Journal of Clinical and Translational Hepatology **2022** vol. 10(4) | 627–641
DOI: 10.14218/JCTH.2021.00216

Original Article

Spindle and Kinetochore-associated Family Genes are Prognostic and Predictive Biomarkers in Hepatocellular Carcinoma

Chenhui Cai, Ying Zhang, Xu Hu, Sizhen Yang, Jiawen Ye, Zihan Wei and Tongwei Chu*

Department of Orthopedics, Xinqiao Hospital, Third Military Medical University (Army Medical University), Chongqing, China

Received: 9 June 2021 | **Revised:** 7 September 2021 | **Accepted:** 8 October 2021 | **Published:** 4 January 2022

Abstract

Background and Aims: Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors. Spindle and kinetochore-associated (SKA) family genes are essential for the maintenance of the metaphase plate and spindle checkpoint silencing during mitosis. Recent studies have indicated that dysregulation of SKA family genes induces tumorigenesis, tumor progression, and chemoresistance via modulation of cell cycle and DNA replication. However, the differential transcription of SKAs in the context of HCC and its prognostic significance has not been demonstrated. ***Methods:*** Bioinformatics analyses were performed using TCGA, ONCOMINE, HCCDB, Kaplan-Meier plotter, STRING, GEPIA databases. qRT-PCR, western blot, and functional assays were utilized for *in vitro* experiments. ***Results:*** We found remarkable upregulation of transcripts of SKA family genes in HCC samples compared with normal liver samples on bioinformatics analyses and *in vitro* validation. Interaction analysis and enrichment analysis showed that SKA family members were mainly related to microtubule motor activity, mitosis, and cell cycle. Immuno-infiltration analysis showed a correlation of all SKA family genes with various immune cell subsets, especially T helper 2 (Th2) cells. Transcriptional levels of SKA family members were positively associated with histologic grade, T stage, and α-fetoprotein in HCC patients. Receiver operating characteristic curve analysis demonstrated a strong predictive ability of SKA1/2/3 for HCC. Increased expression of these SKAs was associated with unfavorable overall survival, progression-free survival, and disease-specific survival. On Cox proportional hazards regression analyses, SKA1 upregulation and pathological staging were independent predictors of overall survival and disease-specific survival of HCC patients. Finally, clinical tissue microarray validation and *in vitro* functional assays revealed SKA1 acts an important regulatory role in tumor malignant behavior. ***Conclusions:*** SKA family members may potentially serve as diagnostic and prognostic markers in the context of HCC. The correlation between SKAs and immune cell infiltration provides a promising research direction for SKA-targeted immunotherapeutics for HCC.

Citation of this article: Cai C, Zhang Y, Hu X, Yang S, Ye J, Wei Z, *et al*. Spindle and Kinetochore-associated Family Genes are Prognostic and Predictive Biomarkers in Hepatocellular Carcinoma. J Clin Transl Hepatol 2022;10(4):627–641. doi: 10.14218/JCTH.2021.00216.

Introduction

Hepatocellular carcinoma (HCC) accounts for 75–85% of primary liver cancers and is associated with high morbidity and mortality.[1] Due to the high propensity for recurrence and metastasis, the overall survival of patients with advanced HCC is extremely poor. Every year, more than 500,000 deaths are attributable to HCC worldwide.[2,3] Efforts have been made to understand the mechanism of the development, progression, and metastasis of HCC. Use of serum markers and advanced medical imaging techniques can facilitate early diagnosis of HCC.[4–6] Besides, molecular targeted therapy, chemotherapy, immunotherapy, and surgical resection are effective treatment methods for HCC.[7,8] However, the molecular characteristics of HCC are not well characterized. The lack of specific markers for tumor type or disease stage poses a significant challenge in the understanding and treatment of HCC.

Mitosis, the basic form of cell division, is a common biological process in eukaryotes. During mitosis, the spindle ensures the division of chromosomes into two equal groups of sister chromatids.[9] The spindle and kinetochore-associated (SKA) complex is composed of SKA1/2/3 proteins and maintains the attachment of intermediate spindle microtubules to the centromere, thus ensuring the completion of mitosis.[10,11] Dysfunction of the SKA complex leads to chromosomal congression failure and subsequent cell death.[12] Recent studies have shown that abnormal cell cycle and uncontrolled cell proliferation can be attributed to the overexpression of SKAs;

Keywords: Spindle and kinetochore-associated genes; Liver hepatocellular carcinoma; Prognostic value; Immune infiltration; Bioinformatics analysis.

Abbreviations: AFP, α-fetoprotein; AUC, area under the curve; CCK-8, Cell Counting Kit-8; DC, dendritic cell; DSS, disease-specific survival; ESCC, esophageal squamous cell carcinoma; FBS, fetal bovine serum; GEO, Gene Expression Omnibus; GEPIA, gene expression profiling interactive analysis; GO, gene ontology; HCC, hepatocellular carcinoma; HCCDB, Integrative Molecular Database of Hepatocellular Carcinoma; ICGC, International Cancer Genome Consortium; IHC, immunohistochemical; KEGG, Kyoto Encyclopedia of Genes and Genomes; LIHC, liver hepatocellular carcinoma; OS, overall survival; PFS, progression-free survival; PPI, protein-protein interaction; ROC, receiver operating characteristic; SKA, spindle and kinetochore-associated; ssGSEA, single sample gene set enrichment analysis; TCGA, The Cancer Genome Atlas; Th2, T helper 2; TILs, tumor-infiltrating lymphocytes; TPM, transcripts per million.

***Correspondence to:** Tongwei Chu, Department of Orthopedics, Xinqiao Hospital, Third Military Medical University (Army Medical University), No.83 Xinqiao Main Street, Shapingba District, Chongqing 400037, China. ORCID: https://orcid.org/0000-0003-0309-7082. Tel: +86-13708388336, E-mail: chtw@sina.com

moreover, studies have also suggested a potential role of SKAs in the genesis, progression, and chemotherapeutic resistance of various types of tumors.[13–18] In an *in vitro* study, G2/M blockade of pancreatic cancer was inhibited by upregulating the *SKA1* gene, which enhanced pancreatic cancer aggressiveness and malignancy.[15] SKA2 expression was found to be significantly upregulated in esophageal squamous cell carcinoma (ESCC) samples and inhibition of SKA2 mRNA level suppressed ESCC cell proliferation and migration.[19] SKA3 overexpression accelerated the cell cycle by activating the PI3K-Akt signaling, thereby promoting cervical cancer cell migration and proliferation.[14] However, the expression profile, molecular biological function, and prognostic significance of SKA in HCC have not been fully elucidated.

In our research, we carried out comprehensive bioinformatics analysis of the expression patterns of SKA family genes in HCC, investigated their association with immune infiltration, assessed their diagnostic and prognostic relevance in HCC. We observed markedly high expression of SKA family genes in HCC, which simultaneously showed good diagnostic significance for HCC. Interaction analysis (i.e. protein interaction and gene interaction) and enrichment analysis showed that SKA family members were mainly related to microtubule motor activity, mitosis, and cell cycle. Comprehensive immune infiltration analysis showed that all SKA family members were correlated with immune cell subsets, especially Th2 cells. We further demonstrated an inverse association between increased expression of SKA family members and the prognosis of HCC patients and identified SKA1 as an independent predictor of HCC patients. Further functional assays indicated that knockdown of SKA1 decreased the proliferation and invasion of HCC cells. Our research aimed to elucidate the specific functions and mechanisms of SKA family genes in the development of HCC from a new perspective; our findings may facilitate a better understanding of the pathogenetic mechanism of HCC and offer insights for the development of SKA-targeted tumor immunotherapy.

Methods

Data resource

We downloaded level three data of liver hepatocellular carcinoma (LIHC) patients from The Cancer Genome Atlas (TCGA) database (cancergenome.nih.gov/), which contains data of 374 samples of HCC and 50 samples of para-carcinoma tissue. The workflow type we chose was HTSeq-FPKM. Corresponding clinical information was also obtained from the TCGA data portal. For the sake of subsequent analysis, we converted the HTSeq-FPKM data into transcripts per million (TPM) reads.

Gene expression profiling interactive analysis (GEPIA)

GEPIA is an online tool for analyses and visualization of RNA sequencing data of 9,700 tumors and 8,500 normal tissues.[20] In this study, transcriptional levels of SKA family genes in 20 distinct cancer samples and nonneoplastic control samples were acquired from GEPIA. Besides, we used a similar gene detection function in the "expression analysis module" to identify the most similar genes of SKA family genes.

Integrative Molecular Database of Hepatocellular Carcinoma (HCCDB)

HCCDB is a specialized database that integrates multiple databases including Gene Expression Omnibus (GEO), International Cancer Genome Consortium (ICGC), etc. It contains expression spectrum analysis of more than 3,000 HCC samples (lifeome.net/database/hccdb/).[21] We applied this comprehensive database to identify the expression of SKA family genes in liver cancer.

ONCOMINE

ONCOMINE database (oncomine.org) is an online data-mining tool that provides an integrated analysis of genome-wide expression in a wide variety of tumor samples and normal control samples.[22] We compared the transcription levels of SKA family members in different HCC samples and normal adjacent tissues. Statistical significance was considered at p-values <0.05. The fold change in our study was set to 2, and thresholds for statistical significance were set at 10%.

STRING

STRING (string-db.org/) is a search tool that predicts a network of genes and proteins that may have an interacting association.[23] A protein-protein interaction (PPI) network analysis of SKAs and their most similar genes were conducted using the STRING and further processed using the visualization tool Cytoscape.

GeneMANIA

GeneMANIA (genemania.org) is a visual database tool with highly accurate prediction algorithms providing information on physical interactions, coexpression, genetic interactions, and co-localization of query genes.[24] We used GeneMANIA to measure the predictive value of SKA family members.

Functional enrichment analysis

To identify the gene ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways where SKAs and similar genes were enriched, functional enrichment analysis was carried out using the R package cluster profiler.[25] Enrichment analysis predicts the functional roles of SKA family genes and their similar genes based on three aspects, i.e., biological process, cellular component, and molecular function, while KEGG analysis defines the related pathways of SKA family genes and their similar genes.

Immune infiltration analysis

The infiltration of 24 tumor-infiltrating immune cells in HCC samples was quantified by single sample gene set enrichment analysis (ssGSEA) methods using the GSVA R package. We scored the enrichment of every immunocyte based on gene signatures unique to 24 tumor-infiltrating lymphocytes (TILs), including B cells, T cells, macrophages, neutrophils, etc. Spearman correlation analysis was used to evaluate the correlation between SKAs and the tumor immune infiltration.

Kaplan-Meier plotter

Kaplan-Meier plotter is an online tool for survival analysis of cancer patients, and it was used to assess the prognostic significance of the expression of SKA family gene mRNA in

HCC patients.[26,27] Survival outcomes included overall survival (OS), progression-free survival (PFS), and disease-specific survival (DSS). The optimal cutoff value was set through the KM plotter algorithm. *P*-values <0.05 were considered significant.

Cell culture and transfection

Normal human liver cell line (L-02 cells) and HCC cell lines (SMMC-7721, LM3, and Hep3) were used in our experimental study. They were purchased from the China Cell Bank (ATCC, Shanghai, China). All cell lines were grown in Dulbecco's Modified Eagle Medium (Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS; Ausbian, Sydney, Australia) and 1% penicillin-streptomycin (Gibco, Carlsbad, CA, USA). Cells were maintained in an incubator at a constant temperature of 37°C with 5% CO_2. siRNA against SKA1 (5′-GCAUGUCAAGGAGCACCACAATTUUGUGGUGCUCCUUGACAUGCTT-3′) and short interfering noncoding oligonucleotides (5′-UUCUCCGAACGUGUCACGUTTACGUGACACGUUCGGAGAATT-3′)

were synthesized by Sangon Biotech (Sangon, Shanghai, China) and transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the product instructions. After 3 days of transfection, the cells were collected to determine the knockdown effects.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from cultured cells by Trizol reagent (Takara, Dalian, China). SYBR Premix Ex Taq II in a PCR detection system (Bio-Rad, Hercules, CA, USA) was used to assay the expression of target genes. cDNA was synthesized using PrimeScript RT reagent kits (Takara Dalian, China). The transcriptional levels were normalized to those of the internal control gene *GAPDH*. The sequences of all target genes are shown in Supplementary Table 5.

Western blotting

The protein concentration of cells lysates were measured with a Bradford protein assay. Total proteins were isolated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. After being blocked with 5% bovine serum albumin at room temperature for 3 h, the membranes were incubated overnight at 4°C with anti-GAPDH (1:1,000; Proteintech, Chicago, IL, USA) and anti-SKA1 (1:1,000; Affinity, Cincinnati, OH, USA) primary antibodies. The membrane was then washed with phosphate buffered saline Tween and incubated with secondary antibodies (1:4,000) for 2 h The protein bands were visualized with an enhanced chemiluminescence detection kit (Biosharp, Hefei, China).

Cell proliferation and invasion assays

Cell Counting Kit-8 (CCK-8) and colony formation assays were performed to assess cell proliferation. Cells maintained in 96-well plates were incubated with 10 μL CCK-8 solution (Dojindo, Kumamoto, Japan) at 37°C for 2.5 h. Cell viability was then determined by at 450 nm absorbance. To assay colony formation, about 2,500 HCC cells were seeded in six-well plates and cultured for 11 days. The colonies were then fixed in 4% paraformaldehyde and stained with crystal violet. Transwell assays were carried out to assess cell invasion. Cell migration was assayed in about 6×10^3 transfected HCC cells that were inoculated in serum-free medium on the Matrigel-coated upper surface of an 8 μm Transwell chamber (Corning, NY, USA). The culture medium in the lower chamber was supplemented with 350 μL of medium containing 10% FBS. The cells that had migrated through the membrane were stained with 0.5% crystal violet and counted by light microscopy.

HCC tissue microarray and immunohistochemical (IHC) staining

Human HCC tissue microarrays (Cat No. IWLT-N-64LV41) containing 10 pairs of HCC and adjacent normal tissues were obtained from Wuhan Saiweier Biotechnology Co; Wuhan, China), and CDT1 expression was assayed by IHC staining using an anti-SKA1 antibody (1:250; Affinity, Cincinnati, OH, USA). And following the kit manufacturer's instructions. The H-score method was used to assess the expression of SKA1 protein. Positivity and H-scores (0–300) were reported as (1×% of cells with weak staining intensity) + (2×% of cells with moderate staining intensity) + (3×% of cells with strong staining intensity).

Statistical analysis

The statistical analysis was performed with R version 3.6.3 (www.r-project.org). One-way analysis of variance was used to compare differences among the three groups, and between-group differences were compared using *t*-tests. Correlations of SKA expression and the clinical parameters of HCC patients were evaluated by the Wilcoxon signed-rank test. Univariate and multivariate Cox analysis was used to evaluate the prognostic significance of SKAs levels in terms of OS and DSS. Correlations of SKA family genes were assessed with Spearman's correlation coefficient. The performance of differentially expressed SKA family genes in distinguishing between HCC samples and normal liver samples was assessed by receiver operating characteristic (ROC) curve analysis using the pROC package (version 1.16.2).[28] An area under the ROC curve (AUC) >0.7 and from 0.5–0.7 indicated good and poor accuracy, respectively. The H-scores of HCC and adjacent healthy tissues were compared using paired *t*-tests. Statistical significance was defined as p <0.05.

Results

Transcription of SKAs in HCC patients

There is accumulating evidence that SKAs are novel tumor biomarkers.[29,30] However, transcriptional analysis and prognostic significance of SKA genes in human HCC have not been well elucidated. Therefore, GEPIA was used to compare the transcription levels of SKA family genes between normal and tumor samples of 33 different cancers. The mRNA levels of SKA family genes were significantly increased compared with normal tissues in the analysis of HCC tissues (p<0.001, Supplementary Fig. 1).

We further compared the transcriptional levels of SKAs between HCC samples and normal control samples in the HCCDB database. The results of the ICGC GEO dataset (GSE22058, GSE22097, GSE54236, GSE64041, GSE25097, and ICGC-LIRI-JP) analysis all suggested abnormally high expression of SKA1/2/3 in HCC (Fig. 1). We also compared the differences of transcription levels of SKAs between HCC cancer samples and normal samples in the TCGA database.

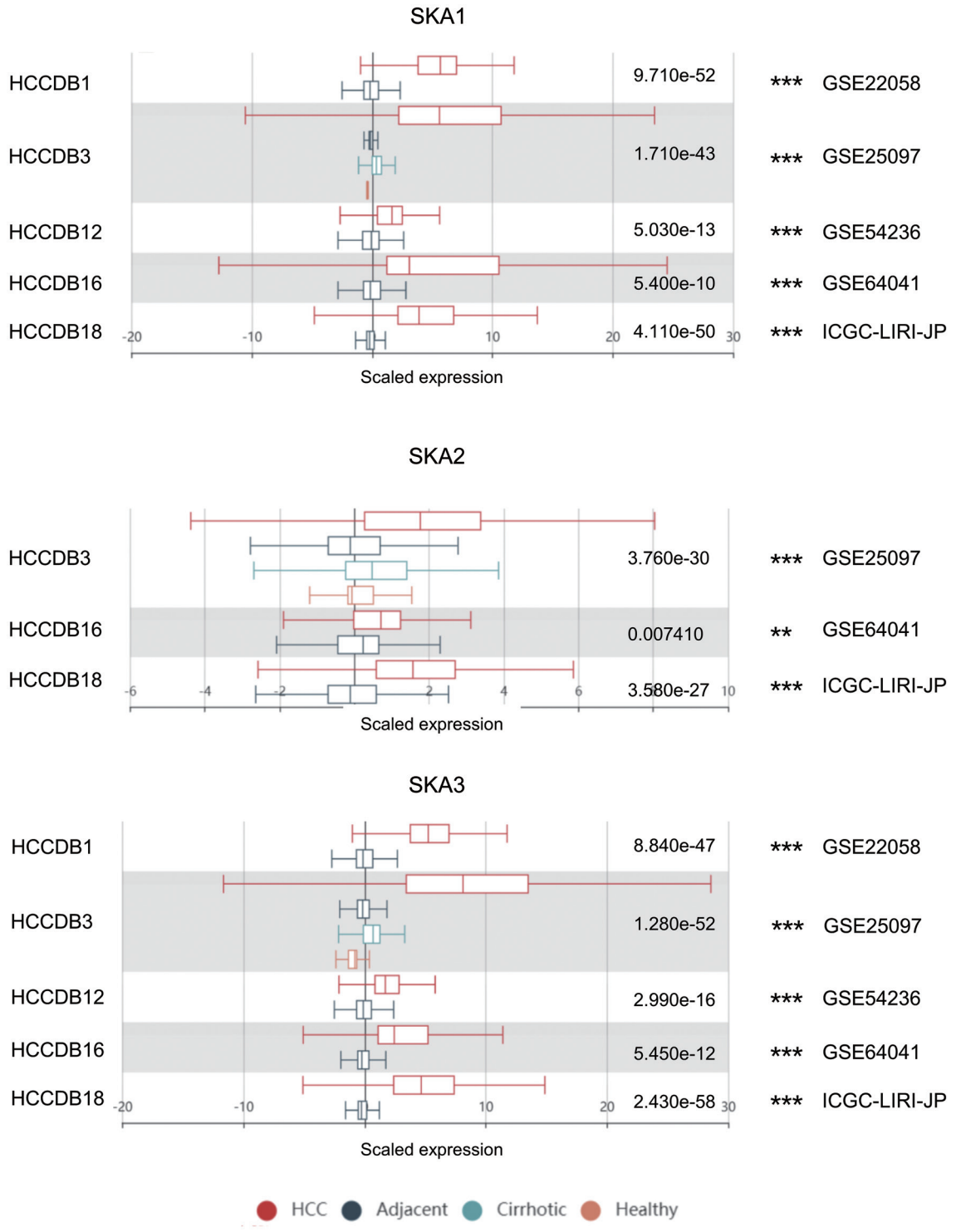

Fig. 1. mRNA level of SKA family genes in different GEO and ICGC datasets (HCCDB). *p<0.05, **p<0.01, ***p<0.001. GEO, Gene Expression Omnibus; HCCDB, Integrative Molecular Database of Hepatocellular Carcinoma; ICGC, International Cancer Genome Consortium; SKA, spindle and kinetochore-associated.

The mRNA levels of SKAs were markedly increased in HCC samples relative to normal liver samples, which was consistent with our previous result (Fig. 2A). The observation was also verified in paired HCC and normal tissues (Fig. 2B). We also compared the transcriptional levels of SKA family members between tumor tissues and normal control tissues in ONCOMINE databases. Specifically, the results from Chen datasets showed upregulation of SKA1 in HCC samples compared with that in normal samples, with fold changes of 1.212–2.534.[31–33] In Wurmbach datasets, HCC

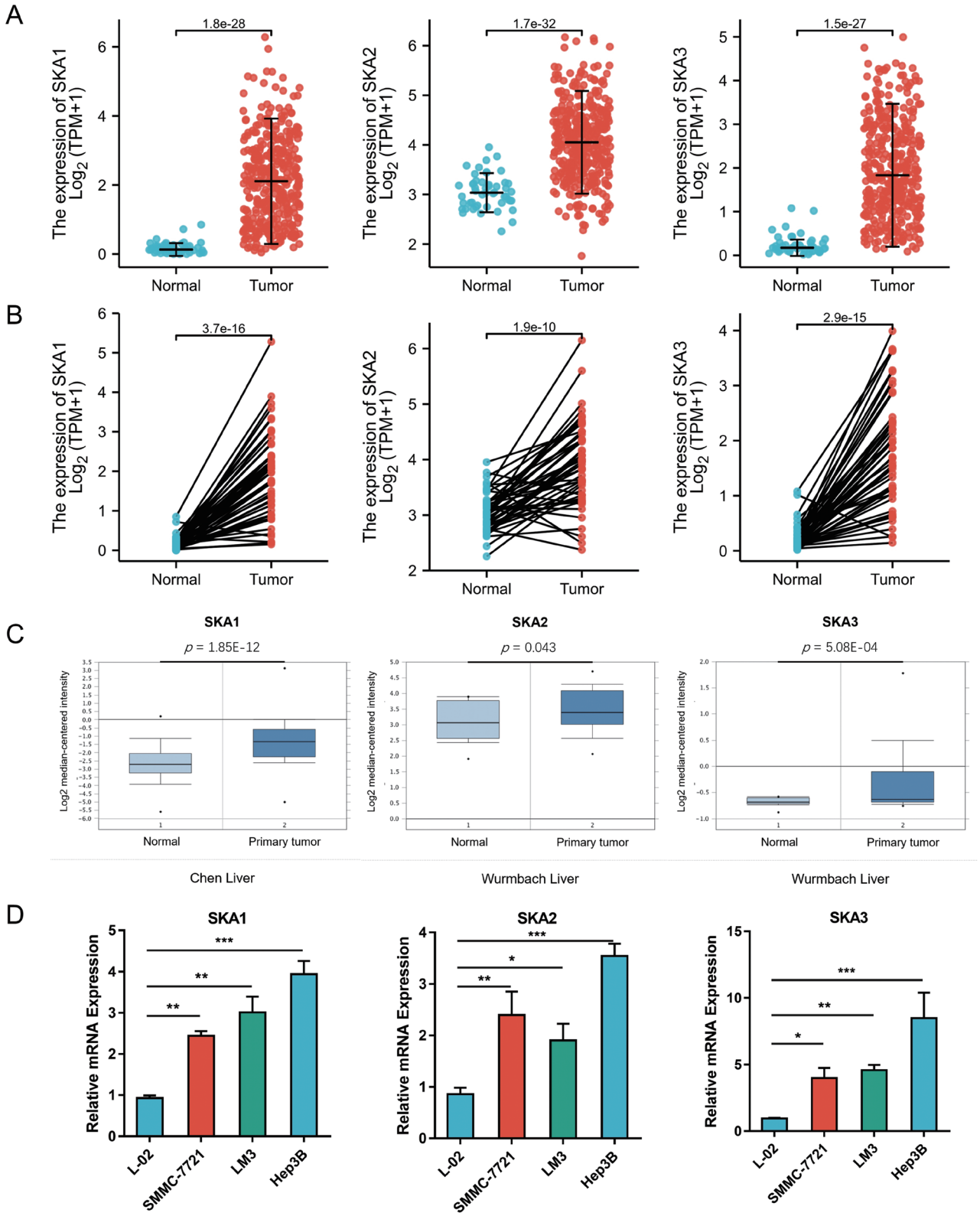

Fig. 2. Transcriptional levels of SKAs in hepatocellular carcinoma samples and normal tissue samples. (A) SKAs mRNA expression in normal and tumor tissues (TCGA). (B) SKA expression in paired tissues (TCGA). (C) SKA mRNA levels in normal and tumor tissues (ONCOMINE) (D) qRT-PCR results of SKA expression in normal human liver cell line and HCC cell lines. *p<0.05, **p<0.01, ***p<0.001. HCC, hepatocellular carcinoma; SKA, spindle and kinetochore-associated; TCGA, The Cancer Genome Atlas.

tissues showed a 1.266–1.331-fold increase in the mRNA expressions of SKA2 and SKA3 (Fig. 2C).[33] Finally, differences in the expression of SKAs were validated by qRT-PCR in normal human liver cell line and HCC cell lines (Fig. 2D).

Correlation and molecular interactions of SKAs in HCC

Correlation between the expression levels of SKA family members in HCC was assessed using Spearman correlation analysis. Scatter plot results indicated a strong correlation between the transcriptional levels of SKA1/3 (r=0.890). Besides, there was a moderate correlation between SKA1 and SKA2 (r=0.590) and between SKA2 and SKA3 (r=0.640, Fig. 3A–C).

Through GEPIA, we identified genes whose expression patterns were similar to those of differentially expressed SKAs in HCC patients. The results of similar gene detection are presented in Supplementary Table 1. To elucidate the potential interactions among SKAs and their most similar genes, we further built a PPI network by STRING dataset analysis and Cytoscape visualization (Fig. 3D). The SKA family genes and their similar genes were associated with microtubule binding, microtubule motor activity, cytoskeletal protein binding, and the cell cycle. The gene-gene interaction network through GeneMANIA also revealed that SKAs and their associated genes (e.g., NUDT5, SPC24, DSN1, NDC80, CENPE, and BUB1) were primarily related to chromosome segregation, mitosis, nuclear division, and microtubule polymerization or depolymerization (Fig. 3E).

GO and KEGG enrichment analyses of SKA family members and their most similar genes

To further investigate the potential mechanisms of SKAs in HCC, GO, and KEGG enrichment analyses were carried out to probe the functions and pathways of SKA family proteins and their most similar genes using the "ClusterProfiler" package in R software. The biological processes for these genes were predominantly enriched in mitotic nuclear division, nuclear division, organelle fission, and microtubule cytoskeleton organization. The molecular functions were mainly microtubule motor activity, motor activity, microtubule binding, and ATP-dependent microtubule motor activity. In terms of the cellular component category, the overexpressed SKA family members and their similar molecules were mainly associated with spindle, chromosome (centromeric region), mitotic spindle, and chromosomal region (Fig. 3F–H). The results of KEGG enrichment revealed several major KEGG pathways of SKAs and their similar genes, and included the p53 signaling pathway, cell cycle, and cell senescence (Fig. 3I). All the enriched pathways were tightly associated with the occurrence and progression of malignant tumors (Supplementary Table 2).[34,35]

Correlation between SKAs levels and immune infiltrates

The complexity of immune cells in the tumor microenvironment influences the biological behavior of the tumor, prognosis, and outcomes of immunotherapy.[36,37] Therefore, we investigated the correlation between various immune cells infiltrating in the HCC microenvironment and SKA family members. All members of the SKA family showed a positive correlation with T helper 2 (Th2) cells [SKA1 (r=0.752, p<0.001), SKA2 (r=0.438, p<0.001), SKA3 (r=0.718, p<0.001)], T follicular helper cells (Tfhs) [SKA1 (r=0.235, p<0.001), SKA2 (r=0.126, p=0.015), SKA3 (r=0.218, p<0.001)], and T helper (Th) cells [SKA1 (r=0.203, p<0.001), SKA2 (r=0.178, p<0.001), SKA3 (r=0.261, p<0.001)], as well as a negative correlation with neutrophils [SKA1 (r=−0.308, p<0.001), SKA2 (r=0.224, p=0.015), SKA3 (r=0.343, p<0.001)] and dendritic cells (DCs) [SKA1 (r=−0.272, p<0.001), SKA2 (r=−0.326, p<0.001), SKA3 (r=−0.328, p<0.001)]. The correlation with Th2 was the most significant (Fig. 4A–C). We further compared the enrichment score of Th2 cells of HCC samples in SKA1-high and SKA1-low groups. Consistently, the results demonstrated that high SKA1 expression samples had a higher enrichment score of Th2 cells than those in low SKA1 expression samples (Fig. 4D–F). All the above analyses indicated the significant correlation between SKA family members and immune cell subsets, especially Th2 cells.

Relationship of mRNA levels of SKAs and clinicopathological features of HCC patients

We further assessed the correlation between transcriptional levels of SKA genes and clinicopathologic features [such as histologic grade, T stage, and α-fetoprotein (AFP)] of HCC patients. The tissues obtained from HCC patients tended to express higher levels of SKAs mRNA as cancer stages advanced [SKA1, (G4 vs. G1, p<0.001), SKA2, (G3 vs. G2, p<0.001), SKA3, (G4 vs. G1, p<0.001)]. The highest mRNA levels of SKA family genes were predominantly found in grade 3 and grade 4 HCC (Fig. 5A–C). T stage has an important prognostic significance. Patients with high-T stage tumors tended to express higher mRNA levels of SKA1/2/3 according to the pathological T stage criterion (p<0.05) (Fig. 5D–F). AFP is a specific diagnostic biomarker for liver cancer. Therefore, we assessed the correlation between SKAs expression and AFP level. As expected, there were significant differences between the high-AFP group and the low-AFP group based on these SKAs expression (p<0.001, Fig. 5G–I). We further analyzed the association of SKAs expression with other clinical characteristics (e.g., age, sex, TNM stage, AFP, Child-Pugh grade, fibrosis Ishak score, vascular invasion, albumin, and prothrombin time) in HCC patients (Supplementary Table 3). High *SKA1* expression was significantly linked to T stage, AFP, and prothrombin time; high *SKA2* expression was significantly linked to age, T stage, AFP, and prothrombin time; high *SKA3* expression was significantly linked to T stage and AFP (p<0.05). The findings indicated the potential prognostic significance of the expression of SKA family members in patients with HCC.

Diagnostic and prognostic value of SKAs expression in HCC patients

To identify the diagnostic role of mRNA expression of SKAs for HCC, the R statistics pROC package was used to construct ROC curves based on transcriptome sequencing and clinical data derived from the TCGA database. The diagnostic potential of mRNA expression of these SKAs for HCC was assessed based on AUC (Fig. 6A–C). The AUCs, optimal cut-off values sensitivity, specificity and Youden index for predicting HCC are shown in Supplementary Table 4. Statistical analysis of the differences in the AUCs of SKAs suggested that the AUC of SKA1 (0.982, 95% CI: 0.970–0.994) was the largest, followed by SKA3 (0.973, 95% CI: 0.957–0.989) and *SKA2* (0.887, 95% CI: 0.852–0.922). We also assessed the diagnostic value of SKA expression for multiple clinical features of HCC patients, such as the pathological stage and TNM stage (Supplementary Fig. 2). The results showed that SKA expression had a certain significance in the diagnosis of TNM staging and pathological staging, which implied that the transcriptional levels of SKAs had a relatively greater sensi-

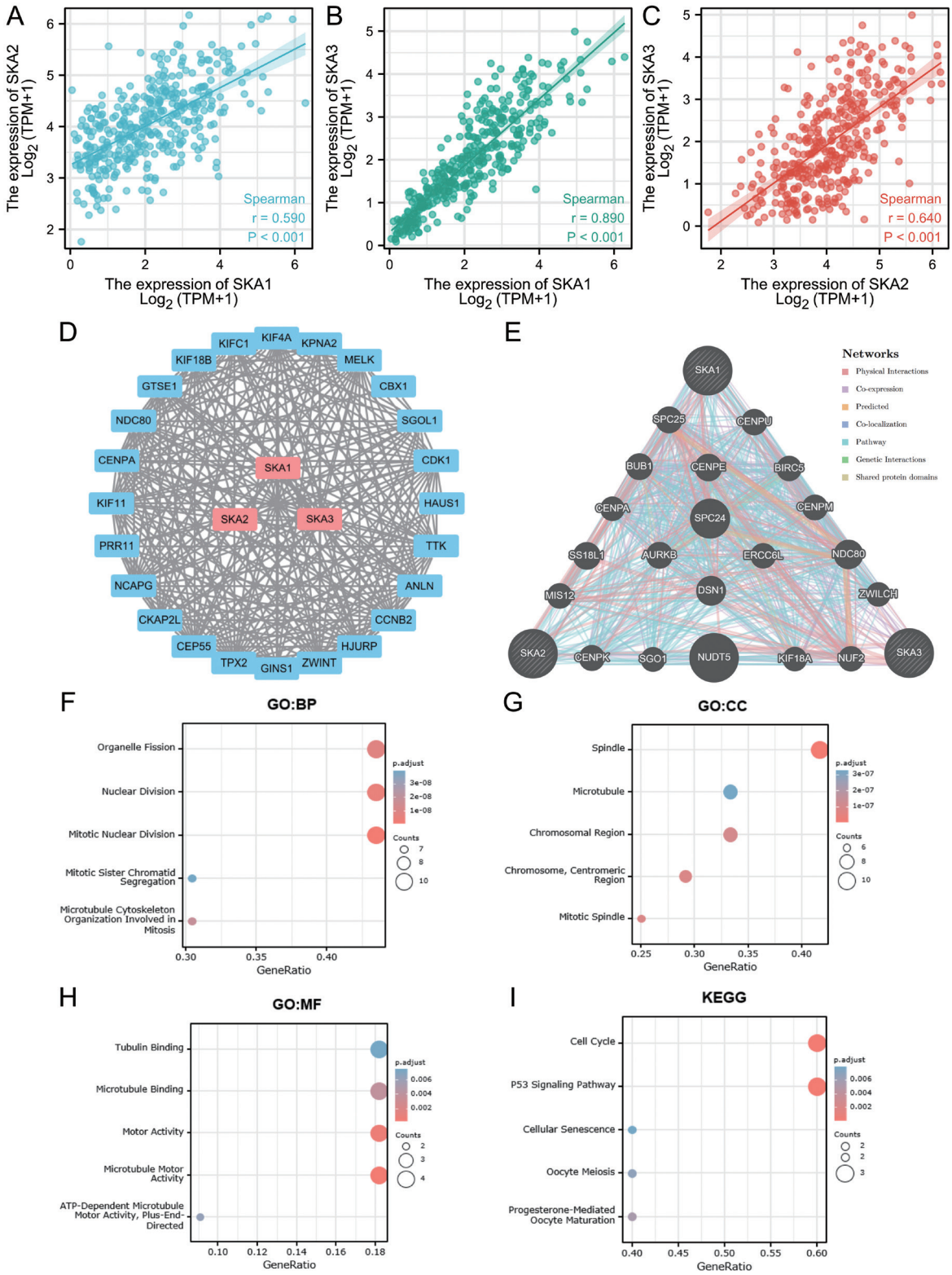

Fig. 3. Expression correlations, interaction analyses and enrichment analysis of SKAs in HCC. (A–C) Correlation scatter diagram of SKA family genes in HCC patients. (D) Interaction network of different expressed SKA family proteins on GeneMANIA datasets. (E) PPI network of different expressed SKAs and the 50 most similar genes based on STRING dataset analysis and Cytoscape visualization. (F–H) Bubble diagram results of GO enrichment analysis. (I) Bubble diagram results of KEGG enrichment analysis. GO, gene ontology; HCC, hepatocellular carcinoma; KEGG, and Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; SKA, spindle and kinetochore-associated.

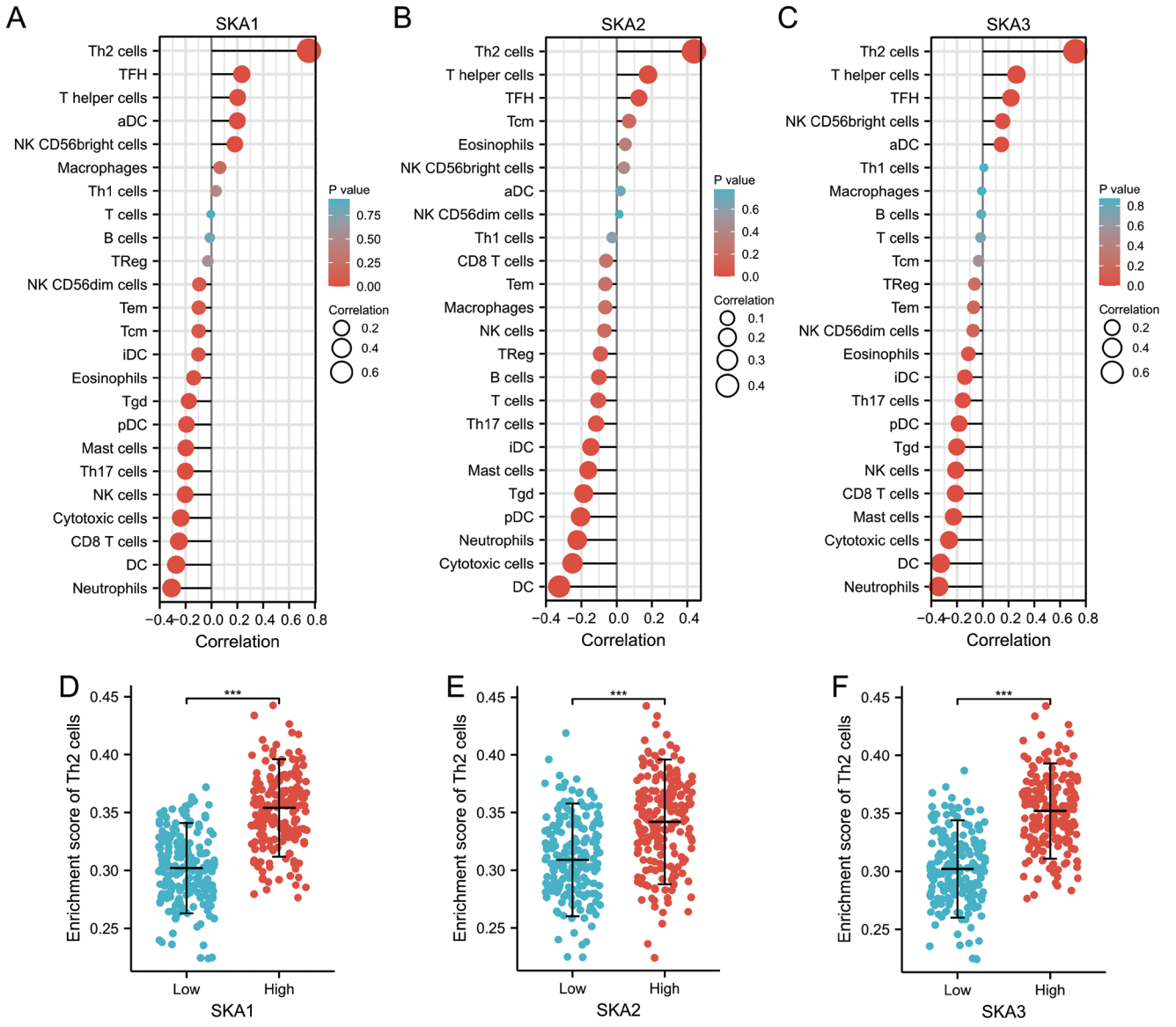

Fig. 4. Association of SKAs with infiltration of tumor immune cells. (A–C) Correlation between differentially expressed SKAs and infiltrating of 24 tumor immune cells (ssGSEA method). (D–F) Enrichment score of Th2 cells in HCC samples in SKA1-high and SKA1-low groups. Data are means ± SD. *p<0.05, **p<0.01, ***p<0.001. Th2, T helper 2; ssGSEA, single sample gene set enrichment analysis; HCC, hepatocellular carcinoma; SKA, spindle and kinetochore-associated.

tivity and specificity for the diagnosis of HCC. Furthermore, we analyzed the influence of the expressions of SKAs on the OS, PFS, and DSS to assess the prognostic significance of SKAs in HCC patients by Kaplan-Meier plotter analysis. The OS of patients with high expressions of SKA1/2/3 was remarkably lower than that of patients with low expression of the corresponding SKAs (p<0.01, Fig. 6D). In addition, increased mRNA expression of SKA1/2/3 were associated with poor PFS and DSS (p<0.05, Fig. 6E–F). The findings indicated the potential prognostic significance of SKA mRNA levels in HCC patients. We further assessed the prognostic value of SKA1/2/3 using univariate and multivariate Cox proportional hazards regression analysis (Table 1). RNA sequencing and clinical data of 374 HCC patients were Level 3 data of the LIHC project in the TCGA database. Univariate analysis showed an association of expression of SKA1/3 and pathologic stage with poor OS and DSS. The Child-Pugh grade was also associated with poor DSS. In multivariate analysis, high transcriptional levels of *SKA1* (HR=2.047, 95% CI: 1.211–3.459, p=0.007) and high pathologic stage (HR=1.920, 95% CI: 1.308–2.818, and p<0.001) were independent predictors of a significantly shorter OS of HCC patients. The expression of SKA1 mRNA, Child-Pugh grade, pathologic stage, and race were independent predictors of DSS. To sum up, we identified transcriptional levels of SKA1 as an independent predictor of OS and DSS of HCC patients.

SKA1 knockdown inhibited the in vitro tumorigenicity of HCC cells

In the previous analysis, SKA1 was confirmed as an independent prognostic factor for HCC. To further validate the role of CDT1 in HCC, we performed IHC staining on HCC

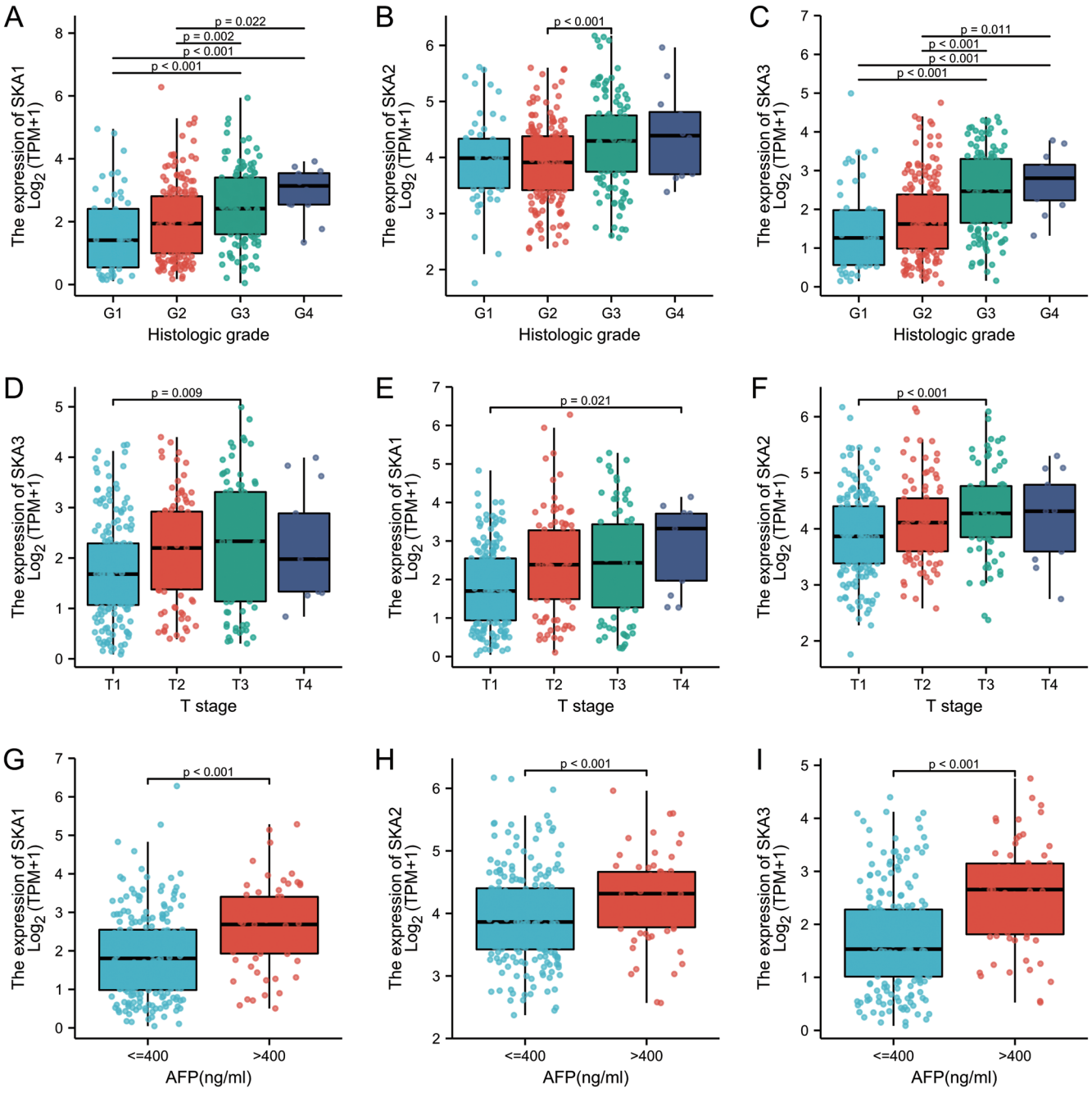

Fig. 5. Association of mRNA levels of SKAs with clinicopathological parameters of patients with hepatocellular carcinoma. (A–C) Relationship of SKA mRNA levels with histologic grade of HCC. (D–F) Correlation of SKA expression with T stage of HCC. (G–I) Relationship of SKA expression with AFP levels of HCC patients. **p*<0.05, ***p*<0.01, ****p*<0.001. AFP, α-fetoprotein; HCC, hepatocellular carcinoma; SKA, spindle and kinetochore-associated.

tissue microarrays. The results of the scatter plot analysis by paired *t*-tests revealed that SKA1 expression in HCC tissues was significantly higher than that in para-carcinoma tissues (Fig. 7A). The functional assays were also performed in LM3 and Hep3B cell lines. Western blot detection showed that SKA1 was markedly knocked down by SKA1 siRNA (Fig. 7B). CCK-8 and colony formation assays demonstrated that CDT1 knockdown significantly inhibited the proliferation and invasiveness of LM3 and Hep3B cells (Fig. 7C, E). CDT1 knockdown also significantly inhibited the invasiveness of HCC cells in Transwell assays (Fig. 7D). The results indicated that SKA1 knockdown suppressed the proliferation and invasiveness of HCC cells.

Discussion

HCC is associated with high mortality and morbidity rates and accounts for approximately 75–85% of all primary liver cancers worldwide. The poor 5-year survival makes it the

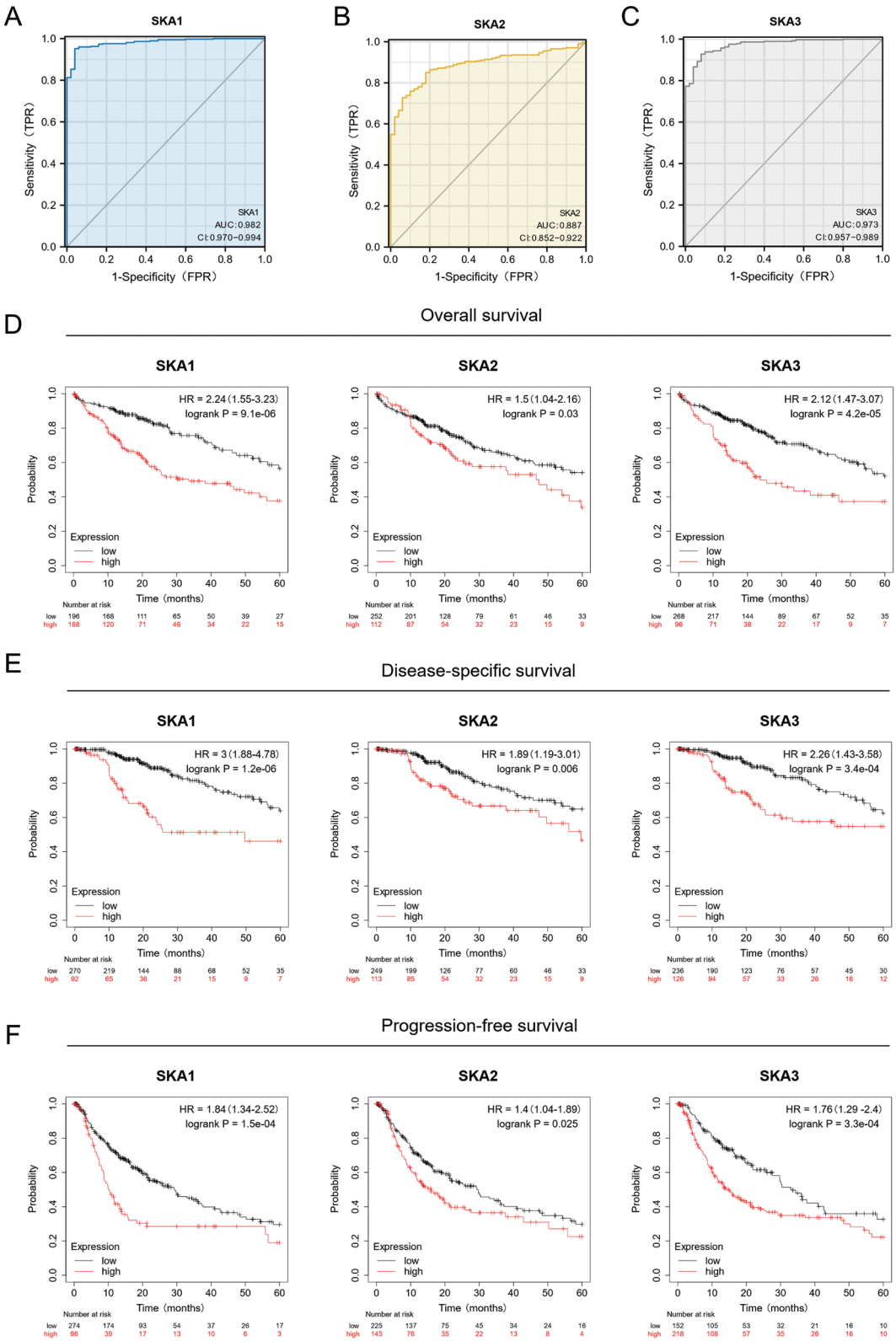

Fig. 6. Diagnostic significance and prognostic value of differentially expressed SKA family genes in HCC patients. (A–C) Diagnostic significance of different SKA mRNA levels in patients with HCC. (D) Correlation of differentially expressed SKAs and OS. (E) Correlation of differentially expressed SKAs and DSS. (F) Correlation of differentially expressed SKA and PFS. DSS, disease-specific survival; OS, overall survival; PFS, progression-free survival; HCC, hepatocellular carcinoma; SKA, spindle and kinetochore-associated.

Table 1. Cox regression analyses of variables for OS and DSS in LIHC patients

Characteristics	OS				DSS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Age (>60 vs. <=60)	1.205 (0.850–1.708)	0.295			0.846 (0.543–1.317)	0.458		
Gender (Male vs. Female)	0.793 (0.557–1.130)	0.200			0.813 (0.516–1.281)	0.373		
Race (White vs. Asian and Black or African American)	1.265 (0.881–1.816)	0.203			1.547 (0.964–2.481)	0.071	2.465 (1.113–5.458)	0.026
Pathologic stage (Stage II and Stage III and Stage IV vs. Stage I)	2.090 (1.429–3.055)	<0.001	1.920 (1.308–2.818)	<0.001	2.909 (1.718–4.925)	<0.001	2.101 (1.043–4.233)	0.038
AFP (ng/ml) (>400 vs. <=400)	1.075 (0.658–1.759)	0.772			0.867 (0.450–1.668)	0.668		
Fibrosis Ishak score (1/2 and ¾ and 5/6 vs. 0)	0.772 (0.465–1.281)	0.316			0.913 (0.474–1.757)	0.784		
Vascular invasion (Yes vs. No)	1.344 (0.887–2.035)	0.163			1.277 (0.707–2.306)	0.418		
Child-Pugh grade (B and C vs. A)	1.643 (0.811–3.330)	0.168			2.560 (1.123–5.834)	0.025	3.090 (1.273–7.499)	0.013
SKA1 (High vs. Low)	1.911 (1.341–2.724)	<0.001	2.047 (1.211–3.459)	0.007	2.505 (1.566–4.008)	<0.001	2.872 (1.266–6.513)	0.012
SKA2 (High vs. Low)	1.241 (0.878–1.752)	0.221			1.489 (0.954–2.325)	0.080	0.534 (0.240–1.189)	0.124
SKA3 (High vs. Low)	1.545 (1.090–2.188)	0.014	0.900 (0.542–1.494)	0.683	1.778 (1.132–2.795)	0.013	1.283 (0.536–3.071)	0.576

LIHC, liver hepatocellular carcinoma; OS, overall survival; DSS, disease-specific survival.

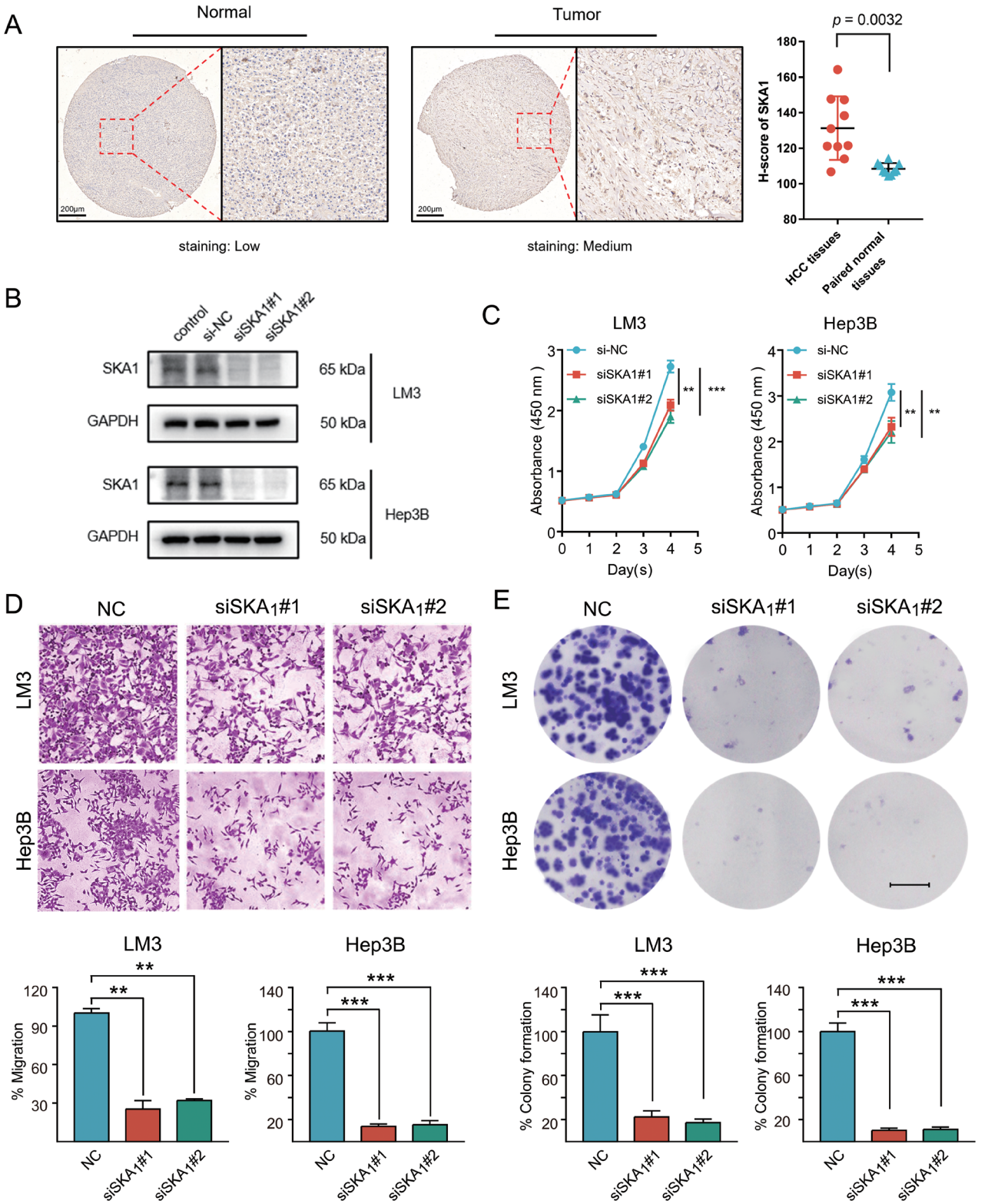

Fig. 7. Knockdown of SKA1 suppressed the proliferation and invasion abilities of HCC cells. (A) IHC staining results of SKA1 protein expression in HCC tissues and paired adjacent normal tissues and the corresponding scatter plot. (B) Western blot assay of SKA1 protein expression in SKA1-knockdown liver cancer cells. (C) The effect of CDT1 knockdown on cell viability was evaluated by CCK-8 assays. (D) Transwell assays reflected the effect of SKA1 knockdown on cell invasion in liver cancer cells. (E) Knockdown of SKA1inhibited clonogenic survival of HCC cells. Scale bars in (E) are 5 mm. Data are means ± SD of three independent experiments. *p<0.05, **p<0.01, ***p<0.001. CCK-8, Cell Counting Kit-8; HCC, hepatocellular carcinoma; IHC, immunohistochemical; SKA, spindle and kinetochore-associated.

second most common cause of death after lung cancer.[1] There are several risk factors for the occurrence and progression of HCC, including hepatitis B or C virus infection, aflatoxin exposure, contamination of drinking water, and excessive alcohol consumption.[38–40] Recent next-generation sequencing studies have demonstrated different evolutionary patterns of liver cancer and confirmed the high molecular heterogeneity in HCC;[1,41] the high incidence of metastasis and recurrence of liver cancer may be attributable to its high molecular heterogeneity. Although numerous studies have probed the molecular mechanisms that contribute to the occurrence, progression, and recurrence of HCC, there are limited therapeutic options to delay tumor progression and improve survival outcomes. Besides, the high metastasis and recurrence rates of HCC are a significant impediment to favorable treatment outcomes. The underlying mechanisms of the clinical characteristics of HCC are not well characterized. SKA family proteins, as essential mediators of mitosis and participate in the coordination of cell cycle and proliferation in eukaryotic cells. Several studies have demonstrated a close association between abnormal expression of SKAs and the occurrence or progression of tumors such as lung carcinoma,[29] invasive breast carcinoma,[17] prostatic intraepithelial neoplasia,[42] and other malignant tumors. However, the molecular mechanisms and functions of different SKA family members in HCC remain unclear. In this study, we used bioinformatics analysis to comprehensively and systematically describe the expression levels, diagnostic and prognostic significance, gene mutation patterns, transcriptional regulation, and immune cell infiltration levels of SKA family genes in HCC.

We found significantly higher expression levels of SKA1/2/3 in HCC tissues relative to nonneoplastic tissues. Significant upregulation of SKA1 has been demonstrated in a variety of tumors, such as non-small cell lung cancer,[43] prostate cancer,[42] and pancreatic ductal adenocarcinoma.[30] An *in vitro* study found that the transcriptional levels of SKA1 were significantly elevated in human non-small cell lung cancer tissues and positively correlated with the proliferation, invasion, and metastatic ability of lung cancer cell lines.[43] Li *et al*. reported that the overexpression of SKA1 induced centrosomal expansion of human prostatic epithelial cells by inducing centriole overduplication, which ultimately leads to spontaneous tumor formation in transgenic mouse models.[42] SKA1 has also been shown to be highly expressed in pancreatic ductal adenocarcinoma tissues compared with noncancer tissues. Survival analysis found that SKA1 expression was an independent prognostic factor for pancreatic cancer but was not related to relapse-free survival.[15] In our study, SKA1 expression was greater in HCC tissues than that in non-tumor tissue. We further explored the transcriptional levels of SKA1 in HCC and assessed their association with clinicopathological characteristics. The results showed a marked correlation of SKA1 expression with histologic grade, T stage, and AFP in HCC patients. Notably, ROC curve analysis showed that the expression of SKA1 had a good diagnostic value for liver cancer. Moreover, high SKA1 expression was linked with unfavorable outcomes in HCC patients. Through Cox proportional hazards regression analysis, we identified transcriptional expression of SKA1 as an independent predictor of poor OS and DSS of HCC patients. To further validate the role of SKA1 in HCC, we performed IHC staining of HCC tissue microarray and found that SKA1 expression in HCC tissues was markedly higher than that in para-carcinoma tissues. *In vitro* functional assays indicated that SKA1 knockdown significantly suppressed the proliferation and invasion abilities of HCC cells. To summarize, we identified SKA1 as an independent marker of survival outcomes in HCC and confirmed its important role in tumor cell malignant behavior.

Multiple studies have also focused on the oncogenic role of SKA2 in a variety of tumors. Wang *et al*. found significant upregulation of the transcriptional levels of SKA2 in breast cancer and that SKA2 knockdown inhibited the proliferation, invasion, and migration of breast cancer cells.[17] In another study, expression of SKA2 mRNA was higher in human ESCC tissues relative to nonneoplastic tissues, and SKA2 overexpression contributed to both proliferation and migration of ESCC cells via activation of Akt signaling *in vitro*.[19] A few studies have investigated the expression pattern, function, and mechanism of SKA2 in liver cancer. SKA2 expression was positively correlated with the expression of β-catenin in liver cancer cells *in vitro* and *in vivo*, and SKA2 knockdown inhibited tumor formation and growth in nude mice.[44] However, further studies are required to investigate the underlying molecular mechanisms, biological functions, and clinical applications of SKA2 in HCC. In the present study, HCC tissues showed higher expression of SKA2 compared with normal tissues, which was significantly linked with unfavorable histologic grade, T stage, and AFP levels. The results of ROC curve analysis suggested that SKA2 may be a potential diagnostic marker in HCC. Moreover, high SKA2 expression in HCC patients was correlated with unfavorable OS, PFS, and DSS. The results indicate the oncogenic role of SKA2 in HCC and its potential prognostic significance.

Similar to SKA1 and SKA2, the carcinogenic role of SKA3 has been demonstrated in multiple human malignancies, such as cervical cancer[14] and laryngeal squamous cell carcinoma.[45] An IHC study of 100 cervical cancer patients revealed overexpression of protein levels of SKA3 in advanced cervical cancer. High SKA3 expression was found to promote cervical cancer cell proliferation and invasion via the PI3K/Akt-dependent signaling.[14] In laryngeal squamous cell carcinoma, Li *et al*. initially revealed a role of SKA3 in regulating tumor proliferation through metabolic reprogramming and demonstrated that targeting SKA3 inhibited the chemotherapy and proliferation resistance of tumor cells by inhibiting glycolysis mediated by the PLK1-Akt axis.[45] The transcriptional levels of SKA3 were found to be significantly higher in pancreatic ductal adenocarcinoma tissues relative to para-carcinoma tissues and had a marked association with unfavorable prognosis and immune infiltration.[30] Consistent with the results of SKA1 and SKA2, our study revealed a higher transcriptional level of SKA3 in HCC samples compared with that in adjacent nonneoplastic tissues, and SKA3 expression showed a strong correlation with clinicopathological characteristics, such as histologic grade, T stage, and AFP levels. Early diagnosis of cancer is crucial for tracking the progression of the disease and early use of antitumor therapy. On ROC curve analysis, transcriptional levels of SKA3 were found to be highly sensitive and specific for the diagnosis of HCC. Predictably, HCC patients with high SKA3 expression showed markedly unfavorable OS, DFS, and DSS. The results revealed that SKA3 may be partially involved in the carcinogenic mechanism of HCC.

To further verify the possible oncogenic function of SKA family genes, we constructed a gene-gene interaction network of SKA family genes through GeneMANIA and a PPI network of SKAs and their most similar genes based on the STRING dataset. The two networks revealed that SKAs were primarily related to microtubule motor activity, cytoskeletal protein binding, and cell cycle, all of which were closely associated with abnormal proliferation of malignant tumors.

Subsequently, we explored the function of highly expressed SKAs and their similar genes through GO and KEGG enrichment analysis. As expected, the results showed that they were significantly related to cell cycle, cellular senescence, and the p53 signaling pathway. It is widely accepted that impaired regulation of the cell cycle leads to uncontrolled growth of normal cells and induces their transformation into tumor cells.[46,47] The p53 signaling pathway, which regulates the initiation of the cell cycle, is the most closely

related pathway found in human tumors.[48,49] Cellular senescence is a universal biological phenomenon, and recent studies have suggested that cellular senescence may contribute to the occurrence and development of tumors by inducing chronic inflammation.[50,51] Therefore, our results demonstrate the participation of SKA family genes in the carcinogenic mechanism of HCC and their potential value as new therapeutic targets.

As an important component of the tumor microenvironment, immune cell infiltration can have a significant impact on tumor progression as well as recurrence and is considered as a key determinant of responses to immunotherapy and clinical outcomes of cancer patients.[52,53] Recent studies have proven the SKA1 expression associated with an abundance of tumor-infiltrating immune cells in adrenocortical carcinoma was closely related with poor prognosis. It has also been reported that SKA1 and SKA3 have a vital role in the recruitment and regulation of immune-infiltrating cells in pancreas ductal adenocarcinoma, which eventually influence overall patient survival. Based on literature research and existing research basis, we analyzed the association of SKA and immune infiltration using the ssGSEA algorithm. We found that the transcriptional levels of SKA family genes were correlated with the infiltration of Th2 cells, Tfhs, and Th cells, neutrophils, and dendritic cells (DCs), especially Th2 cells. Helper T cells, both Th1 and Th2 cells, are important immune regulatory cells, and normally there is a dynamic equilibrium between Th1 and Th2 subtypes.[54] Increased secretion of Th2 cytokines in patients with malignant tumors induces Th1/Th2 drift, resulting in the imbalance of Th1/Th2.[55] Many tumors, including lung cancer, liver cancer, and gastric cancer, have a Th1/Th2 balance shift that is often dominated by Th2 cells in the body, which may be related to the immune escape of tumors.[56] Consistent with the above assumptions, we found a positive association of SKAs expression with Th2 cell infiltration in this study. We observed a significant positive correlation between mRNA levels of SKAs and markers of Th2 cells, thus revealing the underlying mechanism by which SKAs influence the immune microenvironment through altering the infiltration state of Th2 cells.

While our study revealed some important findings, there are some limitations. Most of the experimental results in this study are based on online bioinformatics data analysis, and further experiments are required to explore the underlying mechanisms. In addition, most of the analyses were based on transcriptome data. We did not explore the association between SKA family protein expression and the clinical outcomes of HCC patients. Finally, the specific mechanism by which SKA promotes the progression of HCC was not discussed in this review. Further study is required to address these issues.

Conclusion

In summary, we performed differential expression profiling of SKA family genes in HCC. We analyzed the association of the expression of SKA family genes with tumor-infiltrating immune cells and found positive correlations with Th2 cells. We also evaluated the diagnostic and prognostic significance of SKA family members, identified SKA1 as an independent marker of survival outcomes in HCC, and confirmed its important role in tumor cell malignant behavior. Further experimental studies are required to confirm our findings.

Acknowledgments

The authors thank the TCGA project and other groups for providing invaluable datasets for statistical analyses.

Funding

This research was funded by the General project of Chongqing Natural Science Foundation of China (No. cstc2020jcyj-msxmX0688), National Key Research and Development Plan Project (2016YFC1101504), and the Clinical Research Project of the Second Affiliated Hospital of the Army Military Medical University (2018XLC2016).

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Development of the idea, design of the research and drafting of the manuscript (CC), analysis of the data (YZ, XH, SY, JY, ZW), obtainment of copies of the studies, revision of the writing and project supervision (TC). All authors read and approved the submitted version.

Data sharing statment

The datasets supporting the conclusions of this article are included within the article and the supplementary information files.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71(3):209–249. doi:10.3322/caac.21660.
[2] Gao Q, Zhu H, Dong L, Shi W, Chen R, Song Z, *et al*. Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. Cell 2019;179(5):1240. doi:10.1016/j.cell.2019.10.038.
[3] Zhang Q, He Y, Luo N, Patel SJ, Han Y, Gao R, *et al*. Landscape and Dynamics of Single Immune Cells in Hepatocellular Carcinoma. Cell 2019;179(4):829–845.e820. doi:10.1016/j.cell.2019.10.003.
[4] Mehta N, Dodge JL, Grab JD, Yao FY. National Experience on Down-Staging of Hepatocellular Carcinoma Before Liver Transplant: Influence of Tumor Burden, Alpha-Fetoprotein, and Wait Time. Hepatology 2020;71(3):943–954. doi:10.1002/hep.30879.
[5] Thein HH, Qiao Y, Zaheen A, Jembere N, Sapisochin G, Chan KKW, *et al*. Cost-effectiveness analysis of treatment with non-curative or palliative intent for hepatocellular carcinoma in the real-world setting. PloS one 2017;12(10):e0185198. doi:10.1371/journal.pone.0185198.
[6] Zhu AX, Kang YK, Yen CJ, Finn RS, Galle PR, Llovet JM, *et al*. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2019;20(2):282–296. doi:10.1016/s1470-2045(18)30937-9.
[7] Kulik L, Heimbach JK, Zaiem F, Almasri J, Prokop LJ, Wang Z, *et al*. Therapies for patients with hepatocellular carcinoma awaiting liver transplantation: A systematic review and meta-analysis. Hepatology 2018;67(1):381–400. doi:10.1002/hep.29485.
[8] Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. Nat Rev Clin Oncol 2018;15(10):599–616. doi:10.1038/s41571-018-0073-4.
[9] Mitchison TJ, Salmon ED. Mitosis: a history of division. Nat Cell Biol 2001;3(1):E17–21. doi:10.1038/35050656.
[10] Gaitanos TN, Santamaria A, Jeyaprakash AA, Wang B, Conti E, Nigg EA. Stable kinetochore-microtubule interactions depend on the Ska complex and its new component Ska3/C13Orf3. EMBO J 2009;28(10):1442–1452. doi:10.1038/emboj.2009.96.
[11] Hanisch A, Silljé HH, Nigg EA. Timely anaphase onset requires a novel spindle and kinetochore complex comprising Ska1 and Ska2. EMBO J 2006;25(23):5504–5515. doi:10.1038/sj.emboj.7601426.
[12] Jeyaprakash AA, Santamaria A, Jayachandran U, Chan YW, Benda C, Nigg EA, *et al*. Structural and functional organization of the Ska complex, a key component of the kinetochore-microtubule interface. Mol Cell 2012;46(3):274–286. doi:10.1016/j.molcel.2012.03.005.
[13] Arai T, Okato A, Kojima S, Idichi T, Koshizuka K, Kurozumi A, *et al*. Regulation of spindle and kinetochore-associated protein 1 by antitumor miR-

10a-5p in renal cell carcinoma. Cancer Sci 2017;108(10):2088–2101. doi:10.1111/cas.13331.
[14] Hu R, Wang MQ, Niu WB, Wang YJ, Liu YY, Liu LY, *et al*. SKA3 promotes cell proliferation and migration in cervical cancer by activating the PI3K/Akt signaling pathway. Cancer Cell Int 2018;18:183. doi:10.1186/s12935-018-0670-4.
[15] Li T, Liu X, Xu B, Wu W, Zang Y, Li J, *et al*. SKA1 regulates actin cytoskeleton remodelling via activating Cdc42 and influences the migration of pancreatic ductal adenocarcinoma cells. Cell Prolif 2020;53(4):e12799. doi:10.1111/cpr.12799.
[16] Wang Y, Weng H, Zhang Y, Long Y, Li Y, Niu Y, *et al*. The PRR11-SKA2 Bidirectional Transcription Unit Is Negatively Regulated by p53 through NF-Y in Lung Cancer Cells. Int J Mol Sci 2017;18(3):534. doi:10.3390/ijms18030534.
[17] Wang Y, Zhang C, Mai L, Niu Y, Wang Y, Bu Y. PRR11 and SKA2 gene pair is overexpressed and regulated by p53 in breast cancer. BMB Rep 2019;52(2):157–162. doi:10.5483/BMBRep.2019.52.2.207.
[18] Zhang Q, Sivakumar S, Chen Y, Gao H, Yang L, Yuan Z, *et al*. Ska3 Phosphorylated by Cdk1 Binds Ndc80 and Recruits Ska to Kinetochores to Promote Mitotic Progression. Curr Biol 2017;27(10):1477–1484.e1474. doi:10.1016/j.cub.2017.03.060.
[19] Chen J, Yang HM, Zhou HC, Peng RR, Niu ZX, Kang CY. PRR11 and SKA2 promote the proliferation, migration and invasion of esophageal carcinoma cells. Oncol Lett 2020;20(1):639–646. doi:10.3892/ol.2020.11615.
[20] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Oncol Lett 2017;45(W1):W98–W102. doi:10.1093/nar/gkx247.
[21] Lian Q, Wang S, Zhang G, Wang D, Luo G, Tang J, *et al*. HCCDB: A Database of Hepatocellular Carcinoma Expression Atlas. Genomics Proteomics Bioinformatics 2018;16(4):269–275. doi:10.1016/j.gpb.2018.07.003.
[22] Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, *et al*. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 2004;6(1):1–6. doi:10.1016/s1476-5586(04)80047-2.
[23] Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, *et al*. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47(D1):D607–d613. doi:10.1093/nar/gky1131.
[24] Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, *et al*. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010;38(Web Server issue):W214–W220. doi:10.1093/nar/gkq537.
[25] Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16(5):284–287. doi:10.1089/omi.2011.0118.
[26] Győrffy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q, *et al*. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat 2010;123(3):725–731. doi:10.1007/s10549-009-0674-9.
[27] Gyorffy B, Lánczky A, Szállási Z. Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients. Endocr Relat Cancer 2012;19(2):197–208. doi:10.1530/erc-11-0329.
[28] Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, *et al*. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12:77. doi:10.1186/1471-2105-12-77.
[29] Chen C, Guo Q, Song Y, Xu G, Liu L. SKA1/2/3 serves as a biomarker for poor prognosis in human lung adenocarcinoma. Transl Lung Cancer Res 2020;9(2):218–231. doi:10.21037/tlcr.2020.01.20.
[30] Liu Y, Jin ZR, Huang X, Che YC, Liu Q. Identification of Spindle and Kinetochore-Associated Family Genes as Therapeutic Targets and Prognostic Biomarkers in Pancreas Ductal Adenocarcinoma Microenvironment. Front Oncol 2020;10:553536. doi:10.3389/fonc.2020.553536.
[31] Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, *et al*. Gene expression patterns in human liver cancers. Mol Biol Cell 2002;13(6):1929–1939. doi:10.1091/mbc.02-02-0023.
[32] Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, *et al*. A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. Cancer Res 2010;70(24):10202–10212. doi:10.1158/0008-5472.Can-10-2607.
[33] Wurmbach E, Chen YB, Khitrov G, Zhang W, Roayaie S, Schwartz M, *et al*. Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. Hepatology 2007;45(4):938–947. doi:10.1002/hep.21622.
[34] Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer 2009;9(2):95–107. doi:10.1038/nrc2584.
[35] Phan TG, Croucher PI. The dormant cancer cell life cycle. Nat Rev Cancer 2020;20(7):398–411. doi:10.1038/s41568-020-0263-0.
[36] Wang S, Zhang Q, Yu C, Cao Y, Zuo Y, Yang L. Immune cell infiltration-based signature for prognosis and immunogenomic analysis in breast cancer. Brief Bioinform 2021;22(2):2020–2031. doi:10.1093/bib/bbaa026.
[37] Xiong Y, Wang K, Zhou H, Peng L, You W, Fu Z. Profiles of immune infiltration in colorectal cancer and their clinical significant: A gene expression-based study. Cancer Med 2018;7(9):4496–4508. doi:10.1002/cam4.1745.
[38] de Martel C, Maucort-Boulch D, Plummer M, Franceschi S. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. Hepatology 2015;62(4):1190–1200. doi:10.1002/hep.27969.
[39] El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132(7):2557–2576. doi:10.1053/j.gastro.2007.04.061.
[40] Schaper M, Rodriguez-Frias F, Jardi R, Tabernero D, Homs M, Ruiz G, *et al*. Quantitative longitudinal evaluations of hepatitis delta virus RNA and hepatitis B virus DNA shows a dynamic, complex replicative profile in chronic hepatitis B and D. J Hepatol 2010;52(5):658–664. doi:10.1016/j.jhep.2009.10.036.
[41] Nault JC, Villanueva A. Intratumor molecular and phenotypic diversity in hepatocellular carcinoma. Clin Cancer Res 2015;21(8):1786–1788. doi:10.1158/1078-0432.Ccr-14-2602.
[42] Li J, Xuan JW, Khatamianfar V, Valiyeva F, Moussa M, Sadek A, *et al*. SKA1 overexpression promotes centriole over-duplication, centrosome amplification and prostate tumourigenesis. J Pathol 2014;234(2):178–189. doi:10.1002/path.4374.
[43] Shen L, Yang M, Lin Q, Zhang Z, Miao C, Zhu B. SKA1 regulates the metastasis and cisplatin resistance of non-small cell lung cancer. Oncol Rep 2016;35(5):2561–2568. doi:10.3892/or.2016.4670.
[44] Jiang J, Xu B, Zheng Y, Guo X, Chen F. Spindle and kinetochore-associated protein 2 facilitates the proliferation and invasion of hepatocellular carcinoma via the regulation of Wnt/β-catenin signaling. Exp Cell Res 2020;395(1):112181. doi:10.1016/j.yexcr.2020.112181.
[45] Gao W, Zhang Y, Luo H, Niu M, Zheng X, Hu W, *et al*. Targeting SKA3 suppresses the proliferation and chemoresistance of laryngeal squamous cell carcinoma via impairing PLK1-AKT axis-mediated glycolysis. Cell Death Dis 2020;11(10):919. doi:10.1038/s41419-020-03104-6.
[46] Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. Nature 2004;432(7015):316–323. doi:10.1038/nature03097.
[47] Swanton C. Cell-cycle targeted therapies. Lancet Oncol 2004;5(1):27–36. doi:10.1016/s1470-2045(03)01321-4.
[48] Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death Differ 2018;25(1):104–113. doi:10.1038/cdd.2017.169.
[49] Mandinova A, Lee SW. The p53 pathway as a target in cancer therapeutics: obstacles and promise. Sci Transl Med 2011;3(64):64rv61. doi:10.1126/scitranslmed.3001366.
[50] Childs BG, Baker DJ, Kirkland JL, Campisi J, van Deursen JM. Senescence and apoptosis: dueling or complementary cell fates? EMBO Rep 2014;15(11):1139–1153. doi:10.15252/embr.201439245.
[51] Herranz N, Gil J. Mechanisms and functions of cellular senescence. J Clin Invest 2018;128(4):1238–1246. doi:10.1172/jci95148.
[52] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol 2013;14(10):1014–1022. doi:10.1038/ni.2703.
[53] Li B, Severson E, Pignon JC, Zhao H, Li T, Novak J, *et al*. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol 2016;17(1):174. doi:10.1186/s13059-016-1028-7.
[54] Ding ZC, Blazar BR, Mellor AL, Munn DH, Zhou G. Chemotherapy rescues tumor-driven aberrant CD4+ T-cell differentiation and restores an activated polyfunctional helper phenotype. Blood 2010;115(12):2397–2406. doi:10.1182/blood-2009-11-253336.
[55] Ruterbusch M, Pruner KB, Shehata L, Pepper M. In Vivo CD4(+) T Cell Differentiation and Function: Revisiting the Th1/Th2 Paradigm. Annu Rev Immunol 2020;38:705–725. doi:10.1146/annurev-immunol-103019-085803.
[56] Matsuzaki J, Tsuji T, Imazeki I, Ikeda H, Nishimura T. Immunosteroid as a regulator for Th1/Th2 balance: its possible role in autoimmune diseases. Autoimmunity 2005;38(5):369–375. doi:10.1080/08916930500124122.