SOCS2 serves as a prognostic indicator and is regulated by miR-7-5p in hepatocellular carcinoma

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Research

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Abstract

Background

Suppressor of cytokine signaling (SOCS) family members are essential components of negative regulation of cytokine signaling known to be involved in occurrence and progression of hepatocellular carcinoma (HCC), while a comprehensive analysis of the correlation between SOCS family members and HCC has not yet been elucidated.

Methods

Differential expression analysis of SOCS genes was performed on TIMER, which was further validated by GSE94660 dataset from Gene Expression Omnibus (GEO). Prognostic values of SOCS genes were analyzed by TIMER and GEPIA. TISIDB was used to assess association between SOCS2 expression, clinical stages and pathological grades of HCC, as well as SOCS2 expression across immune subtypes and iClusters. Differential expression of genes (DEGs) identification was tested by two-tail student's t test using The Cancer Genome Atlas (TCGA) RNA-seq of HCC. And functional annotation of the DEGs was performed by Metascape. Fraction of Immune cells was estimated by CIBERSORT, and infiltration difference were compared by two-tail student's t test. Genetic alteration identification and promoter methylation evaluation were analyzed by cBioPortal and DNMIVD, respectively. Starbase was used to predict potential miRNAs that target SOCS2. Differential expressions of candidate miRNAs were analyzed by dbMEMC, which was further validated by GSE22058 from GEO. Survival analysis of miRNA was performed with KM Plotter.

Results

Differential expression analysis showed SOCS2 and SOCS3 were significantly downregulated, while SOCS5 and SOCS7 were upregulated in HCC. Survival analysis revealed only SOCS2 mRNA had significant prognostic value in terms of overall survival and disease-free survival in HCC. Specifically, higher SOCS2 predicted improved outcome. Significant correlations were found between SOCS2 and pathological stage, grade, molecular subtypes and immune subtypes. When comparing $SOCS2^{high}$ versus $SOCS2^{low}$ patients, the DEGs were functionally enriched in metabolism of RNA, organic cyclic compound catabolic process, and rRNA processing in the nucleus and cytosol. Immune cell infiltration analysis showed resting memory CD4 T cells, γδ T cells, follicular helper T cells, regulatory T cells and M0 macrophages were associated with SOCS2 expression. Mechanistically, miR-7-5p was the potential contributor responsible for downregulation of SOCS2.

Conclusions

SOCS2 could be a promising prognostic indicator and a potential therapeutic target for HCC.

1. Background

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and represents a major health challenge worldwide. As one of the leading causes of cancer-related deaths, HCC leads to more than half a million deaths across the world annually (1, 2). Diagnosis at late stages, high incidence of metastasis, and postoperative recurrence are the main contributors of high mortality of HCC (3). Although significant advances have been made in recent decades, the prognosis of patients with HCC remain unfavorable. Therefore, identifying prognostic biomarkers and molecules related to the response to the current treatments of HCC is still urgent to be explored.

Chronic nonresolving inflammation has been recognized as a distinctive feature and an important driver of human malignancies, including HCC (4, 5). In HCC microenvironment, many inflammatory cytokines were demonstrated to make a contribution to poor prognosis of HCC, such as macrophage colony-stimulating factor (6), interleukin 2, interleukin 15 (7), and TNF-α (8). However, the cellular and molecular mechanism between chronic inflammation and HCC genesis and prognosis remains unclear. Among all the molecules and signaling pathway studied most widely, JAK/STAT signaling pathway, a major downstream pathway of pro-inflammatory cytokines, has been implicated in HCC development (9, 10). Protein tyrosine phosphatases, protein inhibitors of activated STATs and suppressor of cytokine signaling (SOCS) family members contribute to the negative regulation of cytokine signaling. Among these inhibitors, the SOCS family is critical negative regulator of JAK/STAT pathway upon cytokine stimulation. Although aberrant expression or promoter methylation of some SOCS genes have been reported in association with vascular invasion, cell growth and migration, and metastasis (11–16), a comprehensive analysis of the correlation between expression of SOCS family members and HCC has not yet been elucidated.

In the current study, we evaluated the expression alteration and prognostic value of SOCS genes in HCC patients using TCGA dataset and Gene Expression Omnibus (GEO) dataset (GSE94660). As the most significant prognostic factor, SOCS2 expression was associated with clinical or immunological features of HCC. We also screened differentially expressed genes according to different SOCS2 expression levels and performed functional enrichment analysis to assess the impact of SOCS2 on biological processes. In addition, we evaluated the association between SOCS2 expression and inferred immune cell infiltration in HCC. Finally, we explored the potential mechanisms that account for down-regulation of SOCS2 in HCC. This study provides novel insight into the potential function of SOCS2 and may offer promising therapeutic target for developing anti-HCC strategy.

2. Materials And Methods

a) Expression and survival analysis of SOCS family members

TIMER (http://timer.cistrome.org/) is a web resource that allows investigators to analyze RNA-seq data of tumors and normal tissues from TCGA project (17). Here TIMER was engaged to perform differential expression analysis of SOCS genes in tumor and normal tissues of HCC patients. In addition, gene expression dataset GSE94660 (18), which contains 21 paired HCC tissues and adjacent normal tissues, was obtained from GEO database to validate the differential expression of the SOCSs. Prognostic values of SOCS genes in HCC were also analyzed by TIMER for overall survival (OS) and GEPIA2 database (http://gepia.cancer-pku.cn/) for disease-free survival (DFS) (19).

b) Correlation analysis

Clinical stages and pathological grades are associated with patients' outcome. TISIDB (http://cis.hku.hk/TISIDB/) (20), an integrated portal for tumor-immune system interactions, was used to assess association between SOCS2 expression and clinical stages as well as pathological grades of HCC. In addition, SCOS2 expression across immune subtypes and iClusters were also evaluated by TISIDB.

c) Differentially expressed genes identification and functional enrichment analysis

RNA-seq data of HCC profiled by TCGA was collected from the FireHose data portal (https://gdac.broadinstitute.org/). Samples were divided into three groups based on their SOCS2 expression (low, intermediate, high), and the 25th and 75th percentiles were used as cutoff thresholds. Differential expression of genes between low and high SOCS2 expression was tested by two-tail student's t test. Metascape (https://metascape.org/gp/index.html) is a powerful web tool that provides a biologist-oriented resource for the analysis of large list of genes and therefore was used to perform functional annotation of the DEGs (21).

d) Immune cell infiltration analysis

CIBERSORT (https://cibersort.stanford.edu/) is an analytical tool that allows estimation of the abundances of immune cells from bulk tissue RNA-seq data (22). Immune cell fractions inferred by CIBERSORT from RNA-seq data of HCC profiled by TCGA were downloaded from Genomic Data Commons (https://gdc.cancer.gov/about-data/publications/panimmune). Immune cell infiltration difference between SOCS2 low and SOCS2 high samples were compared by two-tail student's t test.

e) Genetic alteration identification and promoter methylation evaluation

cBioPortal (http://www.cbioportal.org/) is an open platform for investigating multidimensional cancer genomics data including these from TCGA project (23). Genetic alteration of SOCS2 and its impact on patients' outcome in TCGA-LIHC were queried in cBioPortal. DNMIVD (http://119.3.41.228/dnmivd/index/) is a comprehensive annotation database for DNA methylation and allows researchers to evaluate promoter methylation levels of a gene across human cancers (24). DNMIVD was engaged to assess promoter methylation levels of SOCS2, correlation between promoter

methylation and mRNA expression of SOCS2, and prognostic value of SOCS2 promoter methylation in TCGA-LIHC.

f) miRNA prediction, expression and survival analysis

Starbase (http://starbase.sysu.edu.cn/) was used to predict potential miRNAs that target SOCS2 (25), and evaluate correlation between miRNA expression and SOCS2 expression in HCC. Differential expressions of candidate miRNAs were analyzed by dbMEMC (https://www.picb.ac.cn/dbDEMC/), an integrated database which designed to retrieve differentially expressed miRNA in human cancers based on data collected from TCGA (26). In addition, gene expression dataset GSE22058 was obtained from GEO database to validate the differential expression of the miR-7-5p (27). Survival analysis of miRNA was performed with KM Plotter (https://kmplot.com/analysis/) (28).

3. Results

3.1 Expression analysis of SOCS family members in HCC

Transcription levels of eight SOCS genes in TCGA database were determined by TIMER, which revealed that CISH, SOCS2, SOCS3, and SOCS6 possessed higher expression levels in normal liver tissues, while expression of SCOS5 and SCOS7 were upregulated in HCC tissues (Fig. 1A). To validate these findings, GSE94660 dataset was downloaded and analyzed. The results demonstrated that SCOS2 and SOCS3 were significantly downregulated in HCC tissues, while SOCS1, SOCS4, SOCS5 and SOCS7 were upregulated in HCC tissues (Fig. 1B). Collectively, SOCS2, SOCS3, SOCS5 and SOCS7 showed consistent expression alteration between TCGA-LIHC dataset and GSE94660 dataset. Therefore, SOCS2, SOCS3, SOCS5, and SOCS7 were subjected to further analysis.

3.2 Survival analysis of the selected SOCS family genes

To assess the prognostic value of SOCS2, SOCS3, SOCS5 and SOCS7 in TCGA-LIHC, TIMER was engaged. Survival analysis demonstrated that high level expression of SOCS2 were associated with favorable overall survival while other family members showed no correlations with overall survival (Fig. 2A). In addition, further analysis revealed that only higher expression of SOCS2 was correlated with good disease-free survival (Fig. 2B). These data indicated that SOCS2 may serve as a suppressor of HCC.

3.3 Correlation between SOCS2 expression and clinical/immunological features of HCC

Given the significant prognostic value of SOCS2, we next explored the association between SOCS2 expression and HCC stage and grade by TISIDB (Fig. 3, A and B). A negative correlation was observed between SOCS2 expression and HCC stage, although stage □ HCC showed relative high SOCS2 expression. In addition, grade of HCC inversely correlated with SOCS2 expression as well. The correlation

between SOCS2 and HCC stage and grade is consistent with the prognostic value of SOCS2 on HCC prognosis. The TCGA project enables us to understand human cancer comprehensively. As a result, the immune landscape of cancer was revealed by Thorsson et al,who classified cancer into six immune subtypes. Furthermore Roessler et al. grouped HCC into three molecular clusters (iCluster1, 2, 3) based on multiple genomic platforms. To evaluate the transcription level of SOCS2 across six immune subtypes and three clusters, TISIDB database was queried. We found that SOCS2 expression altered significantly among different immune subtypes and different clusters. The highest SOCS2 expression was observed in immune subtype C3 and iCluster2, which were reported correlated with favorable prognosis (Fig. 3, C and D). All together, these findings strengthen the notion that SOCS2 potentially acted as a negative regulator of HCC.

3.4 Impact of SOCS2 on biological processes

To understand the impact of SOCS2 on biological process, we evaluated the transcriptomes of TCGA-LIHC patients with varying SOCS2 expression and analyzed the most significantly differentially expressed 500 genes in $SOCS2^{high}$ versus $SOCS2^{low}$ patients (adjusted p value < 2.58E-05) (Additional file 1: Supplementary table1). The main biological process clusters associated with SOCS2 expression were presented in Fig. 4A, and the top 5 clusters were Metabolism of RNA, Organic cyclic compound catabolic process, Blood vessel development, Lipid biosynthetic process, Response to starvation. Specifically, the biological processes correlated with SOCS2 expression were listed in Additional File 2: Supplementary Table 2, the top 5 processes were Metabolism of RNA, Organic cyclic compound catabolic process, rRNA processing in the nucleus and cytosol, Ribonucleoprotein complex biogenesis, and Aromatic compound catabolic process (Fig. 4B; Additional file 3: Supplementary Fig. 1).

3.5 SOCS2 expression was associated with immune cell infiltration

Concerning the critical roles of cytokines on the immune response and T cell differentiation and specialization, we speculate that SOCS2 may influence the immune cell infiltration through regulating cytokines signaling. To identify association between SOCS2 expression and estimated immune cell infiltration in HCC, immune populations of TCGA-LIHC RNA-seq data inferred using CIBERSORT method were analyzed (Fig. 5). High SOCS2 expression was associated with increased memory resting CD4 T cells and γδ T cells infiltration, which have been shown to play an anti-cancer role. While increased infiltration of follicular helper T cells, regulatory T cells and macrophages M0 were associated with Low SOCS2 expression.

3.6 SOCS2 expression was potentially downregulated by miR-7-5p in HCC

Since SOCS2 expression decreased in HCC, we therefore explored the underlying mechanism. Usually, regulation of specific gene involves two aspects, genetics and epigenetics. Firstly, we queried cBioportal and found that in TCGA-LIHC patients, only one case with missense mutation with unknown significance was observed (Fig. 6A), and no significant survival difference was found between patients with SOCS2 mutation and those without SOCS2 mutation (Fig. 6B). Promoter methylation usually downregulates gene expression, we then queried DNMIVD to evaluated the methylation levels of SOCS2's promoter. The results demonstrated that promoter methylation of SOCS2 in normal liver tissues was comparable to that of HCC tissues (Fig. 6C), and no correlation was found between SOCS2 promoter methylation and SOCS2 expression (Fig. 6D). In addition, methylation levels of SOCS2 promoter did not affect HCC patients' overall survival (Fig. 6E). Thus, we proposed that SOCS2 expression was downregulated potentially by specific miRNA(s). TargetScan, microT-CDS, and PITA were then employed to predict miRNAs that target SOCS2 (Fig. 7A). Among the 5 common predicted miRNAs, miR-7-5p and miR-655-3p were negatively associated with SOCS2 expression (Fig. 7B; Additional file 4: Supplemental Fig. 2). Then we compared the expression level of miR-7-5p and miR-655-3p between HCC tissues and normal live tissues, and found that only miR-7-5p was upregulated in patients with HCC (Fig. 7C), which was further validated by GSE22058 dataset (Fig. 7D). Moreover, survival analysis demonstrated that high expression of miR-7-5p correlated with poor prognosis of HCC patients (Fig. 7E). Collectively, these findings indicated that low expression of SOCS2 in HCC may result from increased expression of miR-7-5p.

4. Discussion

Initiation and progression of HCC is considered a multi-step process, including chronic inflammation, hepatic fibrosis and cirrhosis, and involving dynamic interactions between multiple cell types. Cytokine signaling plays a significant role in cell-cell communication, growth, differentiation, and immune function (29). Uncoordinated regulation of cytokine signaling has been linked to a variety of inflammatory and neoplastic diseases. Suppressor of cytokine signaling family, the key negative regulators of cytokines and growth factor signaling, consists of eight structurally similar proteins, SOCS1-7, and cytokine-inducible SH2-containing protein (CISH). Among them, SOCS 1–3 and CISH are strictly associated with the control of cytokine signaling (30). Several studies have reported that some SOCS proteins emerged as potential tumor suppressor-like proteins and immune checkpoint molecules, suggesting that SOCS family may modulate tumor progression and immunotherapeutic effect (31). However, the involvement of different SOCS members in tumor progression, especially in HCC is still unclear.

In this study, by integrating the expression profiling from TCGA-LIHC dataset and GSE94660 dataset, we demonstrated that SOCS2 and SOCS3 were significantly downregulated, while SOCS5 and SOCS7 were upregulated in HCC tissues. Furthermore, survival analysis revealed only SOCS2 mRNA had significant prognostic value in terms of OS and DFS, and patients with higher expression level of SOCS2 had improved OS and DFS. These findings were consistent with the study by Xinyu et al., which detected SOCS2 level in 106 HCC patients and found reduced SOCS2 expression correlated with tumor progression (32). However, the biological function and the downregulation mechanism of SOCS2 remain to be investigated.

We next analyzed the association between SOCS2 expression and HCC clinical and immunological features by TISIDB. As tumor stage and grade increased, the mRNA expression of SOCS2 tended to be lower. The mRNA expression of SOCS2 in stage Ⅳ seemed to be lower than that in stage Ⅲ, possibly due to the small sample size (only six HCC patients were at stage Ⅳ). Meanwhile, SOCS2 expression significantly correlated with different immune subtypes and molecular subtypes. Concretely, the highest SOCS2 expression was observed in immune subtype C3 and iCluster2, which were recognized as good prognostic indicators (33, 34), suggesting SOCS2 potentially functioned as a tumor suppressor in HCC.

We then evaluated the effects of SOCS2 on biological processes. By analyzing the most significantly differentially expressed 500 genes in $SOCS2^{high}$ versus $SOCS2^{low}$ patients, the results of functional enrichment indicated SOCS2 may contribute to RNA metabolism, which plays an essential role in the regulation of immune responses through its effects on cytokine production (35, 36). Besides, accumulating evidences have revealed SOCS family proteins affected the behavior and activation state of many immune cell types (37), we further explored the association between SOCS2 level and infiltration of various immune cell subtypes. The results showed that HCC patients with higher SOCS2 mRNA level had higher resting memory CD4 T cells and γδ T cells infiltration but lower follicular helper T cells, regulatory T cells and M0 macrophages infiltration. These immune subtypes were important components of the tumor microenvironment. Memory CD4 T cells and γδ T cells are reported to have anti-tumor response while regulatory T cells are known as one key immunosuppressive subset (38–40). Follicular helper T cells are considered to possess both oncogenic and tumor suppressor properties (41, 42). So are M0 macrophages, which can exert different biological effects by M1/M2 polarization (43). Taken together, these findings indicated that SOCS2 may act as a potent tumor suppressor by altering RNA metabolism and immune responses.

Finally, we investigated the molecular mechanism by which SOCS2 is down-regulated in HCC. In terms of genetic alteration, only one case with missense mutation with unknown significance was observed in the TCGA-LIHC cohort. Since several studies had reported that promoter methylation was associated with the downregulation of some SOCS proteins (12, 44), we then evaluated the methylation levels of SOCS2's promoter in HCC but found SOCS2 expression was independent of the methylation level. In addition, there was no significant difference in promoter methylation of SOCS2 between HCC tissues and normal ones, which didn't seem to affect the OS of patients with HCC. Thus, we suspect that this process could involve microRNA. The analysis of three datasets suggested negative association between miR-7-5p and miR-655-3p with SOCS2. Further comparison showed only miR-7-5p was upregulated in HCC tissues, and patients with lower miR-7-5p level had longer OS. All of these results indicated that miR-7-5p may be a potent regulator of the low expression of SOCS2 in HCC.

There were some limitations in our study need to be recognized. All the data analyzed in this study was retrieved from the online databases, and further in vitro and in vivo studies should be performed to validate our results.

5. Conclusion

By integrated bioinformatic analysis, we confirmed that low expression of SOCS2 was significantly associated with favorable prognosis among SOCS family in patients in HCC. And our results of bioinformatics also showed that SOCS2 could affect RNA metabolism and immune state. Furthermore, the expression pattern of SOCS2 was predicted to be regulated by miR-7-5p. Taken together, our study indicated that SOCS2 could be a prognostic biomarker for patients with HCC, as well as a potential drug target for anti-cancer therapy.

List Of Abbreviations

HCC, hepatocellular carcinoma;

SOCS, suppressor of cytokine signaling;

GEO, Gene Expression Omnibus;

DEGs, differential expression of genes;

TCGA, The Cancer Genome Atlas;

OS, overall survival;

DFS, disease-free survival;

CISH, cytokine-inducible SH2-containing protein.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read and approved the content and agree to submit for consideration for publication in the journal.

Competing interests

The authors declare that they have no competing interests.

Data availability

The datasets TCGA-LIHC, GSE94660(18) and GSE22058(27) for this study can be found in the TCGA (https://portal.gdc.cancer.gov) and GEO database (https://www.ncbi.nlm.nih.gov/geo/).

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Authors' contributions

Conception and design: JZ, CH, YK. Foundation support: JZ, CH. Acquisition and analysis of data: LL, YK, HZ, MH. HZ, XL. Interpretation of data: LL, YK, HZ, MH. Drafting the manuscript and revising for submission quality: JZ, CH, LL, YK. Reviewing and approving the final vision: All authors. Study supervision: JZ, CH. All authors read and approved the final manuscript.

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Supplementary Information Legends

Additional file 1: Supplementary Table 1 The most significantly differentially expressed 500 genes in $SOCS2^{high}$ versus $SOCS2^{low}$ patients, two-tail student's t test.

Additional file 2: Supplementary Table 2 List of the enriched biological processes by functional enrichment analysis.

Additional file 3: Supplementary Figure 1 The functional enriched biological processes correlated with SOCS2 expression, colored by p-value.

Additional file 4: Supplementary Figure 2 Correlation between miR-129-5p, miR-153-3p, miR-194-5p and SOCS2 expression level in HCC tissues (Starbase).

Figures

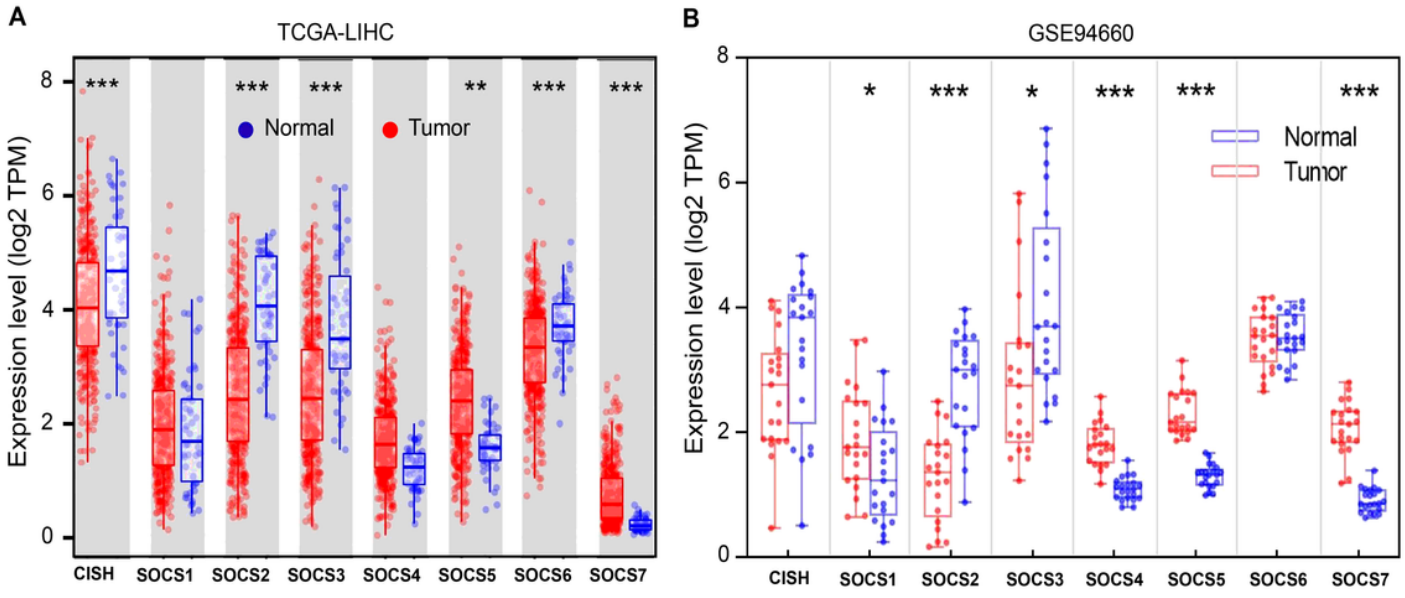

Figure 1

mRNA levels of SOCS family members in HCC. A, The TCGA-LIHC data analyzed by TIMER. B, The GSE94660 data. *P < 0.05, **P <0.01, ***P <0.0001.

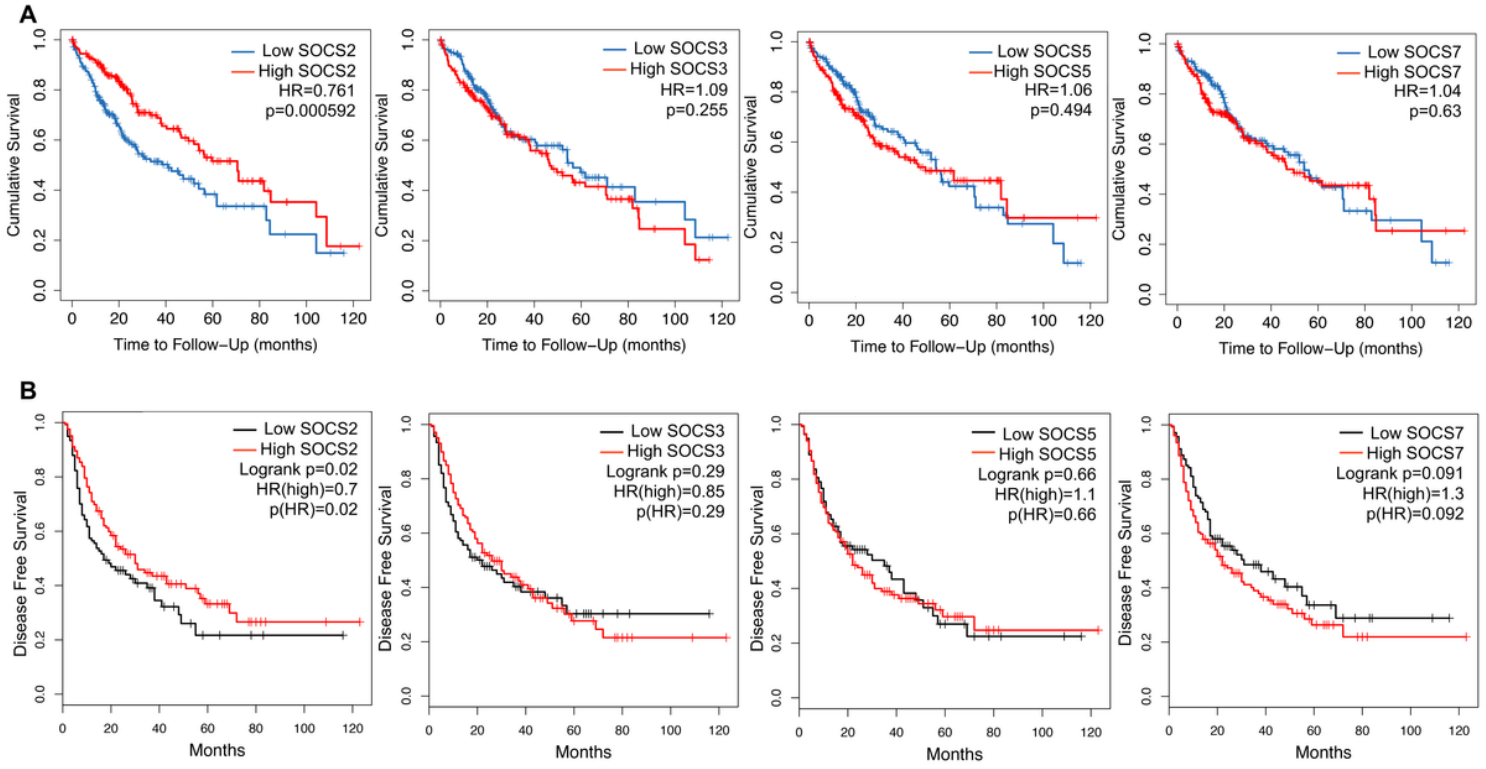

Figure 2

Survival analysis of SOCS2/3/5/7 in patients with HCC. A, Overall survival curve (TIMER). B, Disease free survival curve (GEPIA). P < 0.05 was as statistically significant.

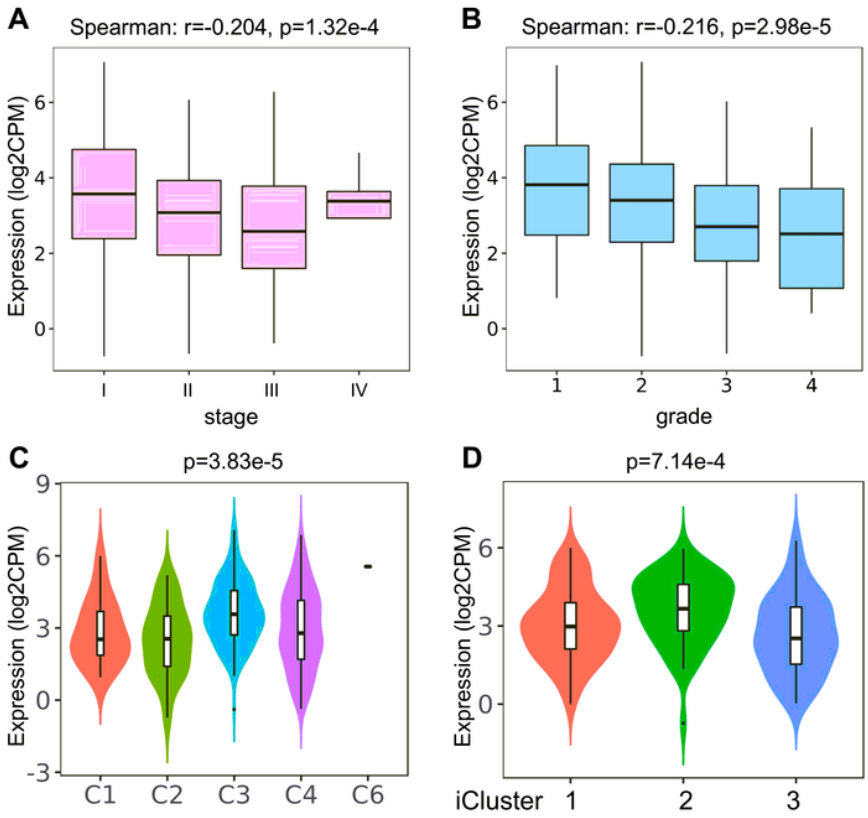

Figure 3

The correlation between SOCS2 and the pathological stage, grade, immune subtypes and molecular subtypes of HCC patients (TISIDB).

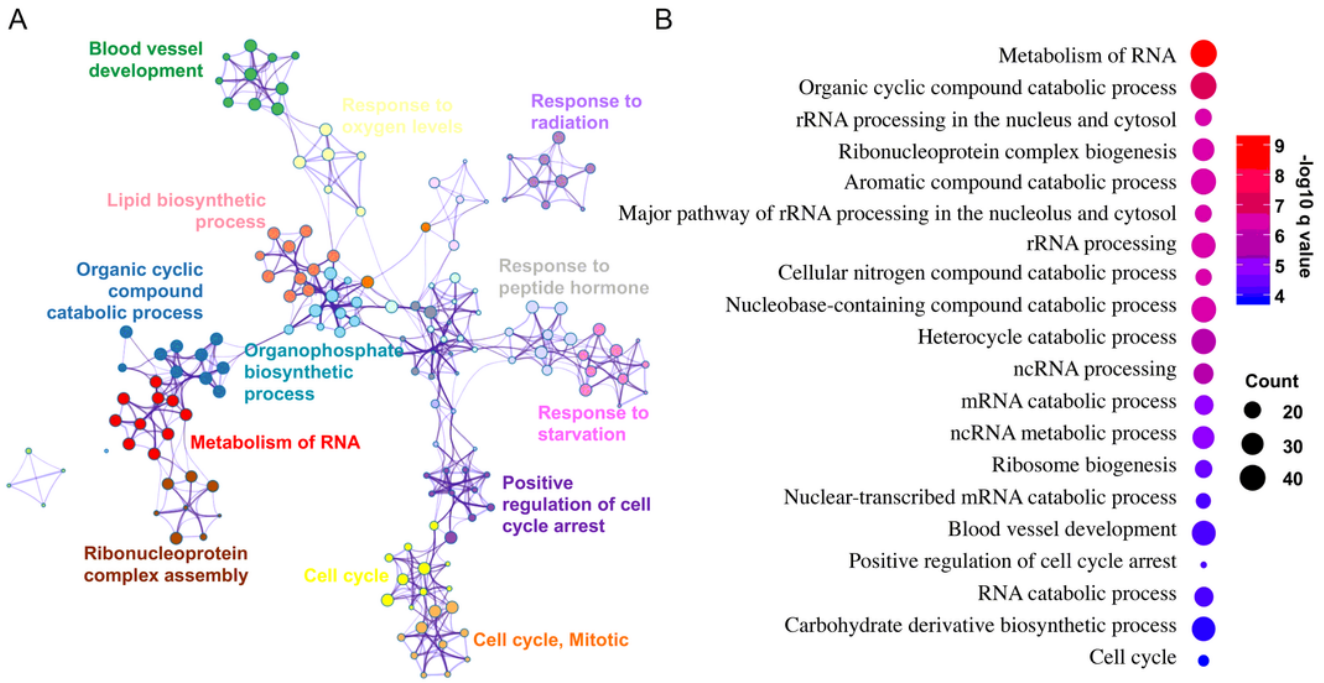

Figure 4

The functional enrichment analysis of SOCS2 in HCC (Metascape). A, Network of enriched terms, colored by biological progress cluster ID. B, The top 20 significantly enriched biological progresses.

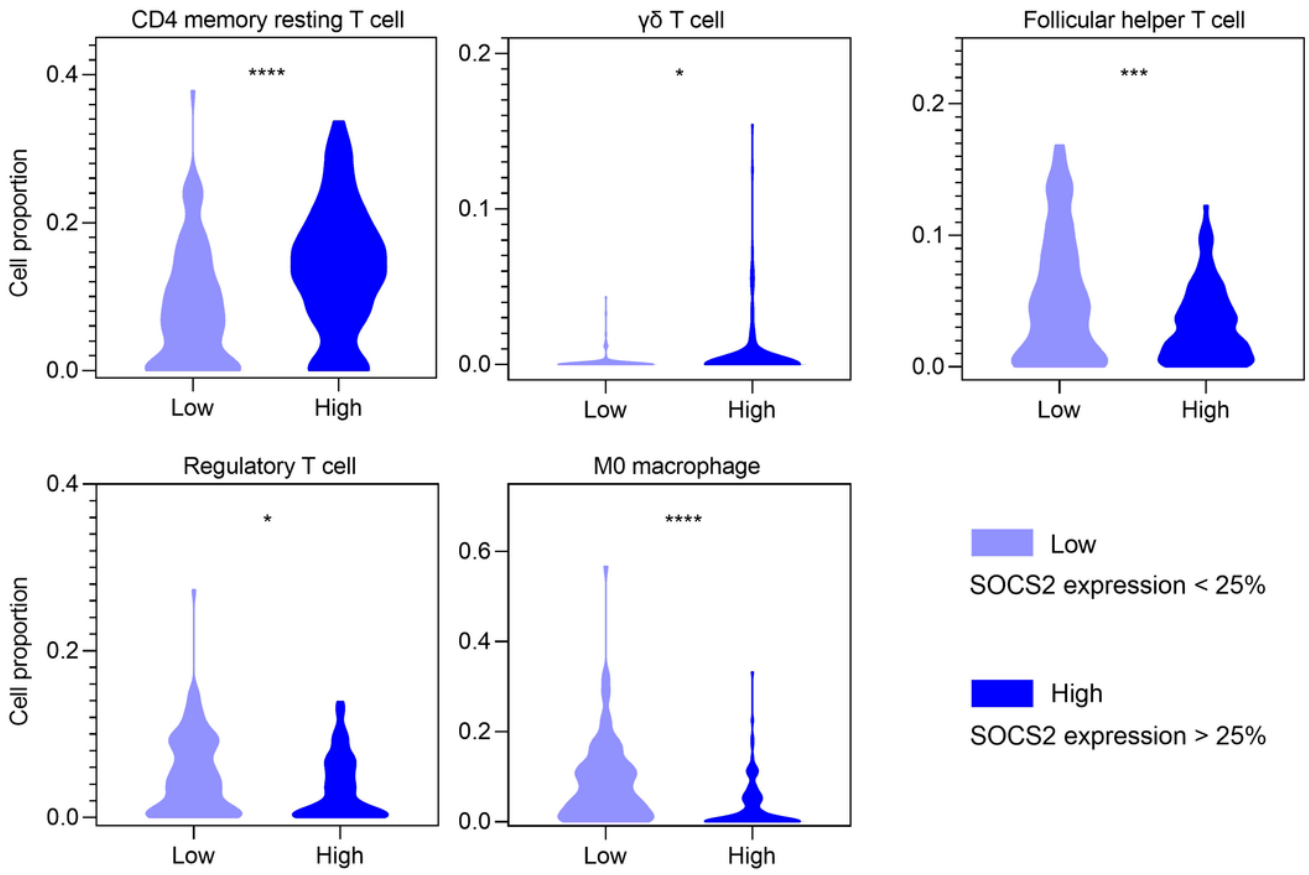

Figure 5

Relative abundance of tumor-infiltrating immune cell populations determined by the CIBERSORT methodology. *P < 0.05, **P < 0.01, ***P < 0.001.

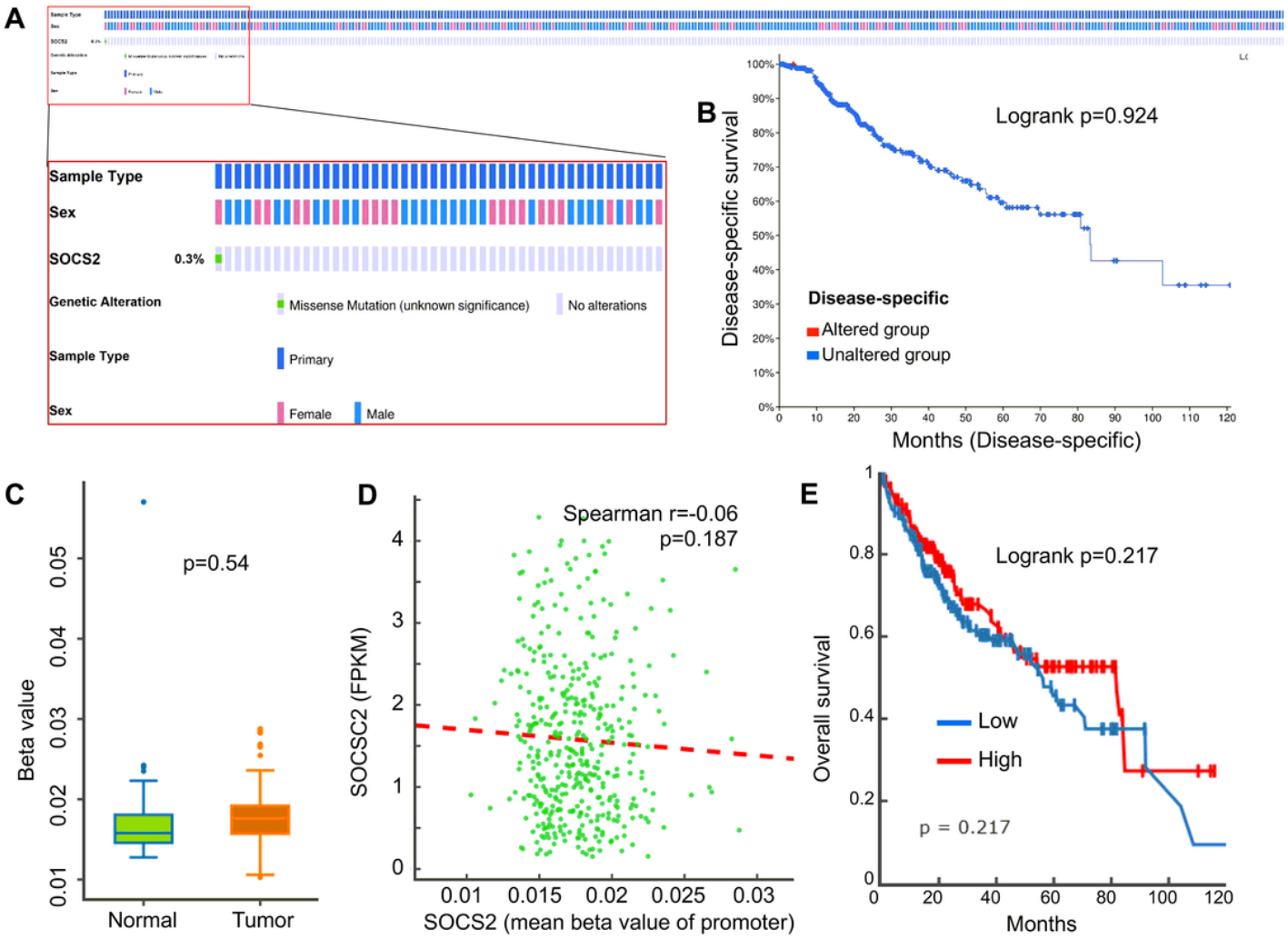

Figure 6

Genetic alteration and promoter methylation of SOCS2 in patients with HCC. A, Alteration frequency analysis of SOCS2 gene in HCC (cBioPortal). B, Survival analysis of SOCS2 gene alteration of TCGA HCC patients with Kaplan-Meier plot (cBioPortal). C, Methylation state of SOCS2 gene promoter in the normal and HCC tissue samples in TCGA-LIHC (DNMIVD). D, Correlation between SOCS2 mRNA expression and promoter methylation (DNMIVD). E, Prognostic value of SOCS2 promoter methylation (DNMIVD).

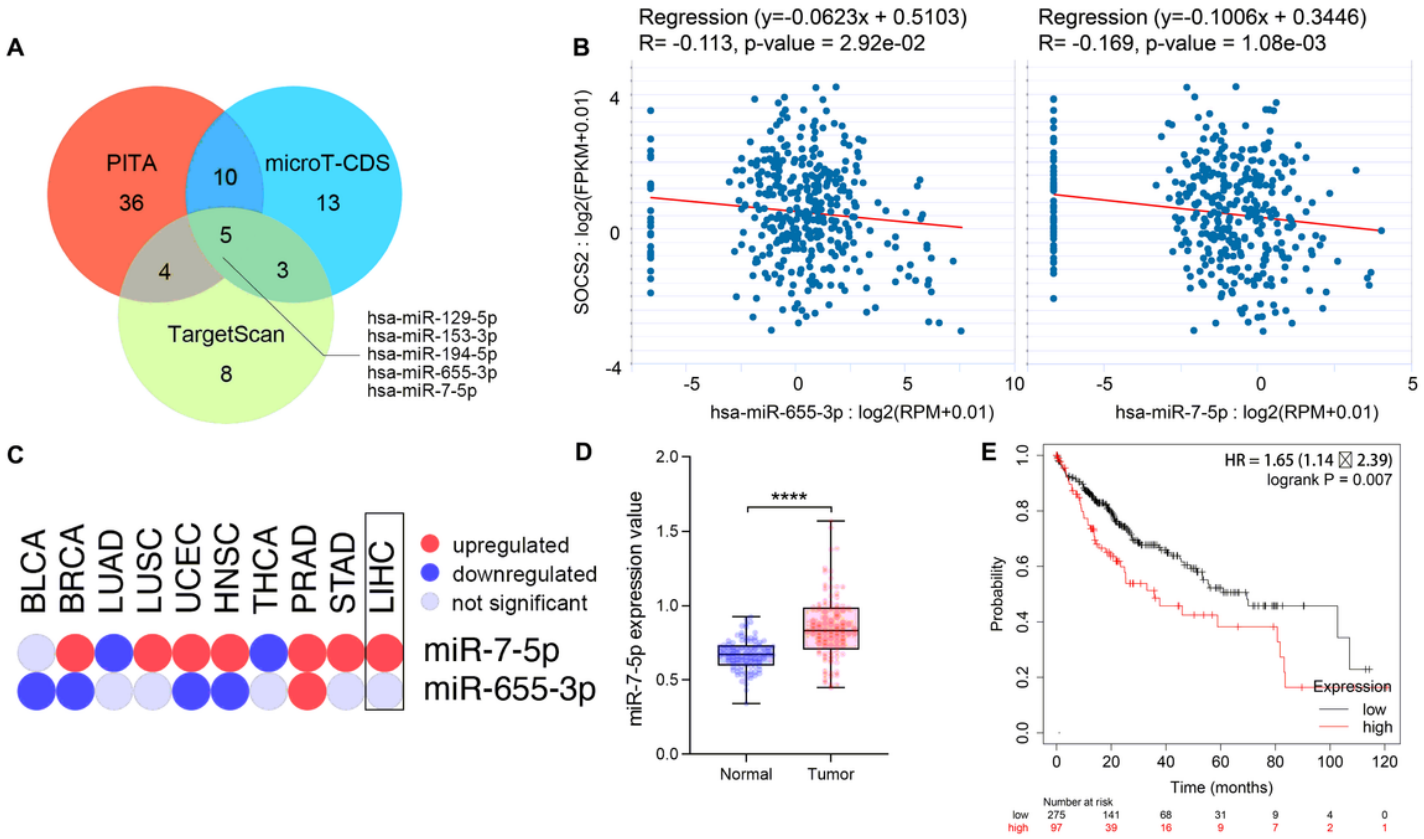

Figure 7

SOCS2 expression was potentially downregulated by miR-7-5p in HCC. A, Venn diagram showing the overlap of predicted miRNA(s) targeted SOCS2 by PITA, microT-CDS and TargetScan. B, Correlation between miR-655-3p, miR-7-5p and SOCS2 expression level in HCC tissues (Starbase). C, The levels of miR-655-3p and miR-7-5p in HCC compared with normal tissues from TCGA-LIHC (dbMEMC). D, miR-7-5p was upregulated in HCC samples based on GSE22058 dataset. ****P < 0.0001. E, The Kaplan-Meier plot of OS and miR-7-5p expression using the online program Kaplan-Meier Plotter (P=0.007).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile4SupplementaryFigure2.tif
- Additionalfile3SupplementaryFigure1.tif
- Additionalfile2SupplementaryTable2.xlsx
- Additionalfile1SupplementaryTable1.xlsx