic Biol Med* 2021; 169: 122-136.
- [29] Li M, Yu X, Chen X, Jiang Y, Zeng Y, Ren R, Nie M, Zhang Z, Bao Y and Kang H. Genkwanin alleviates intervertebral disc degeneration via regulating ITGA2/PI3K/AKT pathway and inhibiting apoptosis and senescence. *Int Immunopharmacol* 2024; 133: 112101.
- [30] Shu Z, Li X, Zhang W, Huyan Z, Cheng D, Xie S, Cheng H, Wang J and Du B. MG-132 activates sodium palmitate-induced autophagy in human vascular smooth muscle cells and inhibits senescence via the PI3K/AKT/mTOR axis. *Lipids Health Dis* 2024; 23: 282.
- [31] Mercurio L, Bailey J, Glick AB, Dellambra E, Scarponi C, Pallotta S, Albanesi C and Madonna S. RAS-activated PI3K/AKT signaling sustains cellular senescence via P53/P21 axis in experimental models of psoriasis. *J Dermatol Sci* 2024; 115: 21-32.
- [32] Gal H, Lysenko M, Stroganov S, Vadai E, Youssef SA, Tzadikvitch-Geffen K, Rotkopf R, Biron-Shental T, de Bruin A, Neeman M and Krizhanovsky V. Molecular pathways of senescence regulate placental structure and function. *EMBO J* 2020; 39: e105972.
- [33] Ren LL, Miao H, Wang YN, Liu F, Li P and Zhao YY. TGF- β as a master regulator of aging-associated tissue fibrosis. *Aging Dis* 2023; 14: 1633-1650.
- [34] Liu W, Zhao Y, Liu Q, Wu D, Li W, Fu Z, Yang L and Liang Y. Systematic bioinformatics analysis reveals the role of shikonin in blocking colon cancer progression by identifying senescence-induced genes. *Front Pharmacol* 2024; 15: 1360587.
- [35] Redmer T, Raigel M, Sternberg C, Ziegler R, Probst C, Lindner D, Aufinger A, Limberger T, Trachtova K, Kodajova P, Högl S, Schleder M, Stoiber S, Oberhuber M, Bolis M, Neubauer HA, Miranda S, Tomberger M, Harbusch NS, Garces De Los Fayos Alonso I, Sternberg F, Moriggl R, Theurillat JP, Tichy B, Bystry V, Persson JL, Mathas S, Aberger F, Strobl B, Pospisilova S, Merkel O, Egger G, Lagger S and Kenner L. JUN mediates the senescence associated secretory phenotype and immune cell recruitment to prevent prostate cancer progression. *Mol Cancer* 2024; 23: 114.
- [36] Zhan Y, Huang Q, Deng Z, Chen S, Yang R, Zhang J, Zhang Y, Peng M, Wu J, Gu Y, Zeng Z and Xie J. DNA hypomethylation-mediated up-regulation of GADD45B facilitates airway inflammation and epithelial cell senescence in COPD. *J Adv Res* 2025; 68: 201-214.