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ORIGINAL RESEARCH

Anticancer Effects of Wild Baicalin on Hepatocellular Carcinoma: Downregulation of AKR1B10 and PI3K/AKT Signaling Pathways

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Introduction: Hepatocellular carcinoma (HCC) is a common and deadly malignancy. Traditional Chinese medicine, such as the compound Astragalus (wild Baicalin), has shown promise in improving outcomes for HCC patients. This study aimed to investigate the effects of wild Baicalin on the human hepatoma cell line HepG2 and elucidate the underlying mechanisms, particularly the role of the AKR1B10 and PI3K/AKT signaling pathways.

Methods: HepG2 cells were treated with varying concentrations of wild Baicalin. Cell proliferation, apoptosis, migration, invasion, and cell cycle were evaluated using CCK-8, flow cytometry, scratch, Transwell, and clonogenic assays, respectively. Transcriptome sequencing was performed to analyze gene expression changes induced by wild Baicalin. Differentially expressed genes were identified and analyzed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The expression of AKR1B10 and PI3K was validated by qPCR.

Results: Wild Baicalin inhibited HepG2 cell proliferation, induced apoptosis, suppressed migration and invasion, and caused cell cycle arrest in a dose-dependent manner. Transcriptome sequencing revealed 1202 differentially expressed genes, including 486 upregulated and 716 downregulated genes. GO analysis indicated that biological processes were pivotal in the anticancer mechanism of wild Baicalin, while KEGG analysis identified metabolic pathways as the most significantly regulated. AKR1B10 and PI3K, key genes in metabolic pathways, were downregulated by wild Baicalin, which was confirmed by qPCR.

Discussion: The findings suggest that wild Baicalin exhibits potent anticancer effects against HepG2 cells by inducing apoptosis, inhibiting proliferation, migration, and invasion, and causing cell cycle arrest. The regulatory effects of wild Baicalin on the AKR1B10 and PI3K/AKT signaling pathways appear to be critical for its inhibitory effects on HCC cell proliferation. These results provide new insights into the mechanism of action of wild Baicalin and support its potential as a therapeutic approach for HCC treatment.

Keywords: hepatocellular carcinoma, wild baicalin, transcriptome sequencing, apoptosis

Introduction

Hepatocellular carcinoma (HCC) ranks 6th among common malignant tumors worldwide[1] and is the second leading cause of cancer death in China.[2] At present, there are problems in the treatment of liver cancer in China, such as high recurrence rate and low survival rate of patients after treatment.[3] Therefore, finding more effective drugs and some liver cancer-specific tumor markers are of great significance for guiding clinical treatment, predicting prognosis, and improving survival rate. Traditional Chinese medicine plays an important role in improving patients' clinical symptoms, improving their quality of life and immune function, and improving prognosis. Yanlong Shie et al, 2022 explored the role of glycosylation-related genes in hepatocellular carcinoma (HCC) and developed a five-gene signature for prognosis.[4] They identified these genes as crucial for HCC patients' survival, with potential implications for

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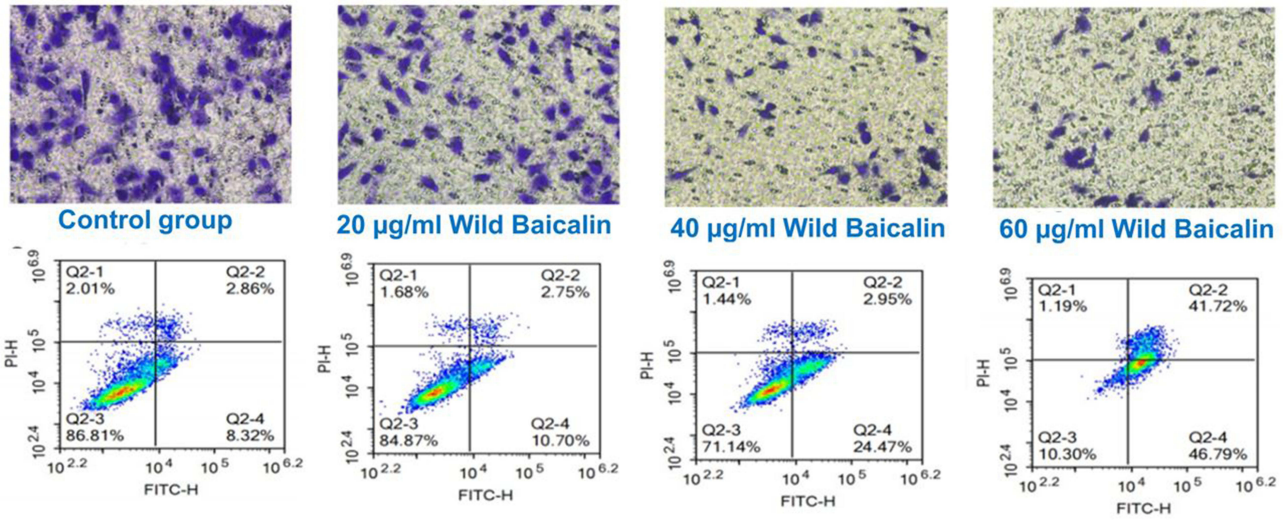
Graphical Abstract



immunotherapy and metabolic processes. Their findings offered valuable insights for therapeutic target prediction and clinical decision-making in HCC treatment.

The chemical substance wild Baicalin extracted from the stems and leaves of Scutellaria baicalensis Georgi and Scutellaria barbata has anti-tumor, anti-inflammatory, antioxidant, and other pharmacological effects, attracting more and more researchers' attention. The generation of tumors is a process in which normal cells, under the stimulation of multiple factors, produce tumorigenic mutations and accumulate, ultimately leading to cancerous transformation. During the process of cell carcinogenesis, the normal cellular signals of cancerous cells become disrupted, and various signals that promote proliferation and invasion, inhibit cell apoptosis, etc. continue to activate, gradually possessing the ability to reproduce indefinitely and ultimately forming a visible tumor to the naked eye.[5] Aldehyde ketone reductase family 1 member B10 (AKR1B10) is a member of the aldehyde ketone (AKR) reductase superfamily. AKR is an oxidoreductase gene superfamily with nicotinamide adenine dinucleotide phosphate (NADPH) as coenzyme, which can catalyze aldehyde and ketone substances into corresponding alcohols, and is a member of aldehyde and ketone reductase family.[6] Studies have shown that AKR1B10 can serve as a biomarker for the diagnosis of liver cancer.[7] Researchers have found that the expression level of AKR1B10 in cancer tissues of hepatocellular carcinoma patients is significantly higher than that in adjacent tissues.[8] Phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway is activated in a variety of normal or abnormal pathophysiological processes, and participates in the regulation of cell proliferation, oxidative stress, inflammatory immunity, and other cell processes.[9,10] Numerous studies have shown that abnormal activation of the PI3K/AKT pathway is involved in the development of tumors.[11–13]

AKR1B10 can activate the PI3K-AKT signaling pathway and promote tumor proliferation and migration, which has been confirmed by many studies.[14] Moreover, the PI3K-AKT signaling pathway has been reported to be closely related to metabolic reprogramming in tumors. It has been reported that AKR1B10 can regulate the expression of total fatty acids, stabilize lipases, indirectly promote the formation of fat, promote cell cloning, and increase the expression of phosphatidylinositol diphosphate (PIP2), the second messenger of lipid, to promote cell proliferation. Overexpression of AKR1B10 induces increased expression of PIP2, diacylglycerol (DAG), and Inositol trisphosphate (IP3).[15] Under the action of PI3K, PIP2 generates lipid second messenger PIP3, which serves as the second messenger to activate AKT. In our gene ontology (GO) analysis of AKR1B10, we found that AKR1B10 had statistical significance in "positive regulation of protein kinase B signal transduction", suggesting that the role of AKR1B10 in tumors might be related to AKT signal pathway.

High expression of AKR1B10 in breast cancer can promote the proliferation and metastasis of breast cancer. AKR1B10 can promote breast cancer metastasis through FAK/Src/RAC1 signaling pathway,[16] up regulate the expression of DAG, and activate protein kinase C (PKC) mediated Raf/MEK/ERK signaling pathway to promote the proliferation of breast cancer. However, the relationship between the expression of AKR1B10 and the PI3K/KAT signaling pathway has not been reported in HCC yet. The second-generation RNA sequencing technology (RNA seq) is a new technology that has emerged in recent years. It can simultaneously analyze the expression of thousands of gene transcription levels in the same or different specimens. It is a high-throughput, fast, effective, high-resolution, and highly sensitive gene expression analysis method that plays an important role in studying the physiological activity patterns and metabolic mechanisms of cells.[17–19] This study used transcriptome sequencing technology to analyze the expression of HepG2 gene in hepatoma cell line under the effect of wild Baicalin, aiming to reveal the changes of wild Baicalin on the molecular level of HepG2 cells, and provide new clues to clarify the mechanism of wild Baicalin inhibiting cell proliferation and promoting apoptosis of HepG2 cells.

Although wild Baicalin is a widely studied compound with multi potent and multi active, its mechanism of effect on cancer is still unclear. We studied how wild Baicalin-induced apoptosis of hepatoma cell line HepG2 through AKR1B10 and PI3K/KAT signaling pathways. This study attempts to reveal the mechanism of wild Baicalin inducing apoptosis of liver cancer cells and clarify the potential therapeutic strategies for HCC treatment.

Materials and Methods

Materials

All cell lines were approved by the institutional research ethics committee of "Cancer Hospital of Shaanxi Province" and were authenticated by STR profile. Human hepatoma cell line HepG2 was presented by the Biomedical Experimental Center of Xi'an Jiaotong University. The number of cells was 1×10^6. Experimental drugs: wild Baicalin (scutellarin, SCU) was purchased from Baoji Chenguang Biotechnology Co., Ltd, purity ≥ 98%, reaching analytical purity level. Reagents: DMEM culture medium was purchased from Gibco Company, batch #14533; Cell RNA rapid extraction kit, batch #CR119, reverse transcription kit, batch #RT205, qPCR kit, batch #QPK109, double antibody (penicillin Streptomycin) batch #PS037 and trypsin cell digestion solution, batch #TS278, containing EDTA without phenol red, were purchased from Biotechnology Co., Ltd; Fetal bovine serum was purchased from BI Company, batch #FB927; anhydrous ethanol was purchased from Biotechnology Co., Ltd, batch #ETH415.

Reagents

The CCK-8 cell proliferation-toxicity test kit was purchased from Wuhan Yilairuite Biotechnology Co., Ltd; The Annexin V-FITC/PI double staining kit and cell cycle kit were purchased from Jiangsu Kaiji Biotechnology Co., Ltd; DMEM culture medium purchased from Gibco Company; PBS buffer, cell RNA rapid extraction kit, reverse transcription kit, qPCR kit, double antibody (Penicillin-Streptomycin), Crystal violet dye, and trypsin cell digestion solution, with EDTA without phenol red, purchased from Biotechnology Co., Ltd; Fetal bovine serum was purchased from BI Company, and anhydrous ethanol was purchased from Biotechnology Co., Ltd. CO_2 incubator (Thermo Company); Multifunctional enzyme-linked immunosorbent assay (BMG LABTECH company); TS2 inverted microscope (Nikon Company); NovoCell Flow Cytometer (Agilent Company).

Cell Culture

Human hepatoma cell line HepG2 was routinely cultured in a 37°C, 5% CO_2 cell incubator with DMEM medium containing 10% fetal bovine serum and 1% Penicillin-Streptomycin, and the medium was changed every 2 days. When the cells grow to 70% −80%, they were digested and passaged with 0.25% trypsin. Cells in the logarithmic growth phase were used for experiments.

CCK-8 Assay

Cells were incubated with wild Baicalin (0, 20, 40, 60 μg/mL) for 24, 48, and 72 h in 96-wells plate. 100 μL medium and 10 μL CCK-8 (5.0 mg/mL) were added and cultured in a humidity incubator (37°C, 5% CO_2) for 2 h, and then the OD

values of each well at a wavelength of 450 nm were measured by an enzyme-linked immunosorbent assay (ELISA). The IC50 value and cell survival rate were calculated.

Apoptosis Assay

HepG2 cells were seeded into a 6-wells plate. After 24 h treatment with different concentrations of wild Baicalin, cells were digested with trypsin (without EDTA), followed by the termination with precooled PBS and centrifuged. The cells were rinsed and re-centrifuged. Prepared cell suspension using V-FITC binding solution, added Annexin V-FITC and incubated at room temperature in the dark for 10 min, and then incubated with PI at room temperature in the dark for another 5 min. Cell apoptosis rate was detected by flow cytometry.

Cell Cycle Assay

HepG2 cells were seeded into a 6-wells plate. After 8 h treatment with wild Baicalin, cells were centrifuged at 1000 g for 5 min and rinsed with precooled PBS for 5 min. The cell precipitation was obtained after centrifuging at 1000 g for 5 min, followed by fixation with precooled 70% anhydrous ethanol. Suspended the cell precipitation with PI solution and incubated at 37°C in the dark for 30 min. Flow cytometry was used to conduct the analysis.

Scratch Assay

HepG2 cells were seeded into a 6-wells plate and cultured for 24 h, with approximately 5×10^5 cells per well. Conduct experiments when cells grew to 90% confluence. Rinsed the cells with serum-free culture medium and then used sterile 100 μL pipette tip to make a scratch at the the central area of the bottom of the 6-wells plate. PBS was used to rinse the cells twice to remove floating cells and debris, and then each well was added with serum-free culture medium containing different concentrations of wild Baicalin (0, 20, 40, 60 ug/mL) for 24 h. The initial scratch width was recorded by taking pictures under the microscope. After 24 h of scratching, took photos and recorded the healing width of the scratch by an inverted microscope. Calculate the percentage of cell scratch healing through image J software analysis (%).

Transwell Invasion Assay

Placed the Transwell chamber into a 24-wells plate, diluted the matrix gel with precooled serum-free DMEM medium in a dilution ratio of 1:8, 50 μL mixture was used to spread on the upper chamber of the Transwell evenly. Placed it in a 37°C incubator overnight for solidification. Removed the solidified matrix from the 37°C incubator and added 200 μL serum-free DMEM to each compartment to hydrate chamber for 4 h. After starving the cells for 12–24 h, digested the cells with trypsin, and the cells were resuspended in serum-free medium and counted. 200 μL serum-free culture medium containing wild Baicalin of different concentrations (0, 20, 40, 60 μg/mL) was added in the upper chamber, with each concentration approximately 6×10^4 cells. 600 μL complete culture medium containing 10% FBS was added to the lower chamber. Discarded the residual culture solution after 24 h of culture in the incubator, wiped the cells that did not pass through the upper layer and rinsed them with sterile PBS solution for twice. After air dry, fixed the cells with 4% paraformaldehyde for 30 min and rinsed them with PBS solution for 3 times. Added Crystal violet solution for staining, rinsed it with clean water and air dry. Under a microscope, randomly selected 5 visual fields to record the images, and calculated cells number.

Plate Cloning Assay

Added an appropriate amount of equal volume complete culture medium to each well of the 6-wells plate in advance. HepG2 liver cancer cell line with logarithmic growth phase was used to digest, centrifuge, and count. Adjusted cell density to 1×10^4 cells/mL, added 100 μL of cell suspension to each well where the culture medium was added in advance, so that the cell density was 1000 cells/well. Paid attention to evenly seeding, so that the cells were evenly laid at the bottom of the 6-wells plate and cultured in the incubator. After the cells adhesion, discarded the previous culture medium, and added complete DMEM culture medium with different concentrations of wild Baicalin (0, 20, 40 μg/mL). Marked the 6-wells plate to record the time, and then incubated it in a cell incubator for 14 days. Took out the 6-wells plate on the 15th day and discarded the old culture medium, rinsed the

cells twice with PBS and added 2 mL 4% paraformaldehyde to each well for 15 min. Discarded paraformaldehyde and added 2 mL Crystal violet solution with concentration of 1% to each well and incubated for 30 min. After staining, discarded Crystal violet and rinsed the 6-wells plate with PBS until there was no more color left in the background, then stayed overnight and dried it. Took photos of each well and counted the number with Image J.

Transcriptome Sequencing

HepG2 cells in logarithmic growth phase were treated with 60 μg/mL wild Baicalin, while the same volume DMSO treated HepG2 cells were used as control group, and total RNA was extracted 48 h later. The foundation for successful gene sequencing experiments is to obtain high-quality RNA. To ensure the success of the experiment, the following methods were used for sample detection: (1) Agarose gel electrophoresis. (2) Qubit 2.0 fluorophotometer. (3) Agilent 2100 biological analyzer. The quality inspection methods for the library are as follows: (1) Qubit 2.0 was used for preliminary quantification. Agilent 2100 was used to detect the insert size of the library. The following experiment could only proceed after the insert size met expectations. (2) qPCR method was used to accurately quantify the effective concentration of the library (the effective concentration of the library >2nM). After the library inspection was qualified, the Illumina platform was used for sequencing (Transcriptome sequencing WAS completed by Maiwei Biology).

2.11. qPCR Assay

qPCR assay was conducted by following the instruction of the kit manual. The primer sequences were as follows: Dll4, upstream primer: 5′ -GTGGGTCAGAACTGGTTATTGGA - 3′, downstream primer: 5′ -CTGGCTGGCACACATAGTGG - 3′; Notch- 1, upstream primer: 5′ - TTTGTGCTTCTGTTCTTCGTGG - 3′, downstream primer: 5′ -GAACTTCTTGGTCTCCAGGTCC- 3′; internal reference: β- actin, upstream primer: 5′- GTCCACCGCAAATGCTTCTA- 3′, downstream primer: 5′-TGCTGTC ACCTTCACCGTTC- 3′. Three replicate wells were made for each target gene and internal reference. The relative expression was calculated by 2-Δ (ΔCt), and the average of 3 times was taken.

Statistical Analysis

All data were analyzed by SPSS 26.0, and the data results were expressed as mean ± standard deviation. The comparison between groups was performed by *T*-test, and the comparison of data between two or more groups was performed by one-way analysis of variance, and $p < 0.05$ was considered to be significantly different.

Results

CCK-8 Assay Result

In the experiment, different concentrations (0, 20, 40, 60 μg/mL) of SCU were used to treat HepG2 cells. After adding CCK-8 reagent every day, the results were detected at the same time. As shown in Figure 1, with the increase of SCU concentration and the incubation time, its inhibitory effect on HepG2 cells became more and more obvious. The half inhibitory concentration (IC50) of SCU at 24 h was 57.86μg/mL, and there was a significant difference between the SCU group and the control group ($p < 0.05$).

Apoptosis Assay Result

Concentrations of 0, 20, 40, and 60 μg/mL of SCU were applied to affect HepG2 cells. Through Annexin V-FITC and PI double staining along with flow cytometry detection, the results indicated the following: as the concentration of SCU increased within the SCU group, the apoptosis rate also exhibited a gradual rise. A significant difference was observed between the SCU group and the control group ($p < 0.05$), suggesting a dose-dependent effect of SCU on HepG2 cell apoptosis, as depicted in Figure 2.

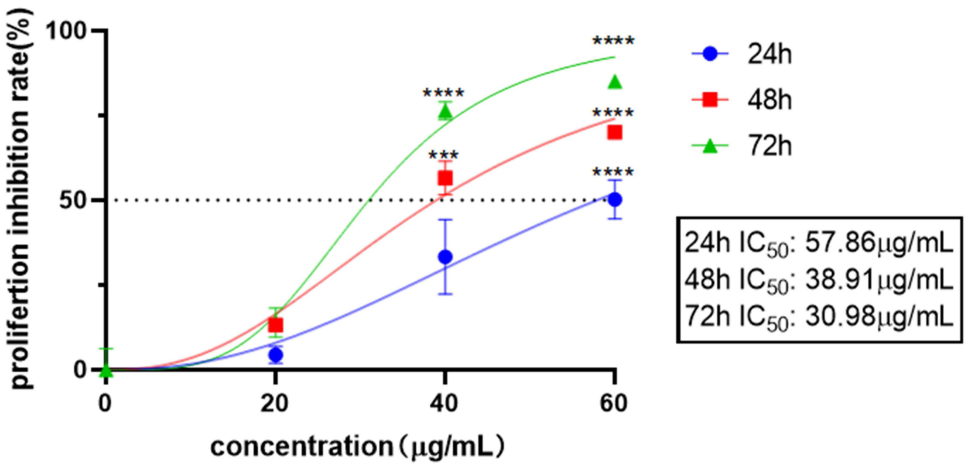


Figure 1 The effect of SCU on the proliferation ability of HepG2 cells.
Notes: ***p < 0.001, ****p < 0.0001.

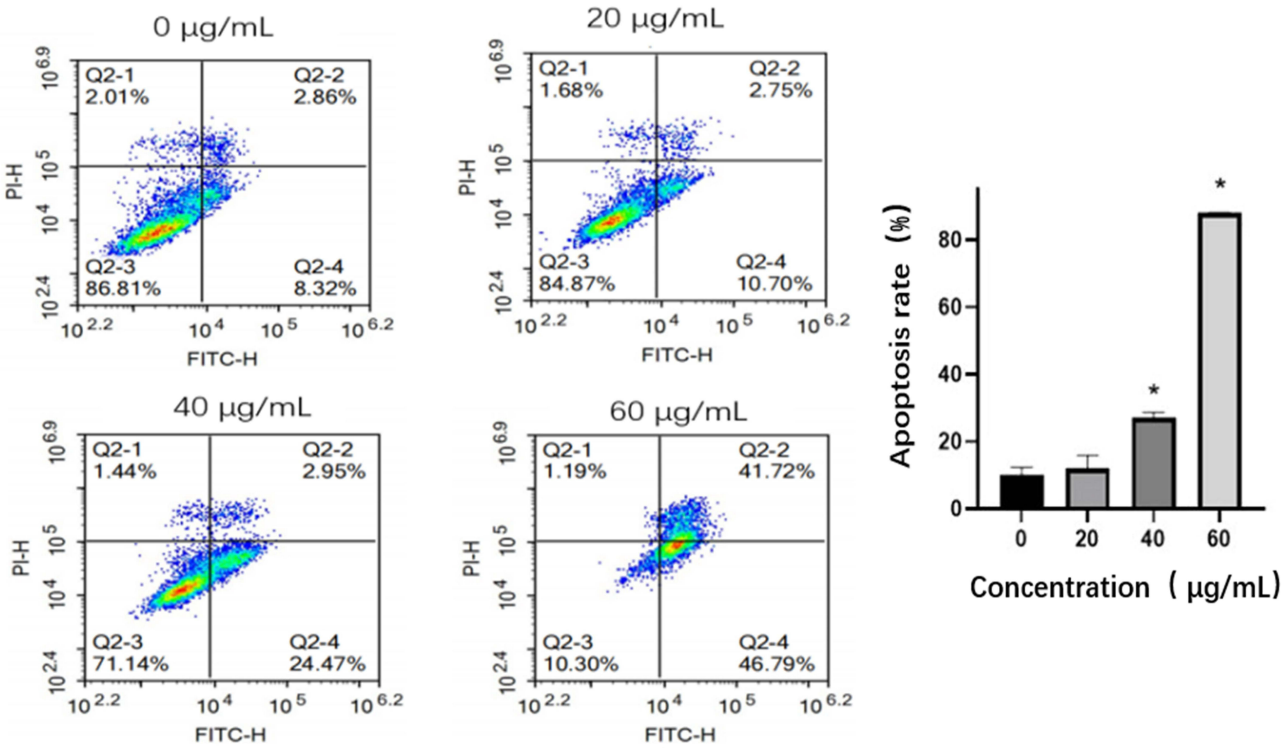


Figure 2 The effect of SCU on the apoptosis ability of HepG2 cells.
Note: *p < 0.05.

Cell Cycle Assay Result

The results of flow cytometry cell cycle test showed that with the increase of drug concentration in the experimental group (0, 20, 40, 60 μg/mL), the number of cells in G0/G1 phase increased significantly, and the number of cells in S phase and G2/M phase decreased gradually, as shown in Figure 3. Through the above experiments, the results showed that SCU had a significant effect on inhibiting the progress of the cell cycle, suggesting that the cells were arrested in the G1 phase. There was a significant difference between the SCU group and the control group ($p < 0.05$).

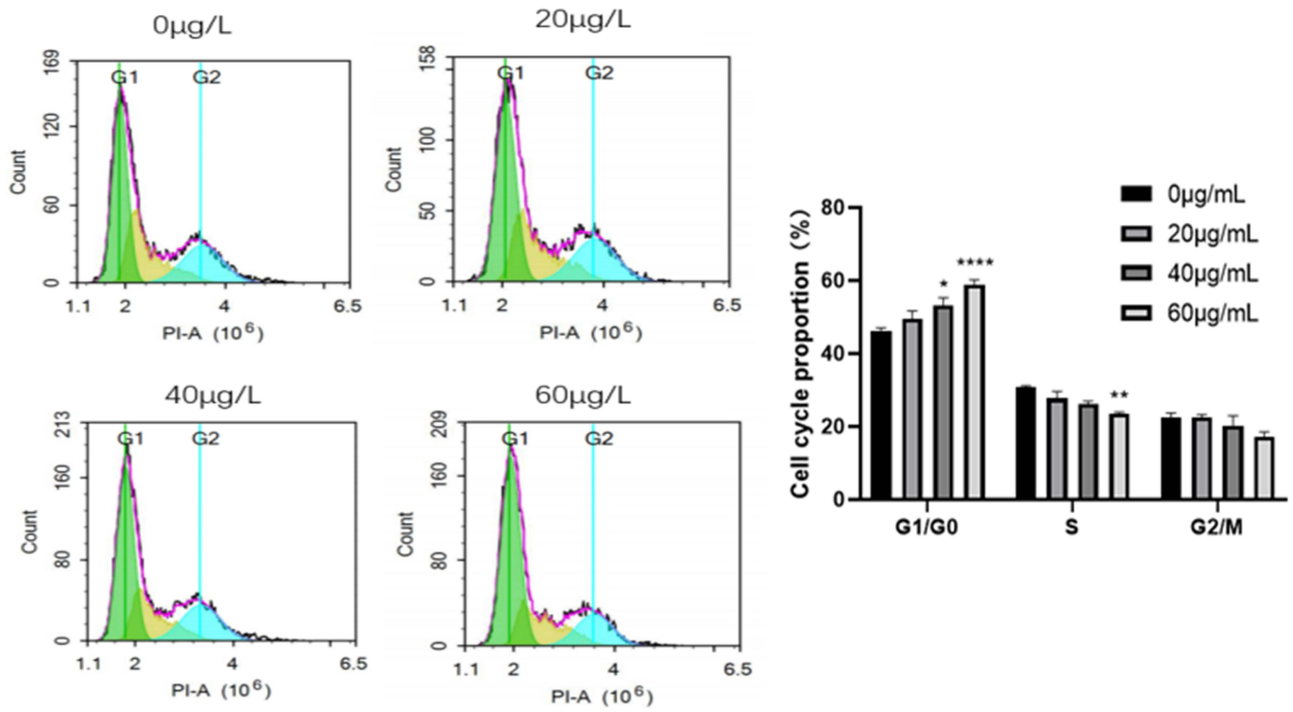


Figure 3 The effect of SCU on the growth cycle of HepG2 cells. *, **, ****Represents $p < 0.05$, $p < 0.01$, $p < 0.0001$, respectively.

Scratch Assay Result

In this experiment, serum-free medium with drug concentrations of 0, 20, 40, and 60 μg/mL was used to act on HepG2 cells, and the migration was observed at 24 h. The use of serum-free medium was to exclude the influence of cell proliferation. The experimental results showed that with the increase of the drug dose, the scratch healing rate gradually decreased, and the difference between the SCU group and the control group was significant ($p < 0.05$), as shown in Figure 4, which confirmed that SCU could significantly inhibit the migration ability of the liver cancer cell line HepG2, which was negatively correlated with the concentration of SCU. The migration ability decreased with the increase of drug concentration.

Transwell Invasion Assay Results

After seeding 7×10^4 HepG2 cells in the upper chamber, the drug solution acted on the HepG2 cells for 24 h (the upper chamber contained the SCU drug prepared in serum-free medium with different concentrations, and the lower chamber contained the culture medium containing 10% FBS). Figure 5 showed that, compared with the control group, the number of cells passing through the small chamber in the experimental group was significantly reduced, and there was a significant difference between the SCU group and the control group ($p < 0.05$).

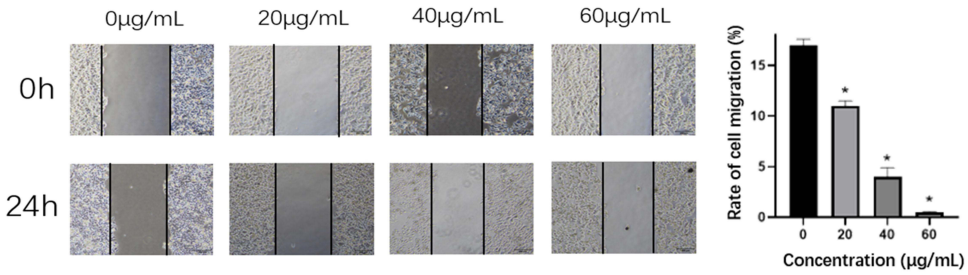


Figure 4 The effect of SCU on the migration ability of HepG2 cells.
Note: *$p < 0.05$.

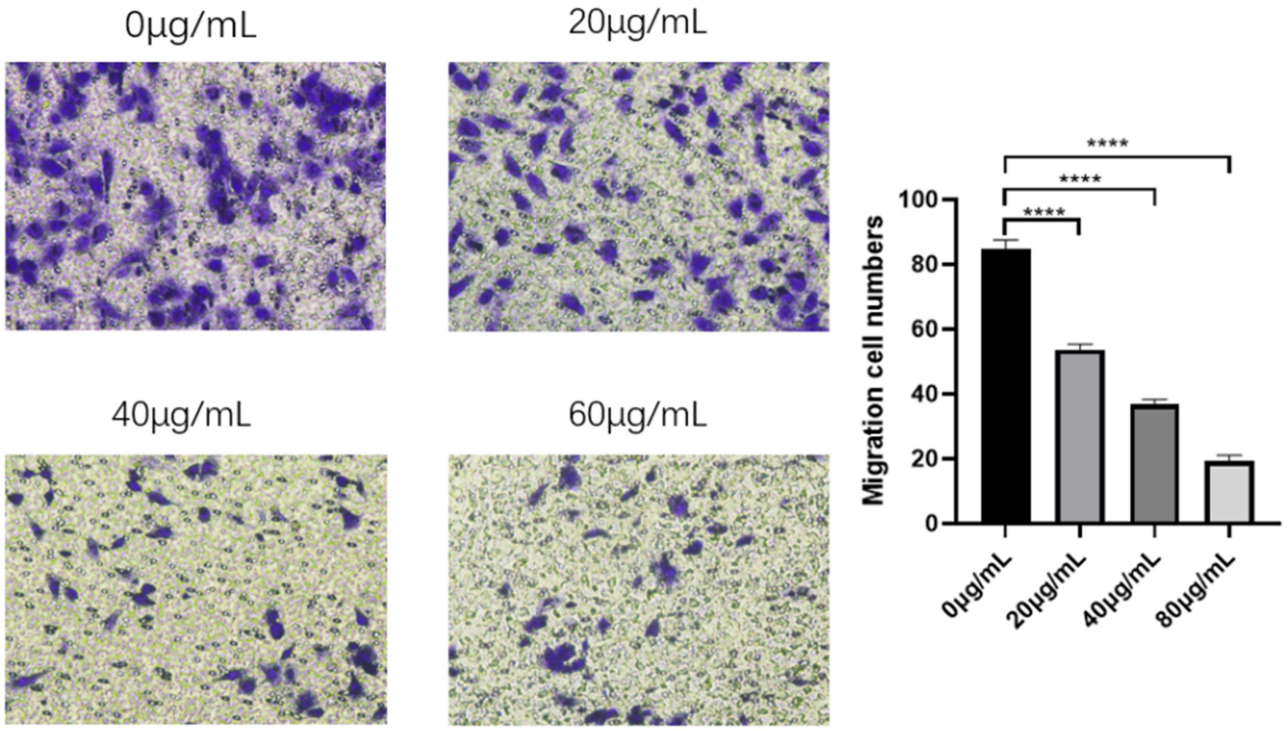


Figure 5 The effect of SCU on the invasion ability of HepG2 cells. ****Represents $p < 0.0001$, respectively.

Plate Cloning Assay Results

The results of the plate cloning experiment showed that when HepG2 cells adhered to the wall, the cells were cultured in HepG2 medium containing 0, 20, and 40 μg/mL SCU for 14 days. Compared with the control group, the cell clone formation rate of the drug group was inversely proportional to the concentration of the SCU. The proliferation and division of cells were significantly inhibited. There was a significant difference between the SCU group and the control group ($p < 0.05$), as shown in Figure 6.

Transcriptome Sequencing Results

Transcriptome sequencing and differentially expressed gene analysis obtained 48,941,306 and 48,641,252 total reads in the control group and treatment group, respectively. The proportions of the filtered clean reads numbers were 97.08% and 98.4%, respectively. A total of 1202 genes with statistically differential expression were detected, including 486 up-regulated genes and 716 down-regulated genes. The cluster diagram and volcano diagram were shown in Figure 7.

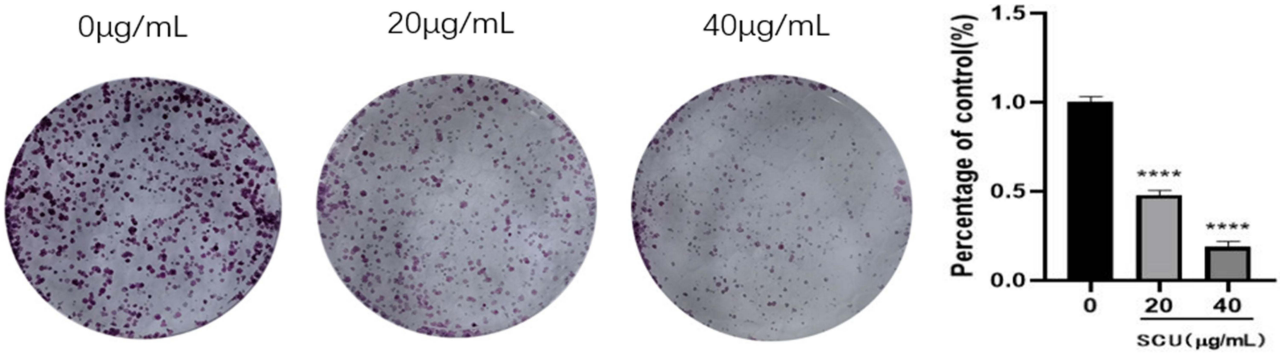


Figure 6 The effect of SCU on the proliferation ability of HepG2 cells. ****Represents $p < 0.0001$, respectively.

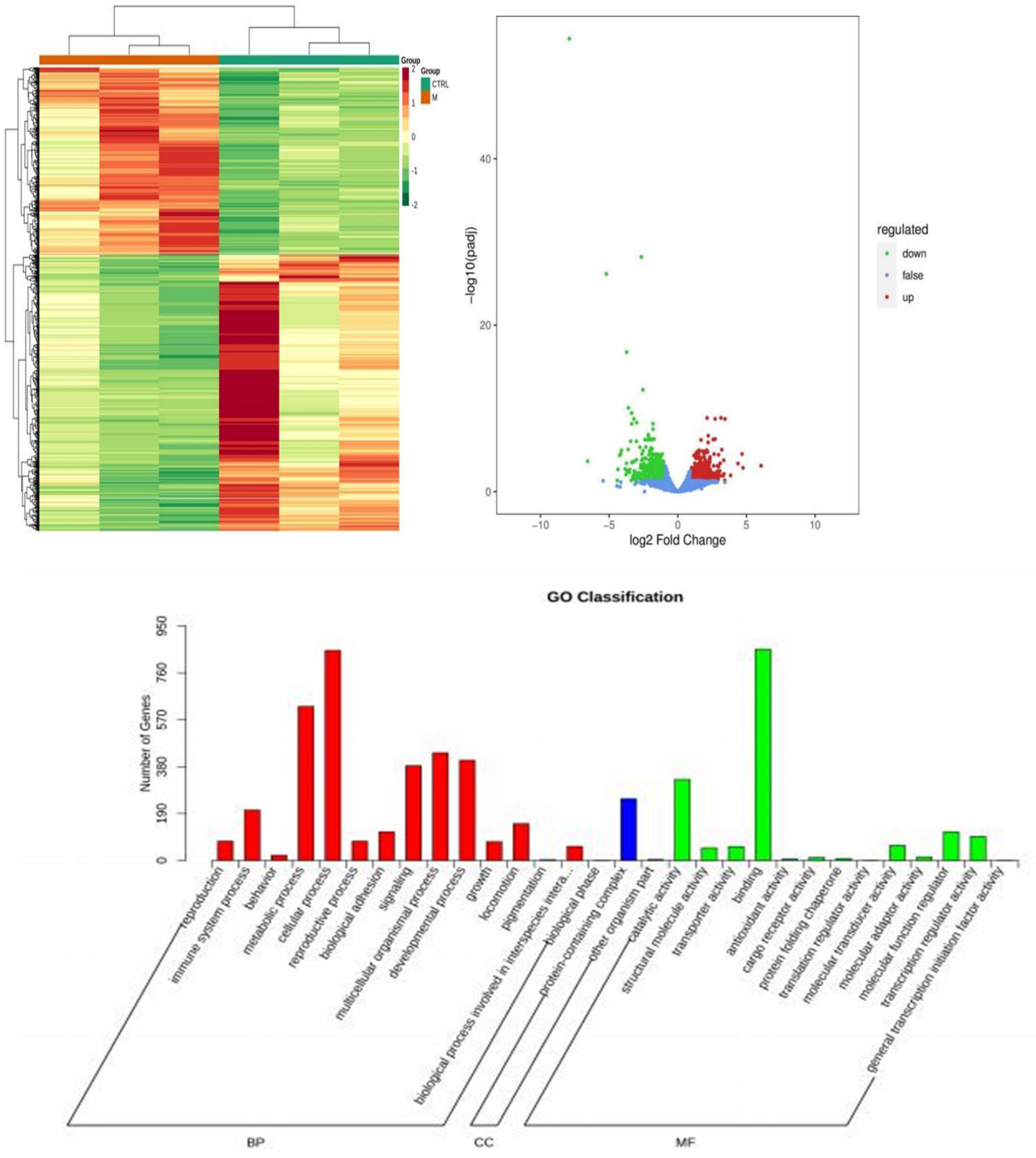


Figure 7 Cluster diagram and volcano diagram.

Gene Ontology Function Prediction of Differentially Expressed Genes

Combined with the Gene Ontology database, the GO functional classification analysis was performed on the genes whose expression levels were differentially changed due to the effect of drug in Hep G2 cells. The results showed that these differential genes were classified into 30 secondary categories under the three major categories of GO (biological process, cellular component and molecular function) in the function prediction. Among the three categories, the biological process category had the largest total annotations,[14] and most genes were mainly enriched in cellular

processes, molecular processes, and multicellular biological processes; followed by the molecular function category,[13] most of which genes were mainly related to binding, structure, molecular activity, and molecular function regulators, while the category of cellular components account for a small amount of total annotations (3 in total), the most obvious ones were protein complexes, as shown in the Figure 8.

Significant Enrichment Analysis of Differential Gene KEGG Pathway

Physiological and biochemical pathways involved in differentially expressed genes could be known through KEGG Pathway significance enrichment analysis. The results showed that the enrichment analysis of differentially expressed genes involved 6 pathways, namely: metabolic pathway, genetic information process pathway, environmental information process pathway, cellular process, transport and alienation pathways, environmental adaptation, endocrine and metabolic diseases. Among them, the differentially expressed genes involved in metabolic pathways were the most, a total of 102 genes, which were the main pathways that mainly affected the liver cancer cells HepG2, as shown in Figure 9.

PCR Results

Wild Baicalin could reduce the expression of PI3K and AKR1B10 in HepG2 cells. Human liver cancer cell line HepG2 cells were treated with different concentrations of wild Baicalin (0 μg/mL, 60 μg/mL) for 48 h. The results showed that

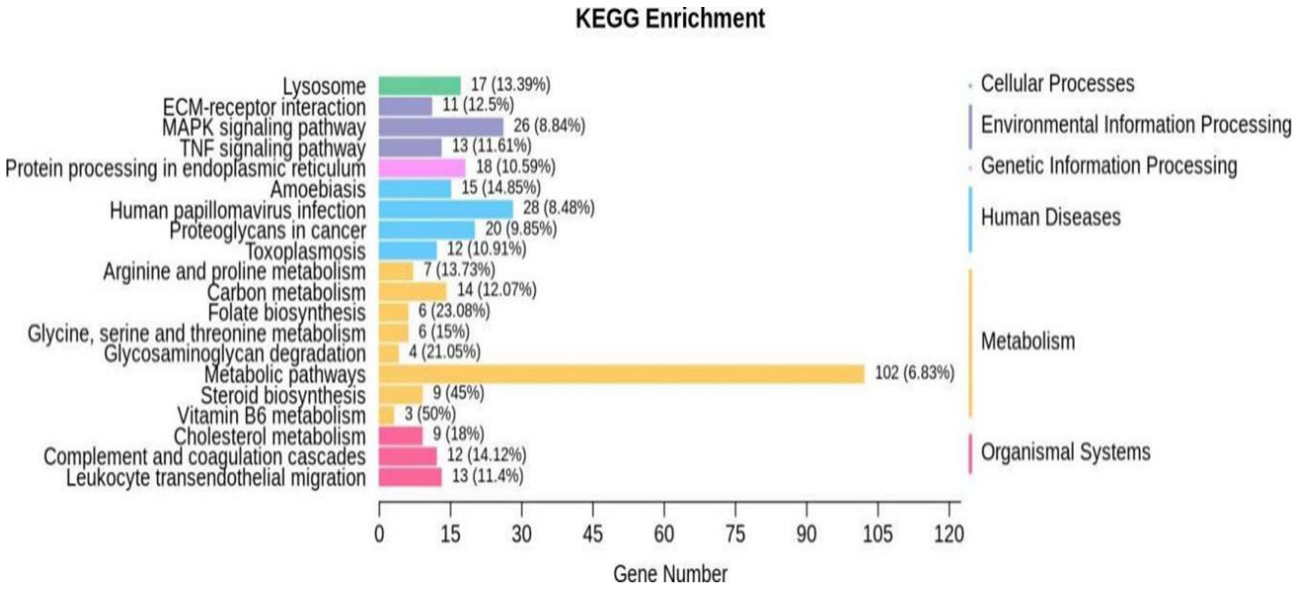


Figure 8 Gene Ontology functional classification of differentially expressed genes in HepG2 cells treated with wild Baicalin.

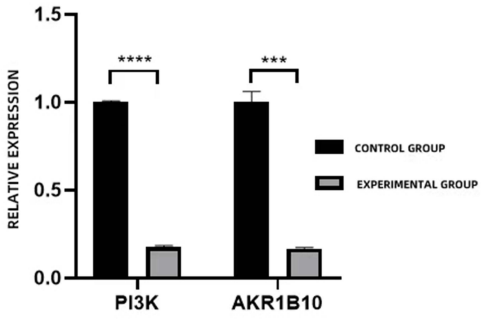


Figure 9 SCU regulates the expression of PI3K and AKR1B10 proteins in HepG2 cells. ***, ****Represents $p < 0.001$, $p < 0.0001$, respectively.

compared with the control group, the PI3K and AKR1B10 mRNA expressions of wild Baicalin intervention group were significantly decreased, and the results were statistically significant (P value <0.001).

Discussion

There are high incidence rate and mortality of primary liver cancer in China.[20,21] In normal people, cell apoptosis and cell proliferation are in a Dynamic equilibrium state. However, the growth and reproduction of tumor cells are not controlled by the normal growth regulation system, and can continue to divide and proliferate which results in a relative reduction of apoptosis. The disruption of the balance between proliferation and apoptosis can lead to the development of tumors.[22,23] Currently, cancer treatments are mainly surgery, chemotherapy, molecular targeted therapy and immunotherapy.[22,23] Despite the anticancer potential of wild Baicalin, the mechanism by which it triggers apoptosis in hepatocellular carcinoma (HCC) remains unclear. Through cell cycle and apoptosis experiments, we found that wild Baicalin could regulate cell cycle progression, arrested cell cycle G0/G1 phase and caused cancer cell apoptosis.[24,25] By CCK-8 analysis, we found that wild Baicalin inhibited the proliferation of human hepatoma cells in a dose-dependent and time-dependent manner. Through cell scratch experiment, Transwell invasion experiment and plate cloning experiment, we concluded that wild Baicalin could inhibit the migration, invasion and proliferation of HepG2 cells. The occurrence and development of liver cancer involves changes in multiple biological processes and signaling pathways. In tumor molecular research, the latest RNA sequencing technology has successfully replaced gene chip technology and has become the mainstream method for exploring gene expression at the genome-wide level. Compared with gene chip technology, it has the advantages of wider dynamic range and higher sensitivity. Consequently, in this study, Illumina sequencing technology was used to study the effect of wild Baicalin on the gene expression of liver cancer Hep G2 cells and find differentially expressed genes. A total of 1202 differentially expressed genes were screened out, of which 486 genes were up-regulated and 716 genes were down-regulated. Through GO enrichment analysis, it was found that biological processes played a key role in the anticancer mechanism of wild Baicalin. Through KEGG enrichment analysis, it was found that metabolic pathways were most significantly regulated by wild Baicalin. The research group selected the two most down-regulated genes AKR1B10 and PI3K in transcriptome sequencing as the targets for further research. Under physiological conditions, there are differences in the expression of AKR1B10 in different tissues and organs, such as high expression in stomach, small intestine, large intestine and gallbladder, and low expression in liver, lung, etc.[26] Under normal physiological conditions, AKR1B10 plays an important protective role for normal cells; however, under pathological conditions, AKR1B10 promotes the formation of tumors and becomes an important tumor-promoting factor.[27] Although AKR1B10 is closely related to the occurrence and development of tumors,[28] especially liver cancer, the high expression of AKR1B10 in liver cancer tissues has been confirmed. AKR1B10 is an important diagnostic marker for early liver cancer, but its mechanism of action in liver cancer is still unknown. The PI3K/AKT signaling pathway is involved in the development of various tumors and affects on cell proliferation and apoptosis. The PI3K/AKT signaling pathway is considered to be the most varied molecular pathway in malignant tumors.[29] Both AKR1B10 and PI3K are key genes in metabolic pathways, and both gene expression are down-regulated. Moreover, studies have shown that AKR1B10 can activate the PI3K/AKT signaling pathway to promote tumor development. In order to verify the results of transcriptome sequencing analysis, we selected differentially expressed genes AKR1B10 and PI3K for qPCR test verification, so as to achieve the purpose of verifying the results of transcriptome sequencing analysis. The qPCR experiment found that wild Baicalin could significantly reduce the mRNA expression of PI3K and AKR1B10 in liver cancer HepG2 cells. This result was consistent with the transcriptome sequencing results, indicating that the transcriptome sequencing results were highly reliable.

Therefore, it is of great significance to further study wild Baicalin as a candidate drug for liver cancer. This study revealed that wild Baicalin could effectively inhibit the proliferation of human hepatoma cell line HepG2, promote apoptosis, inhibit migration and invasion, as well as block the cell cycle and inhibit migration and invasion. This provides a certain theoretical and experimental basis for our future research on wild Baicalin in liver cancer.

Conclusion

In conclusion, this study demonstrated that wild Baicalin has potential as a candidate drug for the treatment of hepatocellular carcinoma (HCC). Wild Baicalin was found to inhibit the proliferation and migration of HepG2 cells, promote apoptosis, and block the cell cycle. Transcriptome sequencing revealed that wild Baicalin affects the expression

of genes involved in biological processes, particularly metabolic pathways. Verification through qPCR confirmed the down-regulation of AKR1B10 and PI3K mRNA expression, suggesting that the regulatory effect of wild Baicalin on the AKR1B10 and PI3K/AKT signaling pathway is critical to its inhibitory effect on HCC cell proliferation. Overall, these findings provide new insights into the mechanism of action of wild Baicalin and support its potential as a therapeutic approach for HCC treatment. Further studies are necessary to explore the clinical implications of these findings and to evaluate the safety and efficacy of wild Baicalin as a potential therapeutic agent for HCC.

Data Sharing Statement

Data will be available on request.

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Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

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