

Research Article

Network Pharmacology and Bioinformatics Approach Reveals the Therapeutic Mechanism of Action of Baicalein in Hepatocellular Carcinoma

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Received 19 October 2018; Accepted 9 January 2019; Published 12 February 2019

Academic Editor: Shao-Hsuan Kao

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Liver cancer is the fourth leading cause of cancer death worldwide, and hepatocellular carcinoma (HCC) accounts for the greatest proportion of these deaths. Baicalein, a flavonoid isolated from the root of *Scutellariae radix*, is considered a potential candidate to treat HCC. However, the underlying molecular mechanisms remain poorly understood. In the present study, a network pharmacological approach was combined with microarray data (GSE95504) acquired from the Gene Expression Omnibus database to reveal the therapeutic mechanisms of action of baicalein at a systemic level. We identified 38 baicalein targets and 76 differently expressed genes (DEGs) following treatment with baicalein, including 55 upregulated and 21 downregulated genes. The DEGs were significantly enriched in the biological functions of apoptosis, endoplasmic reticulum stress, and PERK-mediated unfolded protein response. Protein-protein interaction (PPI) network construction and topological screening revealed a core module of PPIs including two baicalein targets, *TP53* and *CDK1*, and two downregulated DEGs, *HSPA1A* and *HSPA1B*. Expression and survival data for these genes in the module derived from Gene Expression Profiling Interactive Analysis (GEPIA) were subjected to Kaplan–Meier analysis of overall survival and disease-free survival. Overexpression of *CDK1*, *BRCA1*, *TUBB*, *HSPA1A*, *HSPA1B*, and *HSPA4* was associated with significantly worse overall survival, while overexpression of *CDK1*, *CLU7*, *BRCA1*, and *TUBB* was associated with significantly worse disease-free survival. These data suggest that baicalein exerts therapeutic effects against HCC via a PPI network involving *TP53*, *CDK1*, *HSPA1A*, and *HSPA1B*.

1. Introduction

Liver cancer is the fourth leading cause of cancer death worldwide according to global cancer statistics for 2018, with about 782,000 deaths annually [1]. Hepatocellular carcinoma (HCC), comprising 75%–85% of cases, is the most common type of primary liver cancer and is strongly associated with chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), consumption of aflatoxin-contaminated foodstuffs, and heavy alcohol intake [2]. Although improvements in patient outcomes were observed in clinical trials with first- or second-line drugs, the lack of improvements in overall survival in some patients and the lack of curative

treatment options must be urgently addressed [3]. Therefore, elucidation of the molecular mechanisms underlying HCC and development of alternative therapies with lower toxicity is critical for achieving more favorable clinical outcomes and reducing treatment morbidity.

The use of traditional Chinese medicine to treat cancer is built on a foundation of more than 2,500 years of Chinese medical practice, including Chinese herbal medicine (CHM), acupuncture, and dietary therapy. A recently published meta-analysis of 20 randomized controlled trials showed that addition therapy with CHM improved overall survival in HCC patients and reduced adverse events related to conventional treatments [4]. To date, ingredients derived from CHM

have been found to exert suppressive effects on the promotion and proliferation of cancer cells, as well as inhibiting angiogenesis in cancer tissues [5]. Baicalein, a compound originally isolated from *Scutellariae radix*, is reported to exert anticancer activity by blocking colony formation, activating apoptosis, inducing autophagy, and modulating molecular signaling pathways such as mTOR and Wnt/ β -catenin in several human liver cancer cell lines [6–8]. Multitarget therapies elicit complex changes in multiple biological networks and mechanisms and are therefore considered an effective approach for treating HCC [9].

In contrast to the traditional “one drug, one target” principle of drug design, network pharmacology aims to investigate the influence or intervention of drugs on diseases as a whole, based on the synergism of multitargeted drugs and through a holistic perspective at a systemic level [10]. This approach encompasses systems biology, network analysis, connectivity, redundancy, and pleiotropy [11]. Recently, a network pharmacological platform was successfully applied for screening effective ingredients and revealing the pharmacological mechanisms of CHM. Whether by studying classic formulae, patented modern Chinese medicines, or bioactive ingredients derived from CHM, the network pharmacological approach provides new insights into the systemic connection between CHM, therapeutic targets, and a disease as a whole and provides a powerful and promising tool for the elucidation of disease mechanisms at a systemic level and the discovery of potential bioactive ingredients [12–14].

In the present study, in order to recognize the mechanisms underlying the anti-HCC effects of baicalein, we used a network pharmacological approach, including the evaluation of its absorption, distribution, metabolism, and excretion (ADME) properties; identification of differentially expressed genes (DEGs) related to baicalein treatment; prediction of baicalein-related targets; and recognition of core functions and modules via the protein-protein interaction (PPI) network approach. We identified a core modulatory network including two important baicalein targets, TP53 and CDK1 that was significantly related to the regulation of cell death and modulation of the apoptotic signaling pathway. To our knowledge, this study is the first to use a network pharmacological approach to study the efficacy and mechanism of baicalein, providing a powerful and promising tool for the development and application of CHM to HCC therapy.

2. Materials and Methods

2.1. Public Data Collection. The Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo>) is a public functional genomics data repository of high-throughput gene expression, chip, and microarray data. We downloaded the data series GSE95504 from the GEO database (species: *Homo sapiens*), which contains samples of the HCC cell line Bel-7402 treated with DMSO and 40 μ M baicalein. All samples were processed on the GPL17586 Affymetrix Human Transcriptome Array 2.0 platform. The probes were represented using the corresponding gene symbol according to the annotation information in the platform.

2.2. Identification of DEGs. The microarray data were preprocessed using RMA with the oligo and limma package in Bioconductor (v3.7; <http://www.bioconductor.org/>) [15]. Background correction, normalization, and calculation of expression were all included in the preprocessing stage. Microarray data probe IDs were transformed into gene symbols with the Bioconductor Annotation Data software packages. When several probes mapped to the same gene symbol, the mean value was set as the final expression value of this gene. P values were acquired using the unpaired Student's *t*-test provided by the limma package and were adjusted using the Benjamin–Hochberg (BH) method. The thresholds for identifying DEGs were set as adjusted (adj.) $P < 0.05$ and $|\log_2 \text{fold change (FC)}| > 1$. Hierarchical clustering analysis of the DEGs was then performed and visualized using *g*-plots as implemented in the R package.

2.3. Evaluation of Drug-Likeness (DL). The TCMSP server (<http://lsp.nwu.edu.cn/tcmsp.php>) is a systems-level pharmacological database for traditional Chinese medicine that can also be used for calculating the ADME-related properties of naturally occurring compounds of interest [16]. It provides an *in silico* ADME-systems evaluation model created by Wang et al., which integrates DL, oral bioavailability (OB), Caco-2 permeability, and other features.

2.4. Prediction of Baicalein Targets. We used the drug targeting *in silico* prediction models developed by Wang and others to identify potential targets for baicalein [17]. In brief, the *in silico* prediction model efficiently integrates chemical, genomic, and pharmacological information for drug targeting on a large scale, based on two powerful methods: random forest (RF) and support vector machines (SVM). In cases in which drug targets are identified, proteins with an output expectation value (E-value) for SVM > 0.7 or RF > 0.8 are listed as potential targets.

2.5. PPI Network Construction. We used BisoGenet [18], a Cytoscape plugin, to construct a PPI network using six currently available PPI databases, including the Biological General Repository for Interaction Datasets (BioGRID), Biomolecular Interaction Network Database (BIND), Molecular INTeraction Database (MINT), Human Protein Reference Database (HPRD), and Database of Interacting Proteins (DIP). After interactive networks for putative baicalein targets and DEGs were constructed using Cytoscape [19], a merged network was constructed based on the intersection data of the two networks.

2.6. Definition of Topological Feature Set for the Network. We used CytoNCA [20], a Cytoscape plugin, to analyze the topological properties of every node in the interaction network in order to calculate two topological properties: betweenness centrality (BC) and degree centrality (DC). More important nodes received higher quantitative values within the network.

TABLE 1: Differently expressed genes from GSE95504.

Gene	log FC	AveExpr	t	P-Value	adj. P-Value	B
FAM129A	2.624683	7.781198	21.34116	3.19E-08	0.000375	8.410651
ASNS	2.267513	7.745739	25.81981	7.31E-09	0.000226	9.168015
DDIT3	2.158061	5.129843	20.13082	5.00E-08	0.000441	8.148515
SLC7A11	1.940169	8.499572	6.547421	0.000196	0.022113	1.219042
CTH	1.859453	6.75212	17.32877	1.58E-07	0.000828	7.413078
SLFN5	1.77625	7.65223	11.24138	4.16E-06	0.004075	4.860584
ULBP1	1.734087	5.290091	14.11018	7.56E-07	0.001523	6.270654
OSBPL6	1.721142	5.858125	18.94741	7.97E-08	0.000586	7.861891
PSAT1P4	1.708273	7.674072	11.01701	4.83E-06	0.004101	4.729785
CLDN1	1.63562	6.888961	14.4617	6.27E-07	0.001383	6.414871
GDF15	1.610927	6.988729	12.48127	1.90E-06	0.002583	5.525689
CLGN	1.536074	4.371125	18.84393	8.31E-08	0.000586	7.835261
TRIB3	1.535661	7.709365	17.24218	1.64E-07	0.000828	7.386996
TUBE1	1.515188	5.96114	15.58245	3.56E-07	0.00132	6.840486
ERRFI1	1.482139	7.451778	21.4253	3.09E-08	0.000375	8.427814
GPRI	1.471302	4.486647	13.82075	8.84E-07	0.001541	6.147765
GFPT1	1.462896	8.845707	17.25109	1.64E-07	0.000828	7.389692
PXK	1.447934	5.986157	13.87282	8.59E-07	0.001541	6.170157
PSAT1	1.443356	10.30946	13.75243	9.18E-07	0.001541	6.118191
NFIL3	1.438356	6.135237	7.852097	5.60E-05	0.011763	2.454479
PMAIP1	1.319246	6.572005	12.40886	1.99E-06	0.002647	5.489364
CHAC1	1.30625	7.242645	9.979721	1.00E-05	0.005625	4.078163
C15orf65	1.290543	6.123697	6.54843	0.000196	0.022113	1.220081
GADD45A	1.289319	7.282376	16.76291	2.04E-07	0.000958	7.238494
WARS	1.275291	9.228478	12.87648	1.51E-06	0.002159	5.718873
SEL1L3	1.274108	7.258999	9.408758	1.54E-05	0.006705	3.683429
STC2	1.238835	6.081823	15.4665	3.77E-07	0.001328	6.798729
SLC22A15	1.22956	6.064722	13.9939	8.05E-07	0.001541	6.221742
CTAGE5	1.220954	6.554274	11.07457	4.64E-06	0.004075	4.763668
JAG1	1.214166	5.838658	8.328888	3.69E-05	0.009608	2.856702
SESN2	1.19303	7.048649	10.73854	5.83E-06	0.004571	4.562614
PHGDH	1.18876	7.266541	11.47118	3.58E-06	0.003764	4.991071
GARS	1.184448	9.965868	14.75109	5.40E-07	0.001333	6.529612
SGTB	1.173656	5.908581	7.658115	6.68E-05	0.013087	2.283709
MOCOS	1.16865	8.951834	15.92122	3.02E-07	0.001224	6.959663
PDE10A	1.122733	6.077276	14.89259	5.02E-07	0.001333	6.584453
RND3	1.118622	6.761436	13.61063	9.92E-07	0.001591	6.056112
PCK2	1.11477	6.688267	15.34161	4.01E-07	0.001333	6.753186
ARG2	1.109017	5.776241	7.100543	0.000113	0.016946	1.768427
CCDC113	1.10665	6.174742	7.187338	0.000104	0.016349	1.851102
ZNF252P	1.100149	6.009432	5.675607	0.000504	0.03647	0.26837
CARS	1.082632	6.759968	14.92228	4.94E-07	0.001333	6.595858
ETV5	1.080051	6.215242	9.459451	1.48E-05	0.00668	3.719593
AARS	1.075095	10.13323	12.06077	2.46E-06	0.002893	5.310541
NR1D2	1.069287	6.525232	6.13772	0.000302	0.02816	0.785742
PYROXD1	1.064665	7.7943	6.357469	0.000239	0.024807	1.021033
TRIM2	1.063894	6.068377	14.25706	6.99E-07	0.001449	6.331573
XPOT	1.052345	9.70088	8.58299	2.98E-05	0.008532	3.061361
HERPUD1	1.051263	8.736178	14.36397	6.60E-07	0.001411	6.375315
XBPI	1.043021	7.951268	13.68627	9.52E-07	0.001561	6.089348
SARS	1.039932	8.565683	12.23496	2.21E-06	0.002784	5.400901
ACAD11	1.036179	5.605899	14.46101	6.27E-07	0.001383	6.414591

TABLE 1: Continued.

Gene	log FC	AveExpr	t	P-Value	adj. P-Value	B
TSEN15	1.027667	6.697341	9.904686	1.06E-05	0.005645	4.02783
BMP6	1.020646	8.133826	9.363714	1.59E-05	0.006705	3.651105
NUPR1	1.018416	8.725291	10.60041	6.42E-06	0.004666	4.477638
SIPR3	-1.00496	5.622551	-7.97262	5.03E-05	0.011553	2.558468
HIST1H1A	-1.03222	5.249701	-5.77463	0.000451	0.03452	0.381872
SCNN1A	-1.03907	7.479328	-8.90881	2.28E-05	0.007694	3.314433
HIST1H2AB	-1.07344	8.065709	-5.58109	0.000562	0.038202	0.158669
GLP2R	-1.11017	7.780635	-11.02	4.82E-06	0.004101	4.731521
MYPN	-1.12719	7.42583	-10.1703	8.70E-06	0.005247	4.203973
HIST1H2BI	-1.13604	6.08771	-5.2416	0.000836	0.046549	-0.24654
CCL2	-1.14721	5.314616	-12.6518	1.72E-06	0.002378	5.610076
SI00A4	-1.15053	6.164204	-10.5789	6.51E-06	0.004688	4.464307
KIF20A	-1.15804	8.626588	-11.5903	3.31E-06	0.003538	5.057388
HSPA1A	-1.18133	9.35104	-11.0628	4.68E-06	0.004075	4.756747
ZNF831	-1.18556	5.812955	-6.95585	0.00013	0.01809	1.628511
HIST1H3A	-1.18869	6.441841	-7.55158	7.37E-05	0.013674	2.1881
HSPA1B	-1.20391	9.316029	-11.1242	4.49E-06	0.004075	4.792689
GATSL2	-1.22061	7.183912	-9.40487	1.54E-05	0.006705	3.680643
METTL7A	-1.241	6.802659	-9.19234	1.82E-05	0.006822	3.526483
NPY4R	-1.3319	6.836449	-8.96261	2.18E-05	0.007407	3.355245
LXN	-1.34896	6.031784	-6.28611	0.000258	0.025948	0.945372
GATSL1	-1.37709	7.131902	-10.0593	9.43E-06	0.005498	4.131046
ALDH1A3	-1.40489	7.11515	-11.6537	3.18E-06	0.003504	5.092274
OLFML1	-1.6252	7.527223	-8.44414	3.35E-05	0.00908	2.950342

2.7. Clusters of Core PPI Networks. We used MCODE, a plugin of Cytoscape, to obtain clusters of core PPI networks by analyzing the corresponding networks [21, 22]. Based on network theory, connected regions in large PPI networks may represent molecular complexes and together disrupt biological functions, resulting in a particular disease phenotype. As the topological module and functional module have the same meaning in the network, the functional module can be recognized by network properties.

2.8. Gene Expression Data for the Core Cluster for HCC. Data were obtained from the Gene Expression Profiling Interactive Analysis (GEPIA) online database (<http://gepia.cancer-pku.cn/>), a web server that provides customizable functions [23]. Tumors and normal samples in the GEPIA database were derived from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects. Correlations of disease-free survival and overall survival rates with the expression levels of *CDK1*, *CUL7*, *BRCA1*, *TUBB*, *HSPA1A*, *HSPA1B*, and *HSPA4* in HCC patients were also computed using the GEPIA database.

2.9. Gene Ontology and Pathway Enrichment Analysis. We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov/>) [24], an online program that provides comprehensive data for high-throughput gene functional analysis for elucidation of biological characteristics, to obtain Gene Ontology (GO)

terms belonging to the biological process (BP), cellular component (CC), and molecular function (MF) categories. Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway functional enrichment analyses were performed for the above DEGs. Results with values of $P < 0.05$ were considered to be statistically significant. We performed KEGG signaling pathway enrichment analysis of the screened candidate targets of baicalein using ClueGO, a Cytoscape plugin, to visualize nonredundant biological terms for large clusters of genes in a functionally grouped network [25]. The ClueGO network was created with kappa statistics and reflects the relationship between the terms based on the similarity of their associated genes.

3. Results

3.1. Identification of DEGs. Gene expression dataset GSE95504 was downloaded from the GEO database. Statistical analysis software R was used for preprocessing and gene differential expression analysis of microarray data. A total of 76 DEGs were identified between HCC BEL-7402 cells treated with and without baicalein, including 55 that were upregulated and 21 that were downregulated upon baicalein treatment, for subsequent bioinformatics analysis (Table 1, Figure 1).

3.2. GO Enrichment Analyses of DEGs. To analyze the biological classifications of DEGs, functional enrichment analyses

were performed using the DAVID server. As shown in Figure 2, GO analysis results indicated that, in the BP category, DEGs were significantly enriched in the intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, PERK-mediated unfolded protein response, tRNA aminoacylation for protein translation, cellular response to oxidative stress, endoplasmic reticulum unfolded protein response, regulation of protein ubiquitination, glutamine metabolic process, cellular response to insulin stimulus, positive regulation of interleukin-8 production, and cellular response to glucose starvation. In the CC category, DEGs were mostly enriched in the cytosol, nucleosome, extracellular exosome, GATOR2 complex, inclusion body, nucleoplasm, and cytoplasm. In the MF category, DEGs were mainly enriched in protein binding involved in protein folding, tRNA binding, C3HC4-type RING finger domain binding, virus receptor activity, ATP binding, protein heterodimerization activity, ATPase activity, transcription regulatory region DNA binding, Notch binding, and unfolded protein binding.

3.3. Baicalein ADME Properties, Target Prediction, and PPI Network Construction. As shown in Table 2, the DL value of baicalin was 0.21, OB value of baicalin was 33.52%, and Caco-2 permeability of baicalin was 0.63, indicating the potential applicability of baicalin as an oral drug agent. We further predicted 38 potential targets of baicalein including TP53 and CDK1 (Table 3). PPI networks were constructed of baicalein targets and DEGs. We further merged the intersection of the two networks and obtained a core network (92 nodes, 2173 edges) according to the DC and BC (Figures 3(a)–3(c)).

3.4. KEGG Signaling Pathway Enrichment and Main Modules of Core PPI Network Recognition. KEGG signaling pathway enrichment using ClueGO indicated that proteins in the core PPI network were statistically enriched for the following pathways: Epstein-Barr virus infection, bladder cancer, antigen processing and presentation, cell cycle, alcoholism, ubiquitin-mediated proteolysis, and transcriptional misregulation in cancer. Other enriched pathways included the p53 signaling pathway, breast cancer, colorectal cancer, hepatitis B, and microRNAs in cancer (Figure 3(d)). A Cytoscape plugin, MCODE, was used to carry out module analysis, resulting in two main modules of the core PPI network (Figure 4). The first module, containing two baicalein targets, was functionally enriched in beta-catenin-TCF complex assembly, response to unfolded protein, and the ERBB2 signaling pathway. The second module, containing two baicalein targets and two DEGs, was enriched in regulation of signal transduction by p53, regulation of cell death, and the intrinsic apoptotic signaling pathway.

3.5. Analysis of HCC Survival and Expression of Proteins in the Module of Interest. We used the GEPIA online database to perform overall survival and disease-free survival analyses for proteins in module 2 using a Kaplan–Meier curve. HCC patients with higher expression of *CDK1*, *BRCA1*, *TUBB*, *HSPA1A*, *HSPA1B*, and *HSPA4* showed significantly worse overall survival (Figure 5), while HCC patients with higher

expression of *CDK1*, *CLU7*, *BRCA1*, and *TUBB* showed significantly worse disease-free survival (Figure 6).

4. Discussion

S. baicalensis is a skullcap plant native to several East Asian countries and Russia, and it is cultivated in many European countries. This plant has been widely used in traditional Chinese medicine since about 200–250 AD for the clinical treatment of hypertension, atherosclerosis, dysentery, common colds, inflammatory disorders, and tumors [26–28]. Baicalein is a major bioactive flavone derived from the dry roots of *S. baicalensis*. It has been shown that treatment with baicalein inhibits cell proliferation and enhances apoptosis and autophagy of many types of cancer, including HCC [29], bladder cancer [30], and colorectal cancer [31]. Previously, studies have also demonstrated that baicalein exerts multiple effects against HCC, mainly involving the inhibition of cell proliferation by arresting the cell cycle, suppression of metastasis, induction of apoptosis, as well as induction of autophagy via the modulation of associated molecules and signaling pathways [32]. However, these studies were designed based on the traditional research idea of “one drug, one target”, without considering a systemic picture. Therefore, in the present study, we used a network pharmacological strategy to explore the molecular mechanisms by which baicalein exerts therapeutic effects on HCC from a systemic perspective, and we sought to establish connections between basic and clinical research.

We used *in silico* approaches to determine whether baicalein is a good candidate for drug discovery for HCC. According to Lipinski’s “rule of five”, which predicts the DL of a chemical compound with a certain biological activity designed for administration via the oral route [33], the properties of baicalein meet the requirements, namely, molecular weight < 500 Da, calculated log P < 5, number of hydrogen-bond donors < 5, and number of hydrogen-bond acceptors < 10, indicating that this compound is a suitable potential candidate for drug discovery for HCC. Based on data from the TCMSP database, the DL of baicalein was calculated to be 0.21, which is above the average DL value of DrugBank compounds (0.18); these data additionally indicate the potential of baicalein as a promising candidate for HCC treatment.

To understand the anti-HCC effect of baicalein at a systemic level, we predicted targets of baicalein and analyzed microarray data from baicalein-treated HCC cell lines derived from the GEO database. Thirty-eight baicalein targets and 76 DEGs were identified and selected for further analysis. GO analysis of DEGs indicated that apoptosis, endoplasmic reticulum stress, and oxidative stress were statistically related to the anti-HCC effect of baicalein. Through PPI network construction and topological screening, we obtained a core PPI network with 92 nodes and 2173 edges. Following KEGG analysis of proteins from the core PPI network using ClueGO, we identified the Epstein-Barr virus infection, cell cycle, alcoholism, hepatitis B, and p53 signaling pathways, as well as some cancer pathways. An increasing number of studies

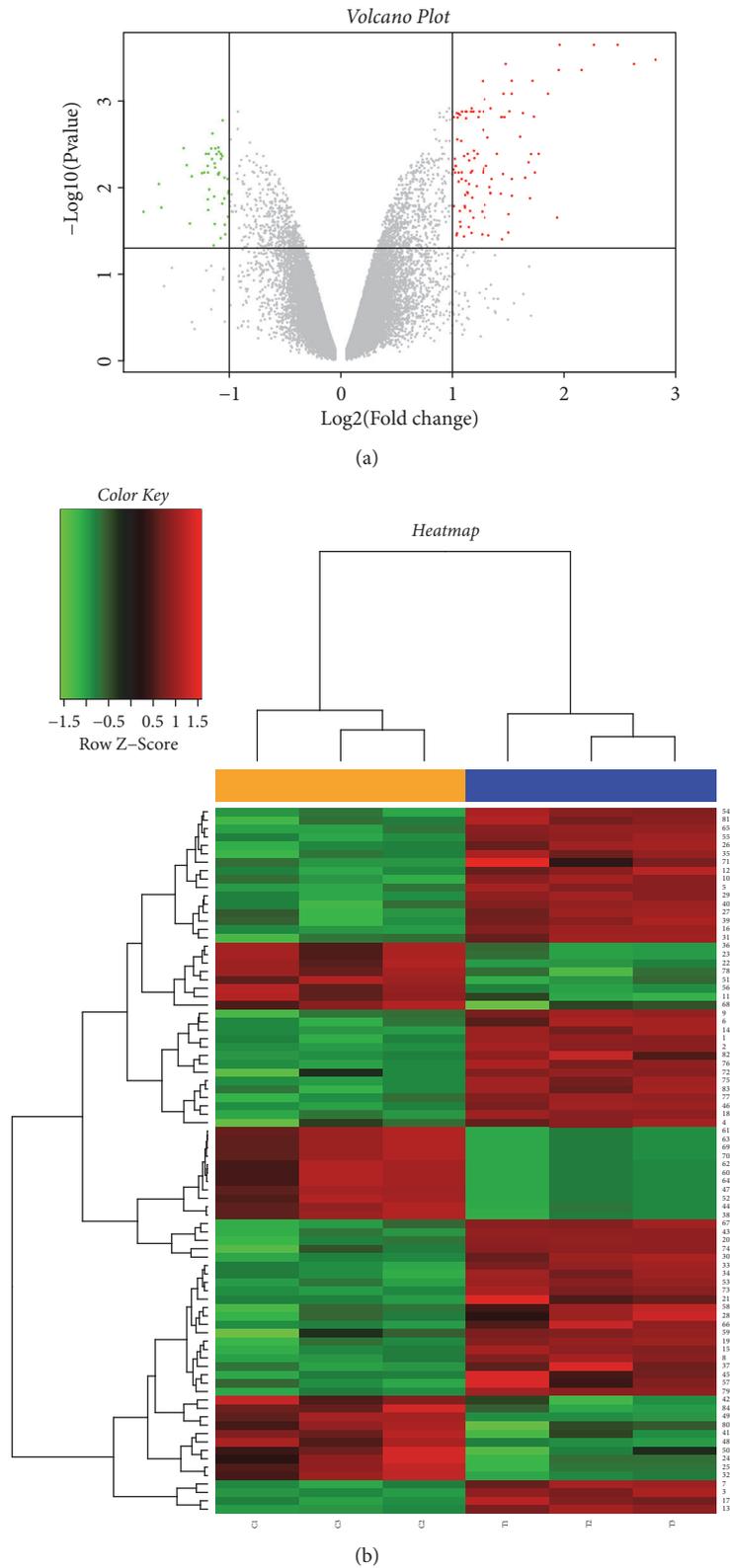


FIGURE 1: Volcano plot of gene expression and heatmap of DEGs from GSE95504. (a) The x-axis specifies the \log_2 fold change (FC), and the y-axis specifies the $-\log_{10}$ of the adj. P-value. Black vertical and horizontal lines reflect the filtering criteria (adj. $P < 0.05$ and $|\log_2\text{FC}| > 1$). (b) Rows represent genes, and columns represent samples. The heatmap is color-coded based on the Z-score; red represents a high expression value and green represents a low expression value.

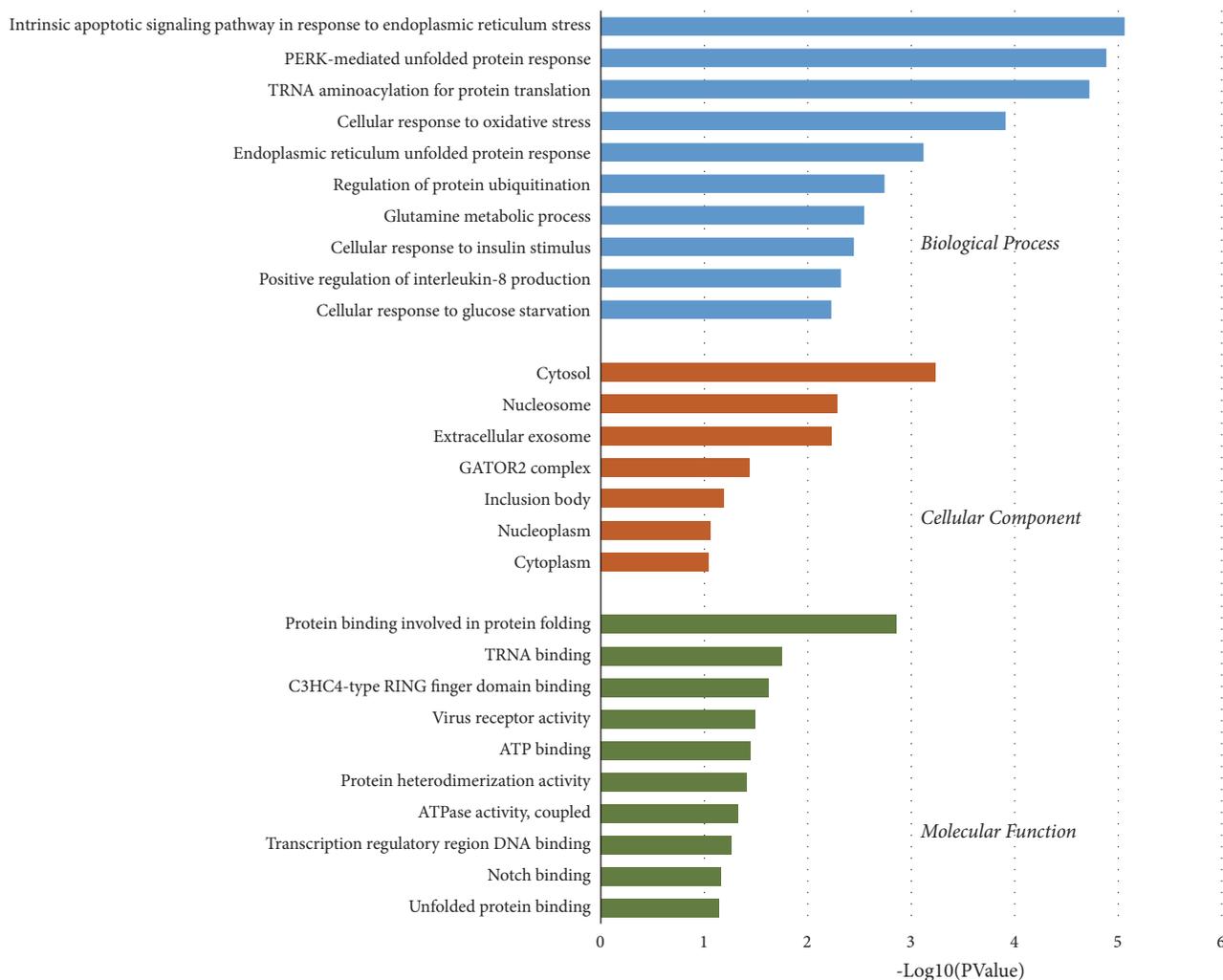


FIGURE 2: GO analysis for biological process, cellular component, and molecular function terms was performed on DEGs; the top 10 terms with $P < 0.05$ are shown.

TABLE 2: Pharmacological and molecular properties of baicalein.

MW	AlogP	Hdon	Hacc	OB (%)	Caco-2	BBB	DL	FASA-	TPSA	RBN	HL
270.25	2.33	3	5	33.52	0.63	-0.05	0.21	0.36	90.9	1	16.25

support the idea that baicalein induces the apoptosis of HCC cells and that the underlying biological mechanisms are strongly related to endoplasmic reticulum stress, reactive oxygen species generation, and the p53 signaling pathway [32, 34, 35]. Baicalein has also been shown to arrest the cell cycle in different HCC cell lines at all three phases: G0/G1 [36], S [37], and G2/M [38]. These findings are consistent with the results from the present *in silico* network pharmacological analysis.

Using a topological approach, we identified an important module containing 14 genes, including *TP53* (baicalein target), *CDK1* (baicalein target), *HSPA1B* (downregulated DEG), *HSPA1B* (downregulated DEG), and other genes in the PPI network; these genes play important roles in the regulation

of signal transduction by p53, regulation of cell death, and the intrinsic apoptotic signaling pathway. We further assessed the expression of these genes in relation to overall and disease-free survival. High expression of *CDK1*, *BRCA1*, and *TUBB* was significantly associated with reductions in both overall and disease-free survival.

Baicalein has been identified as a selective inhibitor of CDK1 by FRET analysis and structure-activity relationship analysis in the Hep G2 and SMMC-7721 cell lines [39, 40]. A549 and H1299 non-small-cell lung cancer cells and primary cultured heart endothelial cells treated with baicalein have additionally been reported to exhibit downregulated expression of CDK1 [41]. CDK1 is a member of the Ser/Thr protein kinase family that is frequently overexpressed in HCC

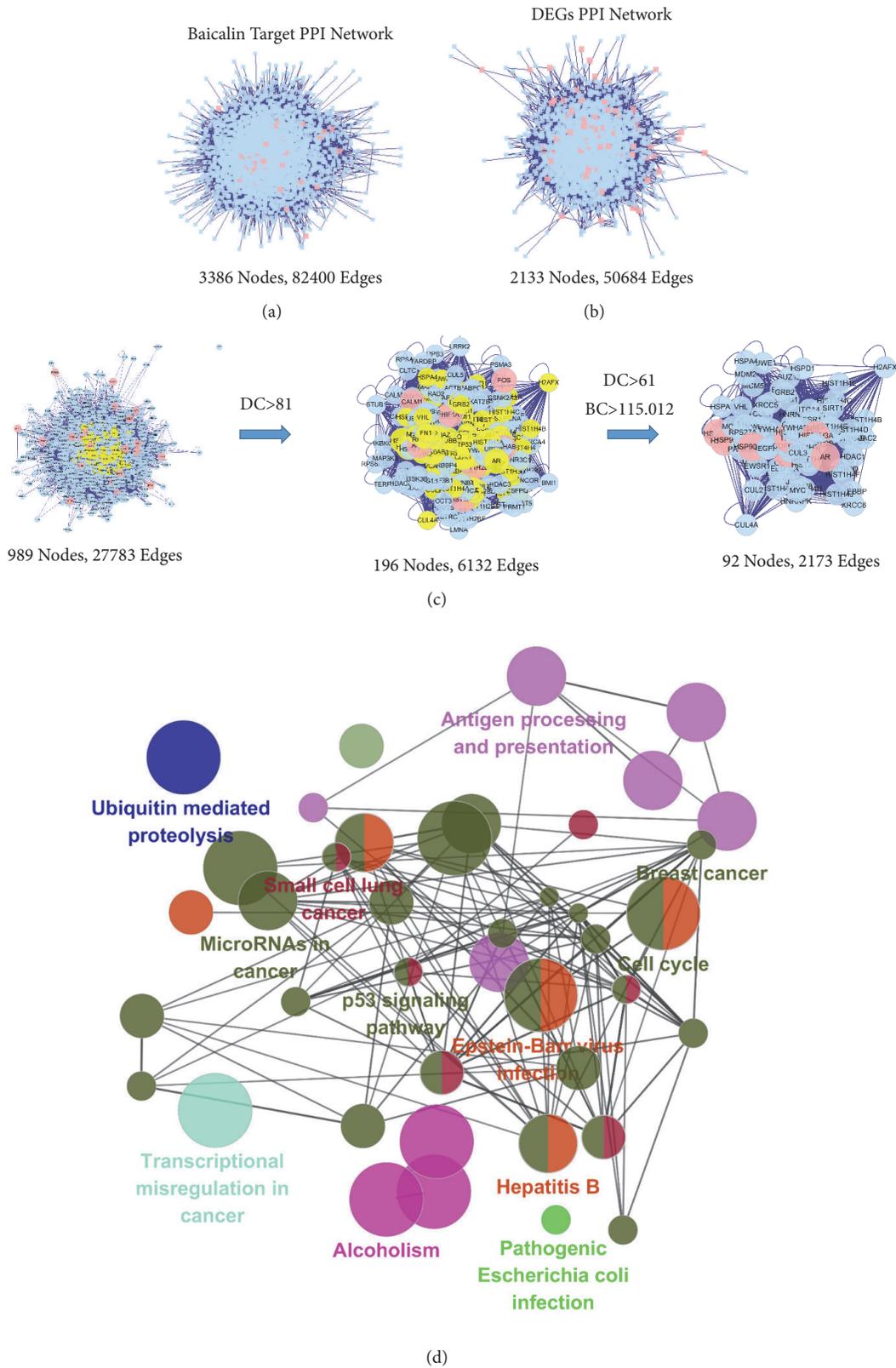


FIGURE 3: Identification and KEGG enrichment analysis of candidate targets for baicalein against HCC. (a) Protein-protein interaction network of baicalein targets. (b) Protein-protein interaction network of DEGs. (c) Topological screening of the interactive PPI network of baicalein targets and DEGs based on degree and betweenness centrality. (d) KEGG enrichment analysis was performed by ClueGO, and the most vital term in the group is labeled.

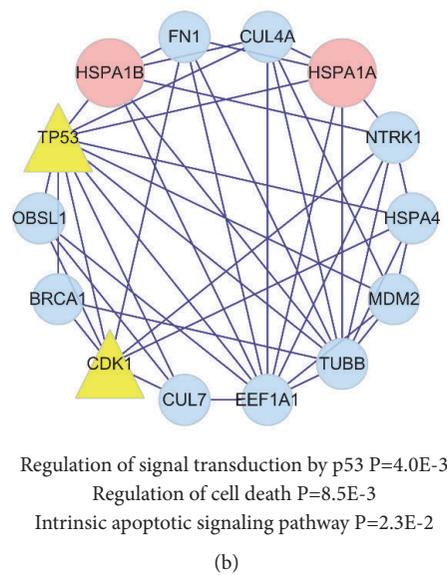
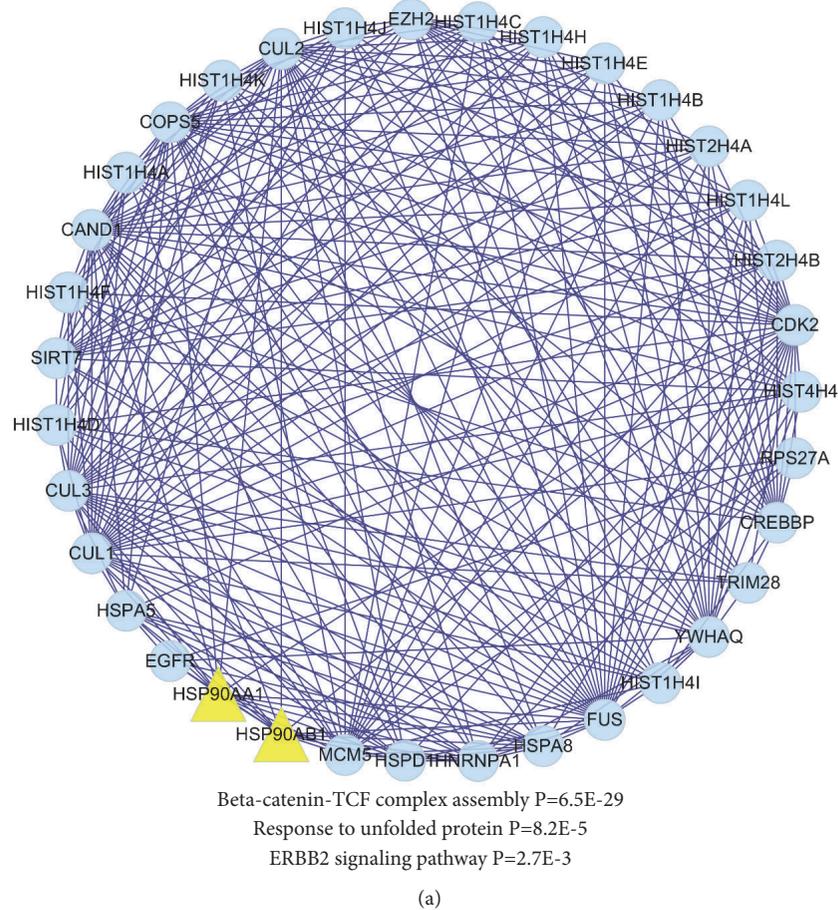


FIGURE 4: Modules of core PPI networks of baicalein against HCC. (a) One module was related to beta-catenin-TCF complex assembly, response to unfolded protein, and ERBB2 signaling pathway. (b) The other module was related to regulation of signal transduction by p53, regulation of cell death, and intrinsic apoptotic signaling pathway.

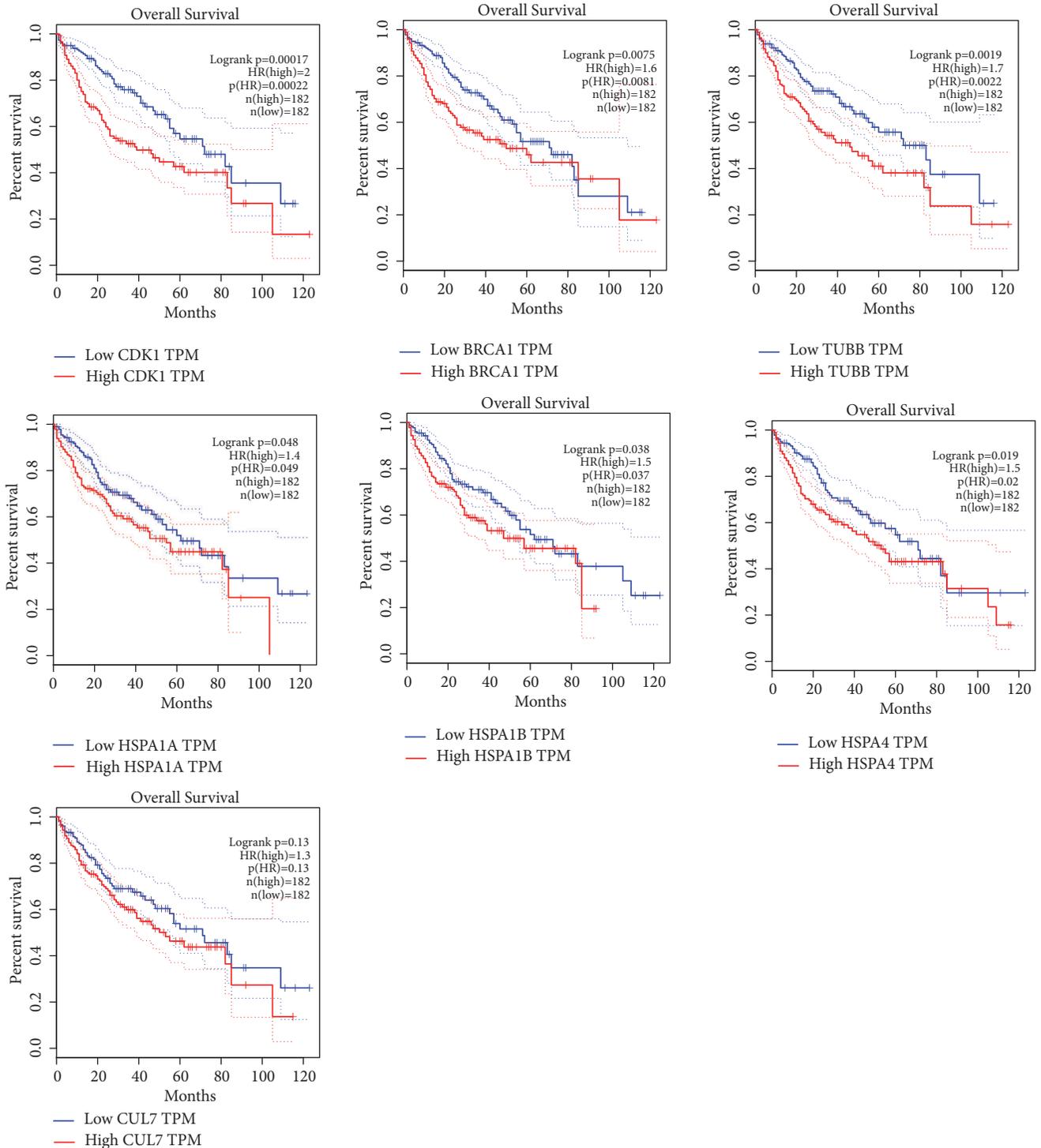


FIGURE 5: Overall survival analyses of *CDK1*, *CUL7*, *BRCA1*, *TUBB*, *HSPA1A*, *HSPA1B*, and *HSPA4* were performed using GEPIA online platform; differences with $P < 0.05$ were considered statistically significant.

and associated with tumor progression [42, 43]. Depletion or inhibition of CDK1 with microRNAs or small molecular compounds has been shown to lead to reduced clonogenicity by arresting cells in the S-G2 and G2-M phases of the cell cycle and inducing apoptosis in HCC cell lines [44-47].

TP53, another baicalein target, is a classic tumor suppressor involved in tumor cell apoptosis, genomic stability, and inhibition of angiogenesis [48]. Previous studies have found that baicalein induces cell cycle arrest and apoptosis in HepG2 cells via a TP53-dependent pathway [49]. Similar

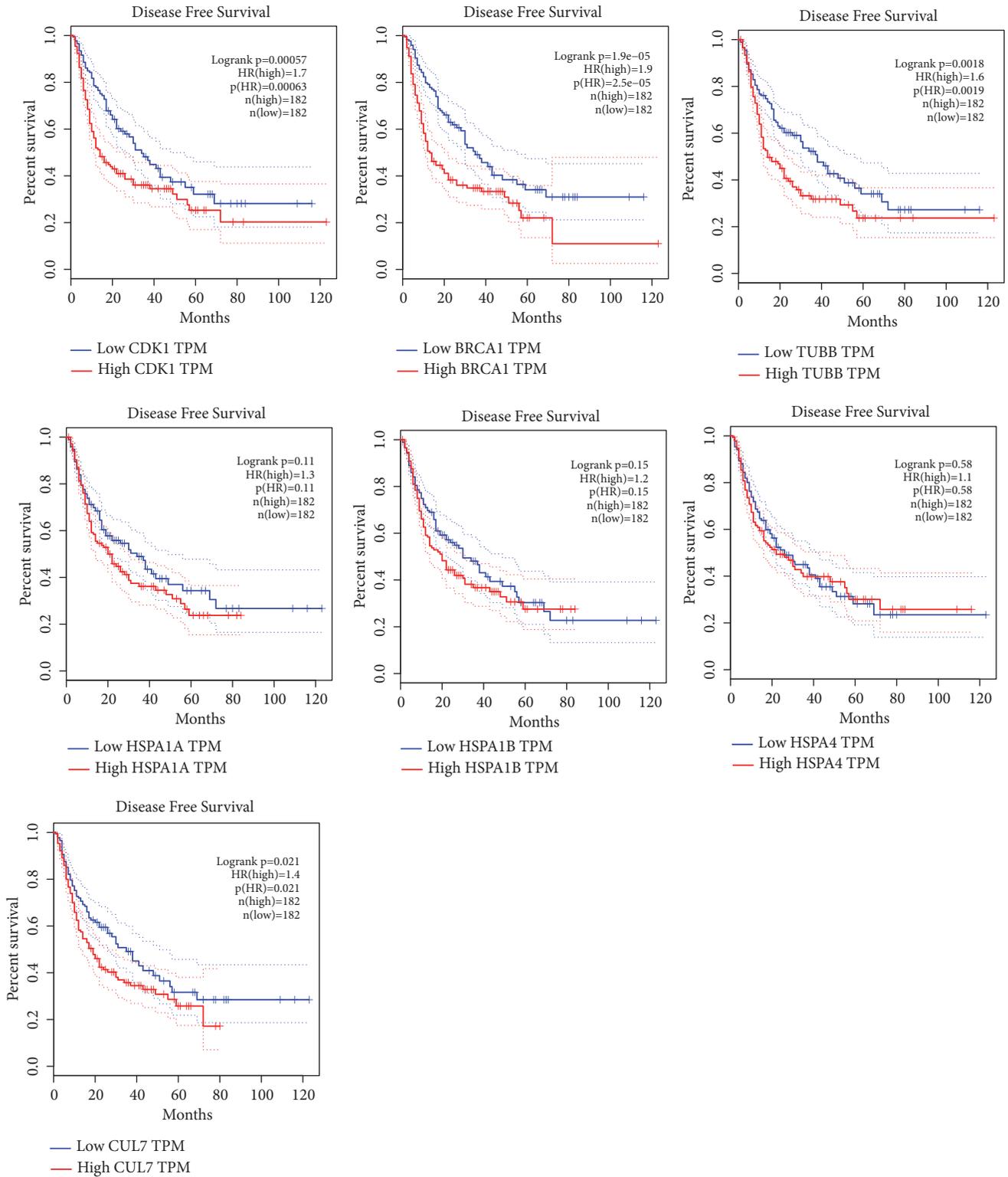


FIGURE 6: Disease-free survival analyses of *CDK1*, *CUL7*, *BRCA1*, *TUBB*, *HSPA1A*, *HSPA1B*, and *HSPA4* were performed using GEPIA online platform; differences with $P < 0.05$ were considered statistically significant.

TABLE 3: Predicted targets of baicalein.

Protein name	Gene symbol
Prostaglandin G/H synthase 1	PTGS1
Androgen receptor	AR
Prostaglandin G/H synthase 2	PTGS2
Heat shock protein HSP 90	HSP90AA1
Heat shock protein HSP 90	HSP90AB1
mRNA of PKA Catalytic Subunit C-alpha	PRKACA
Dipeptidyl peptidase IV	DPP4
Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform	PIK3CG
CGMP-inhibited 3',5'-cyclic phosphodiesterase A	PDE3A
Trypsin-1	PRSS1
Nuclear receptor coactivator 2	NCOA2
Nuclear receptor coactivator 1	NCOA1
Calmodulin	CALM1
Transcription factor p65	RELA
RAC-alpha serine/threonine-protein kinase	AKT1
Vascular endothelial growth factor A	VEGFA
Apoptosis regulator Bcl-2	BCL2
Proto-oncogene c-Fos	FOS
Apoptosis regulator BAX	BAX
Matrix metalloproteinase-9	MMP9
Caspase-3	CASP3
Cellular tumor antigen p53	TP53
Hypoxia-inducible factor 1-alpha	HIF1A
Fos-related antigen 1	FOSL1
Fos-related antigen 2	FOSL2
Cell division control protein 2 homolog	CDK1
G2/mitotic-specific cyclin-B1	CCNB1
Myeloperoxidase	MPO
Aryl hydrocarbon receptor	AHR
Insulin-like growth factor II	IGF2
Cytochrome c	CYCS
Arachidonate 12-lipoxygenase, 12S-type	ALOX12
Nuclear factor of activated T-cells, cytoplasmic 1	NFATC1
Tudor domain-containing protein 7	TDRD7
Egl nine homolog 1	EGLN1
NADPH oxidase 5	NOX5
Fatty acid-binding protein, epidermal	FABP5
Apolipoprotein D	APOD

results were observed in human lung [50], colorectal [51], and prostate [52] cancer cells.

Heat shock proteins (HSPs) and molecular chaperones are considered to play important roles in protein homeostasis, cell physiology, and protection against stressors [53]. In the present study, HSPA1A and HSPA1B represented two downregulated DEGs present in the PPI module network of baicalein. As members of the heat shock protein 70 (Hsp70) family, they were similarly expressed in both tumor

and nontumor tissues of HCC patients, with higher expression levels in HCC tumor tissues [54]. Previous studies have shown that the knockdown of HSP70 inhibits the proliferation of two HCC cell lines, namely SMMC-7721 and Hep3B cells [55]. *In vitro* evidence showed that high levels of HSP70 promote cell migratory ability and suppress apoptosis [56]. Of note, it has been reported that HSP70 plays significant roles in endothelial cell migration and lumen formation and is considered an important

molecule in angiogenesis and the immune response in HCC [57].

To further understand the roles of the ten other proteins in the potential module, based on our current knowledge, we propose that EEF1A1, MDM2, CUL7, and BRCA1 are strongly related to HCC. These proteins are considered to play important roles in the pathophysiology of tumors and participate in cell growth, cell cycle regulation, and the maintenance of cell survival [58–61].

In conclusion, our *in silico* study showed that the anti-HCC effect of baicalein was related to endoplasmic reticulum stress, apoptosis, oxidative stress, and the p53 signaling pathway. We identified a PPI network module containing 14 proteins, and we speculate that baicalein targets CDK1 and TP53 to downregulate the expression of HSP70, thereby exerting preventive effects against HCC via this module. Biological experiments are needed to verify these *in silico* results in the future.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the submission of this manuscript.

Authors' Contributions

Chongyang Ma and Tian Xu contributed equally. Xiaoguang Sun and Shuang Zhang are responsible for data curation. Shuling Liu contributed to formal analysis. Qingguo Wang, Xueqian Wang, and Fafeng Cheng contributed to funding acquisition. Shuning Fan, Changming Zhai, and Changxiang Li contributed to investigation. Fafeng Cheng and Xueqian Wang contributed to methodology. Feifei Tang and Chaofang Lei contributed to project administration. Chongyang Ma is responsible for software. Juan Luo is responsible for validation. Tian Xu is responsible for visualization. Chongyang Ma and Tian Xu contributed to writing the original draft. Fafeng Cheng and Wei Wei contributed to review and editing of the paper.

Acknowledgments

This article was supported by the National Natural Science Foundation of China (Grants nos. 81430102, 81774122, 81774030, and 81874448) and the Beijing University of Traditional Chinese Medicine Independent Subject Selection Project (Grant no. 2017-JYB-XS-014).

References

- [1] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA: A Cancer Journal for Clinicians*, vol. 68, no. 6, pp. 394–424, 2018.
- [2] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2018," *CA: A Cancer Journal for Clinicians*, vol. 68, no. 1, pp. 7–30, 2018.
- [3] J. M. Llovet, R. Montal, D. Sia, and R. S. Finn, "Molecular therapies and precision medicine for hepatocellular carcinoma," *Nature Reviews Clinical Oncology*, vol. 15, no. 10, pp. 599–616, 2018.
- [4] Z. Yang, L. Xian, Y. Lu et al., "Add-on therapy with traditional chinese medicine improves outcomes and reduces adverse events in hepatocellular carcinoma: a meta-analysis of randomized controlled trials," *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, no. 4, Article ID 3428253, 13 pages, 2017.
- [5] Y. Hu, S. Wang, X. Wu et al., "Chinese herbal medicine-derived compounds for cancer therapy: a focus on hepatocellular carcinoma," *Journal of Ethnopharmacology*, vol. 149, no. 3, pp. 601–612, 2013.
- [6] S.-R. Chen, H.-C. Qiu, Y. Hu, Y. Wang, and Y.-T. Wang, "Herbal medicine offered as an initiative therapeutic option for the management of hepatocellular carcinoma," *Phytotherapy Research*, vol. 30, no. 6, pp. 863–877, 2016.
- [7] J. Li, B. Duan, Y. Guo et al., "Baicalein sensitizes hepatocellular carcinoma cells to 5-FU and Epirubicin by activating apoptosis and ameliorating P-glycoprotein activity," *Biomedicine Pharmacotherapy*, vol. 98, p. 806, 2018.
- [8] Y.-F. Wang, T. Li, Z.-H. Tang et al., "Baicalein triggers autophagy and inhibits the protein kinase B/mammalian target of rapamycin pathway in hepatocellular carcinoma HepG2 cells," *Phytotherapy Research*, vol. 29, no. 5, pp. 674–679, 2015.
- [9] T. Li, Y. Xue, G. Wang et al., "Multi-target siRNA: Therapeutic strategy for hepatocellular carcinoma," *Journal of Cancer*, vol. 7, no. 10, pp. 1317–1327, 2016.
- [10] A. L. Hopkins, "Network pharmacology," *Nature Biotechnology*, vol. 25, no. 10, pp. 1110–1111, 2007.
- [11] P. Bawa, P. Pradeep, P. Kumar, Y. E. Choonara, G. Modi, and V. Pillay, "Multi-target therapeutics for neuropsychiatric and neurodegenerative disorders," *Drug Discovery Therapy*, vol. 21, no. 12, pp. 1886–1914, 2016.
- [12] Z. Chen and Q. Wang, "Activation of PPAR γ by baicalin attenuates pulmonary hypertension in an infant rat model by suppressing HMGB1/RAGE signaling," *FEBS Open Bio*, vol. 7, no. 4, pp. 477–484, 2017.
- [13] H. Y. Fang, H. W. Zeng, L. M. Lin et al., "A network-based method for mechanistic investigation of Shexiang Baoxin Pill's treatment of cardiovascular diseases," *Scientific Reports*, vol. 7, Article ID 43632, 2017.
- [14] L. Zeng and K. Yang, "Exploring the pharmacological mechanism of Yanghe Decoction on HER2-positive breast cancer by a network pharmacology approach," *Journal of Ethnopharmacology*, vol. 199, pp. 68–85, 2017.
- [15] R. C. Gentleman, V. J. Carey, D. M. Bates et al., "Bioconductor: Open software development for computational biology and bioinformatics," *Genome Biology*, vol. 5, no. 10, article no (R80), 2004.
- [16] J. Ru, P. Li, J. Wang et al., "TCMSP: a database of systems pharmacology for drug discovery from herbal medicines," *Journal of Cheminformatics*, vol. 6, no. 1, article 13, 2014.
- [17] H. Yu, J. Chen, X. Xu et al., "A systematic prediction of multiple drug-target interactions from chemical, genomic, and pharmacological data," *PLoS ONE*, vol. 7, no. 5, Article ID e37608, 2012.

- [18] A. Martin, M. E. Ochagavia, L. C. Rabasa, J. Miranda, J. Fernandez-de-Cossio, and R. Bringas, "BisoGenet: a new tool for gene network building, visualization and analysis," *BMC Bioinformatics*, vol. 11, article 91, 2010.
- [19] P. Shannon, A. Markiel, O. Ozier et al., "Cytoscape: a software Environment for integrated models of biomolecular interaction networks," *Genome Research*, vol. 13, no. 11, pp. 2498–2504, 2003.
- [20] Y. Tang, M. Li, J. Wang, Y. Pan, and F.-X. Wu, "CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks," *BioSystems*, vol. 127, pp. 67–72, 2015.
- [21] G. D. Bader and C. W. V. Hogue, "An automated method for finding molecular complexes in large protein interaction networks," *BMC Bioinformatics*, vol. 4, no. 1, p. 2, 2003.
- [22] A. Barabási, N. Gulbahce, and J. Loscalzo, "Network medicine: a network-based approach to human disease," *Nature Reviews Genetics*, vol. 12, no. 1, pp. 56–68, 2011.
- [23] Z. Tang, C. Li, B. Kang, G. Gao, C. Li, and Z. Zhang, "GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses," *Nucleic Acids Research*, vol. 45, no. 1, pp. W98–W102, 2017.
- [24] D. W. Huang, B. T. Sherman, and R. A. Lempicki, "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources," *Nature Protocols*, vol. 4, no. 1, pp. 44–57, 2009.
- [25] G. Bindea, B. Mlecnik, H. Hackl et al., "ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks," *Bioinformatics*, vol. 25, no. 8, pp. 1091–1093, 2009.
- [26] R. Shi, D. Zhu, Z. Wei et al., "Baicalein attenuates monocrotaline-induced pulmonary arterial hypertension by inhibiting endothelial-to-mesenchymal transition," *Life Sciences*, vol. 207, pp. 442–450, 2018.
- [27] Q. Zhao, X.-Y. Chen, and C. Martin, "Scutellaria baicalensis, the golden herb from the garden of Chinese medicinal plants," *Chinese Science Bulletin*, vol. 61, no. 18, pp. 1391–1398, 2016.
- [28] I. Hussain, S. Waheed, K. A. Ahmad, J. E. Pirog, and V. Syed, "Scutellaria baicalensis targets the hypoxia-inducible factor-1 α and enhances cisplatin efficacy in ovarian cancer," *Journal of Cellular Biochemistry*, vol. 119, no. 9, pp. 7515–7524, 2018.
- [29] X. Yu, W. Tang, Y. Yang et al., "Long noncoding RNA NKILA enhances the anti-cancer effects of baicalein in hepatocellular carcinoma via the regulation of NF- κ B signaling," *Chemico-Biological Interactions*, vol. 285, pp. 48–58, 2018.
- [30] L. Jiang, H. Song, H. Guo, C. Wang, and Z. Lu, "Baicalein inhibits proliferation and migration of bladder cancer cell line T24 by down-regulation of microRNA-106," *Biomedicine & Pharmacotherapy*, vol. 107, pp. 1583–1590, 2018.
- [31] Z. Chen, R. Hou, S. Gao, D. Song, and Y. Feng, "Baicalein inhibits proliferation activity of human colorectal cancer cells HCT116 through downregulation of ezrin," *Cellular Physiology and Biochemistry*, vol. 49, no. 5, pp. 2035–2046, 2018.
- [32] B. Bie, J. Sun, Y. Guo et al., "Baicalein: A review of its anti-cancer effects and mechanisms in Hepatocellular Carcinoma," *Biomedicine & Pharmacotherapy*, vol. 93, pp. 1285–1291, 2017.
- [33] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings," *Advanced Drug Delivery Reviews*, vol. 64, no. 1-3, pp. 4–17, 2012.
- [34] Z. Wang, C. Jiang, W. Chen et al., "Baicalein induces apoptosis and autophagy via endoplasmic reticulum stress in hepatocellular carcinoma cells," *Biomed Research International*, vol. 2014, Article ID 732516, 13 pages, 2014.
- [35] Y. Wang, E. Han, Q. Xing et al., "Baicalein upregulates DDIT4 expression which mediates mTOR inhibition and growth inhibition in cancer cells," *Cancer Letters*, vol. 358, no. 2, pp. 170–179, 2015.
- [36] Y.-H. Zheng, L.-H. Yin, T. H. M. Grahn, A.-F. Ye, Y.-R. Zhao, and Q.-Y. Zhang, "Anticancer effects of baicalein on hepatocellular carcinoma cells," *Phytotherapy Research*, vol. 28, no. 9, pp. 1342–1348, 2014.
- [37] C.-H. Chen, L. L. H. Huang, C.-C. Huang, C.-C. Lin, Y. Lee, and F.-J. Lu, "Baicalein, a novel apoptotic agent for hepatoma cell lines: A potential medicine for hepatoma," *Nutrition and Cancer*, vol. 38, no. 2, pp. 287–295, 2000.
- [38] M. Kuo, H. C. Tsai, Y. L. Lin et al., "Mitochondrial-dependent caspase activation pathway is involved in baicalein-induced apoptosis in human hepatoma J5 cells," *International Journal of Oncology*, vol. 35, no. 4, article no 717, 2009.
- [39] S. Zhang, Y. Bao, X. Ju et al., "BA-j as a novel CDK1 inhibitor selectively induces apoptosis in cancer cells by regulating ROS," *Scientific Reports*, vol. 5, no. 1, pp. 13626–13626, 2015.
- [40] L. Ha, Y. Qian, S. Zhang et al., "Synthesis and biological evaluation of scutellaria flavone cyclanaminol mannich base derivatives as novel CDK1 inhibitors," *Anti-Cancer Agents in Medicinal Chemistry*, vol. 16, no. 7, pp. 914–924, 2016.
- [41] G. Su, H. Chen, and X. Sun, "Baicalein suppresses non small cell lung cancer cell proliferation, invasion and Notch signaling pathway," *Cancer Biomarkers*, vol. 22, no. 1, pp. 13–18, 2018.
- [42] R. Ohashi, C. Gao, M. Miyazaki et al., "Enhanced expression of cyclin E and cyclin A in human hepatocellular carcinomas," *Anticancer Research*, vol. 21, pp. 657–662, 2001.
- [43] C. X. Wu, X. Q. Wang, S. H. Chok et al., "Blocking CDK1/PDK1/ β -Catenin signaling by CDK1 inhibitor RO3306 increased the efficacy of sorafenib treatment by targeting cancer stem cells in a preclinical model of hepatocellular carcinoma," *Theranostics*, vol. 8, no. 14, pp. 3737–3750, 2018.
- [44] D. Cai, V. M. Latham Jr., X. Zhang, and G. I. Shapiro, "Combined depletion of cell cycle and transcriptional cyclin-dependent kinase activities induces apoptosis in cancer cells," *Cancer Research*, vol. 66, no. 18, pp. 9270–9280, 2006.
- [45] C. Haider, M. Grubinger, E. Řezníčková et al., "Novel inhibitors of cyclin-dependent kinases combat hepatocellular carcinoma without inducing chemoresistance," *Molecular Cancer Therapeutics*, vol. 12, no. 10, pp. 1947–1957, 2013.
- [46] S.-J. Cho, S.-S. Lee, Y.-J. Kim et al., "Xylocyline, a novel Cdk inhibitor, is an effective inducer of apoptosis in hepatocellular carcinoma cells in vitro and in vivo," *Cancer Letters*, vol. 287, no. 2, pp. 196–206, 2010.
- [47] J. Zhou, S. Han, W. Qian et al., "Metformin induces miR-378 to downregulate the CDK1, leading to suppression of cell proliferation in hepatocellular carcinoma," *Oncotargets & Therapy*, vol. 11, pp. 4451–4459, 2018.
- [48] A. J. Levine, "p53, the cellular gatekeeper for growth and division," *Cell*, vol. 88, no. 3, pp. 323–331, 1997.
- [49] C. H. Chen, T. S. Huang, C. H. Wong et al., "Synergistic anti-cancer effect of baicalein and silymarin on human hepatoma HepG2 Cells," *Food & Chemical Toxicology*, vol. 47, no. 3, pp. 638–644, 2009.

- [50] J. Gao, W. A. Morgan, A. Sanchez-Medina, and O. Corcoran, "The ethanol extract of *Scutellaria baicalensis* and the active compounds induce cell cycle arrest and apoptosis including upregulation of p53 and Bax in human lung cancer cells," *Toxicology and Applied Pharmacology*, vol. 254, no. 3, pp. 221–228, 2011.
- [51] S.-J. Kim, H.-J. Kim, H.-R. Kim et al., "Antitumor actions of baicalein and wogonin in HT-29 human colorectal cancer cells," *Molecular Medicine Reports*, vol. 6, no. 6, pp. 1443–1449, 2012.
- [52] D.-H. Lee, C. Kim, L. Zhang, and Y. J. Lee, "Role of p53, PUMA, and Bax in wogonin-induced apoptosis in human cancer cells," *Biochemical Pharmacology*, vol. 75, no. 10, pp. 2020–2033, 2008.
- [53] C. Wang, Y. Zhang, K. Guo et al., "Heat shock proteins in hepatocellular carcinoma: Molecular mechanism and therapeutic potential," *International Journal of Cancer*, vol. 138, no. 8, pp. 1824–1834, 2016.
- [54] Z. Yang, L. Zhuang, P. Szatmary et al., "Upregulation of heat shock proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumour tissues is associated with poor outcomes from HBV-related early-stage hepatocellular carcinoma," *International Journal of Medical Sciences*, vol. 12, no. 3, pp. 256–263, 2015.
- [55] J. Xiong, X.-M. Jiang, S.-S. Mao, X.-N. Yu, and X.-X. Huang, "Heat shock protein 70 downregulation inhibits proliferation, migration and tumorigenicity in hepatocellular carcinoma cells," *Oncology Letters*, vol. 14, no. 3, pp. 2703–2708, 2017.
- [56] B.-P. Huang, C.-S. Lin, C.-J. Wang, and S.-H. Kao, "Upregulation of heat shock protein 70 and the differential protein expression induced by tumor necrosis factor-alpha enhances migration and inhibits apoptosis of hepatocellular carcinoma cell HepG2," *International Journal of Medical Sciences*, vol. 14, no. 3, pp. 284–293, 2017.
- [57] H. Yukawa, K. Suzuki, K. Aoki et al., "Imaging of angiogenesis of human umbilical vein endothelial cells by uptake of exosomes secreted from hepatocellular carcinoma cells," *Scientific Reports*, vol. 8, no. 1, Article ID 6765, 2018.
- [58] J. Huang, C. Zheng, J. Shao, L. Chen, X. Liu, and J. Shao, "Overexpression of eEF1A1 regulates G1-phase progression to promote HCC proliferation through the STAT1-cyclin D1 pathway," *Biochemical and Biophysical Research Communications*, vol. 494, no. 3-4, pp. 542–549, 2017.
- [59] G. Liao, D. Yang, L. Ma et al., "The development of piperidinones as potent MDM2-P53 protein-protein interaction inhibitors for cancer therapy," *European Journal of Medicinal Chemistry*, vol. 159, pp. 1–9, 2018.
- [60] S. Shafique, W. Ali, S. Kanwal, and S. Rashid, "Structural basis for Cullins and RING component inhibition: Targeting E3 ubiquitin pathway conductors for cancer therapeutics," *International Journal of Biological Macromolecules*, vol. 106, pp. 532–543, 2018.
- [61] R. Alsiary, S. C. Brownhill, A. Brüning-Richardson et al., "Expression analysis of the MCPH1/BRIT1 and BRCA1 tumor suppressor genes and telomerase splice variants in epithelial ovarian cancer," *Gene*, vol. 672, pp. 34–44, 2018.