Elevated STIL predicts poor prognosis in patients with hepatocellular carcinoma

Huangqin Song
Shanxi Medical University
Haichao Zhao
Shanxi Medical University
Changzhou Chen
Fudan University
Donglin Zhang
Shanxi Medical University
Xiaoxiao Wang
Shanxi Medical University
jiefeng he (hejiefeng1995@163.com)
Shanxi Academy of Medical Sciences: Shanxi Bethune Hospital

Research

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Abstract

Background

Overexpression of STIL centriolar assembly protein (STIL) has been observed in a variety of cancers. However, the functional significance of STIL in hepatocellular carcinoma remains unknow.

Results

STIL expression were abnormal in HCC tissues, and HCC patients with increased STIL expression had poor prognosis. In addition, increased STIL expression was correlated with T stage, pathologic stage, histologic grade, AFP, age, tumor status. STIL was an independent predictor of poor prognosis in HCC patients, as verified with a nomogram based on a Cox regression model. STIL was involved in HCC progression by modulating the cell cycle, DNA replication, oocyte meiosis, etc. Correlation analysis indicated TUBG1 mRNA expression was correlated with immune infiltrates.

Conclusion

STIL plays a vital role in HCC progression and prognosis; it may, therefore, serve as an effective biomarker for the prediction of patient survival.

Background

Liver cancer is the seventh most common malignant tumor and forth common cause of death of tumor worldwide, with about 900,000 new cases and over 830,000 deaths in 2020[1]. 90% cases of primary liver cancer were hepatocellular carcinoma (HCC) and chronic HBV infection is a prominent cause of hepatocellular carcinoma (HCC) [2, 3]. Most HCC patients are usually detected at an advanced stage, with a 5-year survival rate of about 12% [4]. Therefore, identification of the new biomarkers for the diagnosis of HCC is critically needed to improve the prognosis.

SCL/TAL1 interrupting locus (STIL) is a critical regulator of mitotic centrosome to promote the centriolar replication and cell cycling. It is notable that STIL expression is elevated in multiple types of cancers, such as lung cancer and pancreatic cancer and correlated with the expression of several checkpoint genes and mitotic indicators. Furthermore, STIL has been reported to be one of the up-regulated 17 genes in primary adenocarcinoma and their elevated expression is associated with metastasis. Hence, STIL acts as an oncogenic factor to promote the progression of several types of cancers. However, the role of STIL in the development and progression of HCC has yet been explored.

In this study, we analyzed the expression of STIL in pan-cancer and HCC, and assessed its association with clinicopathological features and survival in HCC. additionally, GO and KEGG were used to explore the function of STIL in HCC. immune infiltration correlation analysis was also performed to analyzed the

correction between STIL and tumor-infiltrating immune cells. Finally, a nomogram prediction models based on independent risk factors was established for HCC prognosis.

Results

Pan-cancer analysis of STIL expression levels

In order to analysis the expression of the STIL in different types of cancer, we queried the online database of Oncomine and found that STIL was over-expressed in most tumor expect for leukemia (Figure 1A). We also use TIMER database to confirm this result (Figure 1B). Given the high expression of STIL in many cancers, STIL may function as oncogene. then, we analyzed the possibility of STIL as prognostic biomarker or therapeutic target in HCC.

Overexpression of STIL in HCC

The expression of STIL was increased in the 371 tumor tissues compared to the 50 normal tissues and the result is consistent with expression analysis of STIL in 50 pairs of matched tumor/normal for HCC samples (Figure 2A and 2B). In GSE121248, STIL was also overexpressed in the tumor than in normal tissue (Figure 2C).

Correlation between the expression of STIL and clinicopathological features

we found that the expression of STIL was associated with age, AFP, tumor status (Figure 3A-C). Moreover, STIL expression was correlated with T stage, histologic grade and pathologic stage (Figure 3D-F). Kaplan-Meier survival analysis indicated STIL-high expression group has poor overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) in HCC(Figure 3G-I).

Biological function & mechanism of co-expressed genes

GO enrichment analysis of STIL co-expressed genes are involved in chromosome segregation, DNA replication, nuclear division (Figure 4A-C). According to the Kyoto Encyclopedia of Genes and Genomes analysis, STIL co-expressed genes play a major role in the cell cycle, spliceosome, DNA replication and other signaling cascades (Figure 4D).

Correlation between STIL expression and immune infiltration

Finally, we analyzed the correlation between the expression level of STIL and immune cell enrichment (generated by ssGSEA) based on the Spearman correlation coefficient. STIL expression was negatively correlated with the abundance of cytotoxic cells, CD8 T cells, DCs, pDCs and Neutrophils, and was positively correlated with the abundance of T helper cells and Th2 cells (Figure 5).

Development and validation of a nomogram

STIL and T stage were identified as independent prognostic factors using Univariable and multivariable Cox regression analyses (Table 1). Then we develop a nomogram, including STIL and T stage as the predictors, to predict the survival probability of 1-,3-,5-year in HCC patients (Figure 6A). the c-index for the model was 0.641 (95% CI: 0.614–0.668). we also developed a calibration plot of the nomogram, which proved that the nomogram was well-calibrated (Figure 6B).

Discussion

SCL/TAL1 interrupting locus has been thought to be an oncogenic factor and its expression is up-regulated in many types of malignancies. However, there is no report on its role in the progression of HCC. In this study, we found that STIL expression is a robust prognostic predictor of HCC.

On the current study, we firstly evaluated the expression levels of STIL and observed that TUBG1 was abnormally expressed in various tumors including HCC. The result in HCC had been verified in multiple databases. In conclusion, STIL is overexpressed in HCC and further studies are necessary to reveal the mechanism of STIL of HCC.

Moreover, overexpression of STIL correlated to poor prognosis and poor clinicopathologic factors in HCC. Our study indicated that high expression of STIL was closely related to poor OS, DSS and PFI in HCC patients. In addition, we established a nomogram including STIL and T stage based on multivariate analysis. Calibration plot was used to verify the nomogram and demonstrated that prediction by the nomogram was consistent with actual observation for the probability of 1-,3-,5-year OS, with the C-indexes of 0.641 (95% CI: 0.614–0.668). Thus, our model could be a novel approach to evaluate prognosis of HCC patients.

To better explore the biological function of STIL in HCC patients, GO and KEGG functional enrichment analysis demonstrated that most enriched GO terms of the co-expressed genes were “chromosome segregation”, “DNA replication” and “nuclear division”. KEGG pathways, such as “DNA replication”, “spliceosome” and “cell cycle”. It is well-known that defects in cell cycle regulation, such as sustaining proliferation and unlimited replication, are fundamental characteristics of cancer pathogenesis[5], and some newly discovered TNBC-associated small molecule inhibitors have been demonstrated to induce cell cycle arrest [6]. Similarly, chromosome segregation with nuclear division in M phase and DNA replication in S phase are essential processes during mitotic cell division[7]. In tumorigenesis, driven by oncogene activation, DNA replication stress and its adverse impact on chromosome segregation are associated with genome instability[8]. Furthermore, oocyte meiosis and progesterone-mediated oocyte maturation pathways are enriched in survival associated miRNAs of ovarian carcinomas[9]. Taken together, the results of our study indicated that STIL might modulate DNA replication and cell cycle to promote the occurrence and development of HCC. However, to better understand the role of STIL in HCC, further research of molecular regulatory mechanism was needed.

Recently, research studies have revealed that the interactions between immune cells and tumor are very important for tumor progression[10]. Furthermore, potential clonal amplification and preferential

enrichment of TIICs are present in HCC[11], and poor prognosis associated with TIICs accumulation in HCC [12]. Our study showed that expression level of STIL was negatively correlated with a variety of immune cells, such as cytotoxic cells and dendritic cells (DCs, iDCs, and pDCs). DCs, known as antigen-presenting cells, play an important role in the initiation and regulation of tumor immune response[13]. Recently, anti-cancer effect of DCs has been reported in HCC[14]. Immature DCs have the function of phagocytosis. However, mature DCs have important regulatory functions and produce and secrete lots of cytokines[15]. Moreover, cytotoxic cells, also known as CD8+T lymphocytes with cytotoxic granules, are the important anti-tumor effector cells[16]. A research study has shown that hepatocellular carcinoma cell inhibited the cytotoxic T cells response to modulate tumor progression and tolerance to PD1 therapy[17]. Furthermore, the expression of STIL was also closely related to T helper cells, Th2 cells, and Tfh in HCC. In conclusion, our results demonstrated that the STIL plays an important role in modulation of immune infiltrating cells in HCC.

However, this study had several limitations that should be considered. Firstly, we could not obtain all types of clinical information in public database, such as the approach of treatment for each patient, to better analysis the role of STIL in progression of HCC. Second, expansion of the clinical sample size is needed to validate the relationship between the expression of STIL and prognosis. Finally, in order to better explore the mechanism of STIL in HCC, we would carry out experimental research on STIL in the sooner future.

Conclusion

the present study revealed that the expression of STIL was higher in HCC and was an essential biomarker with prognostic value. High STIL might promote the development of HCC by modulating cell cycle, DNA replication and immune infiltration. Nevertheless, further validation by experimental investigations is needed to analyze the biological functions and the underlying mechanism of STIL for HCC patients.

Materials And Methods

Data resource

The mRNA expression data of 424 cases involving HTSeq fragments per kilobase million were retrieved from the TCGA data resource, including 50 cases involving normal liver tissue and 374 cases involving HCC tissue. Patients with missing or incomplete clinical data and lack of follow-up prognosis data were filtered. Next, level 3 HTSeq-FPKM data were transformed into TPM (transcripts per million reads), and the TPM data of 371 HCC patients were used for further analyses; then TPM information of 371 HCC samples was applied for the next analyses. The expression data of 50 nonmalignant liver tissues and 50 HCC tissues belonged to the same patient. STIL expression in HCC tissues was analyzed in the TCGA and GEO data resource. The corresponding patients' clinical data was also used from the TCGA database.

Screening co-expressed genes of STIL in the TCGA database

Pearson correlation coefficient (r) represented the correlation between the two genes and exhibited the biological association linking the two genes. STIL co-expressed genes were obtained via the R package using HCC tissues from the TCGA database. Screening criteria were $p < 0.05$ and $|r| > 0.6$, which identified the STIL co-expressed genes as showing moderate or greater expression.

Biological function & mechanism analysis

KEGG and GO analyses were carried out for co-expressed genes of STIL. The gene data of 374 HCC tissues were stratified into two expression groups depending on the STIL median value. A $p < 0.05$ was considered as the standard for TUBG1 involvement in biological functions and signaling cascades.

Immune infiltration analysis

We performed immune infiltration analysis using the ssGSEA method implemented in the R package GSVA for 24 types of immune cells. Spearman correlation analysis was performed to assess the correlation between STIL expression and immune cells infiltration.

Statistical analysis

STIL expression and its relationship with clinicopathological characteristics were explored via the one-way ANOVA test, the T test and Wilcoxon signed-rank test. Cox regression and Kaplan–Meier survival analysis were used to investigate the association between STIL and the clinicopathological features and OS of HCC patients. Co-expressed genes of STIL were filtered via Pearson correlation analysis.

Abbreviations

STIL: SCL/TAL1 interrupting locus; HCC: hepatocellular carcinoma; TCGA: cancer genome atlas; GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; BP: biological processes; CC: cellular components; MF: molecular functions; OS: over survival

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

The author(s) read and approved the final manuscript.

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Authors' information

1 Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Taiyuan, 030032, China

2 Department of Liver Surgery and Transplantation, Liver Cancer Institute, Zhongshan Hospital, Fudan University; Key Laboratory of Carcinogenesis and Cancer Invasion (Fudan University), Ministry of Education; Institute of Biomedical Sciences, Fudan University, Shanghai 200032, China.

3 Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, 030032, China

4 Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China.

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Tables

TABLE1 Correlation between the expression of STIL and clinicopathological features

Characteristic	Low expression of STIL	High expression of STIL	p
n	187	187	
T stage, n (%)			**0.002**
T1	109 (29.4%)	74 (19.9%)	
T2	38 (10.2%)	57 (15.4%)	
T3	32 (8.6%)	48 (12.9%)	
T4	5 (1.3%)	8 (2.2%)	
N stage, n (%)			0.624
N0	122 (47.3%)	132 (51.2%)	
N1	1 (0.4%)	3 (1.2%)	
M stage, n (%)			0.361
M0	130 (47.8%)	138 (50.7%)	
M1	3 (1.1%)	1 (0.4%)	
Pathologic stage, n (%)			**0.002**
Stage I	103 (29.4%)	70 (20%)	
Stage II	38 (10.9%)	49 (14%)	
Stage III	32 (9.1%)	53 (15.1%)	
Stage IV	4 (1.1%)	1 (0.3%)	
Tumor status, n (%)			**0.005**
Tumor free	115 (32.4%)	87 (24.5%)	
With tumor	63 (17.7%)	90 (25.4%)	
Gender, n (%)			0.185
Female	54 (14.4%)	67 (17.9%)	
Male	133 (35.6%)	120 (32.1%)	
Race, n (%)			**0.048**
Asian	67 (18.5%)	93 (25.7%)	
Black or African American	8 (2.2%)	9 (2.5%)	
White	102 (28.2%)	83 (22.9%)	
Age, n (%)			**0.034**

Characteristic	Low expression of STIL	High expression of STIL	p
<=60	78 (20.9%)	99 (26.5%)	
>60	109 (29.2%)	87 (23.3%)	
Histologic grade, n (%)			**< 0.001**
G1	38 (10.3%)	17 (4.6%)	
G2	100 (27.1%)	78 (21.1%)	
G3	43 (11.7%)	81 (22%)	
G4	4 (1.1%)	8 (2.2%)	
AFP(ng/ml), n (%)			**< 0.001**
<=400	127 (45.4%)	88 (31.4%)	
>400	21 (7.5%)	44 (15.7%)	
Vascular invasion, n (%)			0.537
No	113 (35.5%)	95 (29.9%)	
Yes	55 (17.3%)	55 (17.3%)	

TABLE 2 Relationship among clinicopathological variables and SCL/TAL1 interrupting locus (STIL) expression and Overall survival in hepatocellular carcinoma patients

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (>60 vs. <=60)	373	1.205 (0.850-1.708)	0.295		
Gender (Male vs. Female)	373	0.793 (0.557-1.130)	0.200		
Race (White vs. Asian&Black or African American)	361	1.265 (0.881-1.816)	0.203		
T stage (T3&T4 vs. T1&T2)	370	2.598 (1.826-3.697)	**<0.001**	2.504 (1.756-3.572)	**<0.001**
STIL (High vs. Low)	373	1.562 (1.104-2.211)	**0.012**	1.446 (1.018-2.054)	**0.039**

Figures

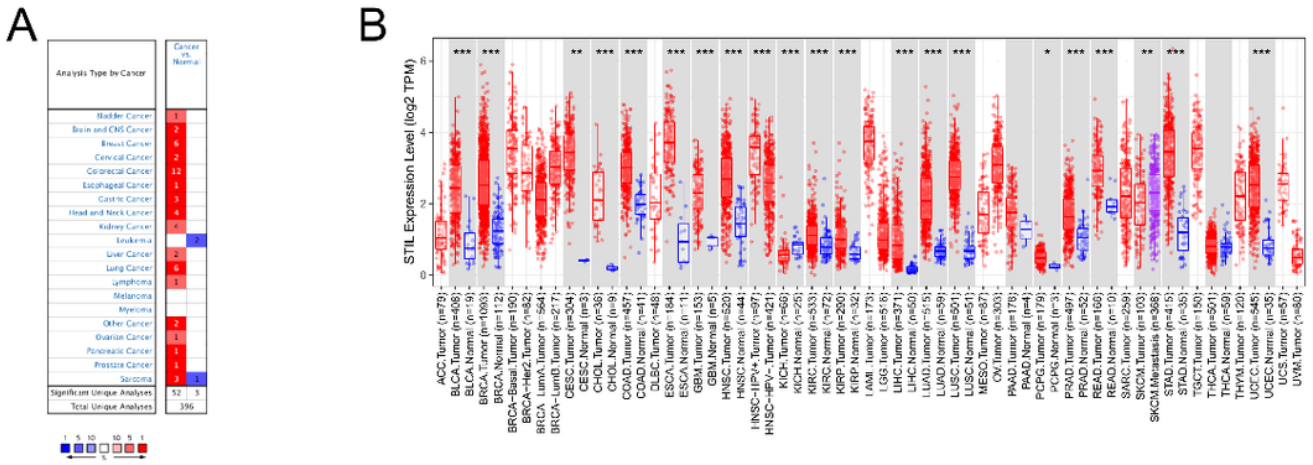

Figure 1

STIL expression in different types of human cancers. (A) the expression of STIL in different human cancer tissues compared with normal tissues using the Oncomine database. (B) The level of STIL expression in different tumor types from the TCGA database in TIMER. Note: *P < 0.05, **P < 0.01, ***P < 0.001.

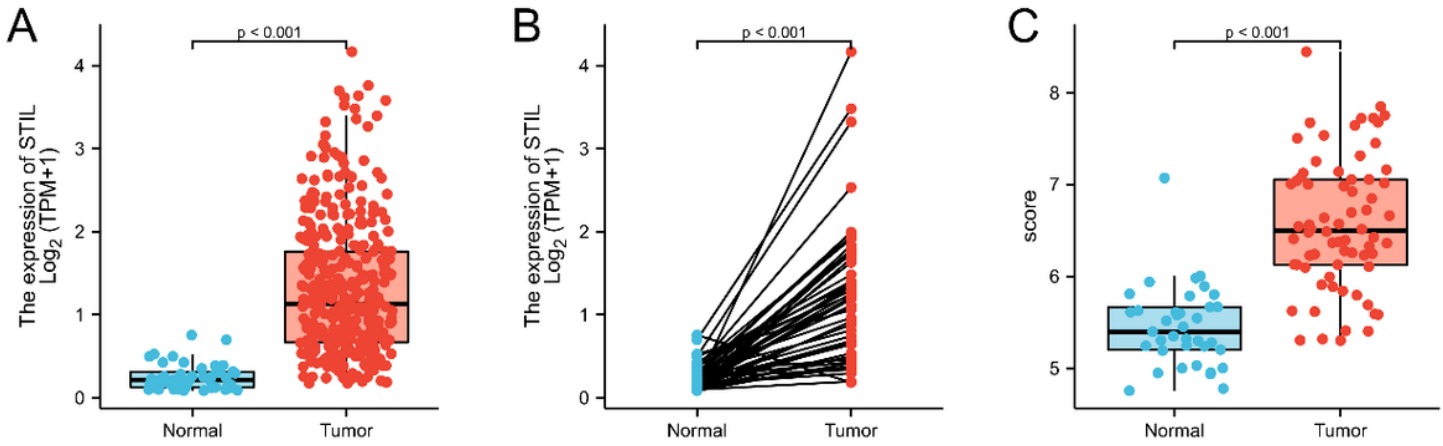

Figure 2

STIL expression in different types of human cancers. (A) the expression of STIL in different human cancer tissues compared with normal tissues using the Oncomine database. (B) The level of STIL expression in different tumor types from the TCGA database in TIMER. Note: *P < 0.05, **P < 0.01, ***P < 0.001.

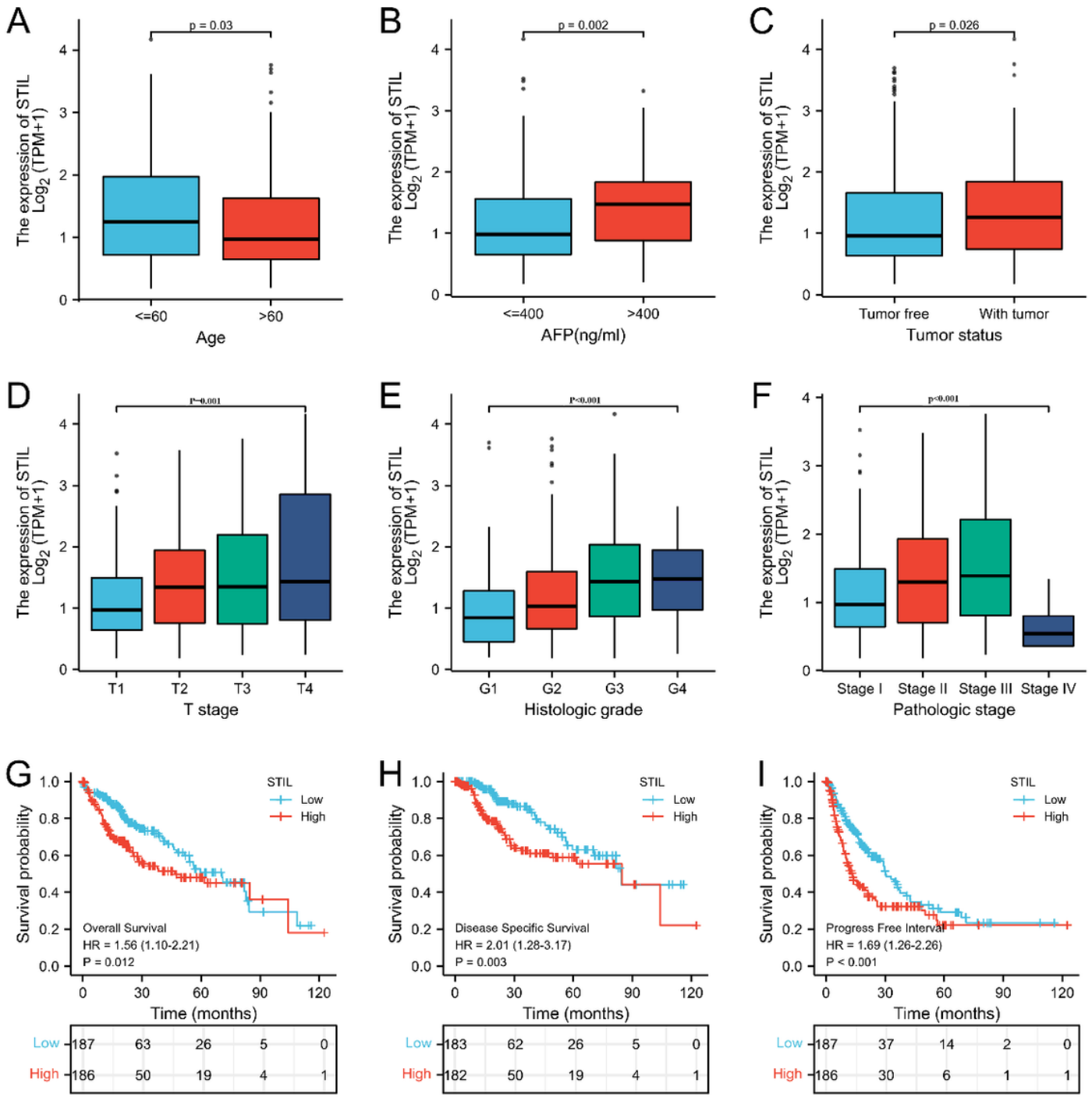

Figure 3

Association of STIL expression with clinicopathologic characteristics. (A) T stage; (B) pathologic stage; (C) Histologic grade; (D) AFP; (E) Prothrombin time; (F) Race□□G-I□Kaplan-Meier survival curves of (G) Overall survival, (H)Disease specific survival and (I) Progression-free interval.

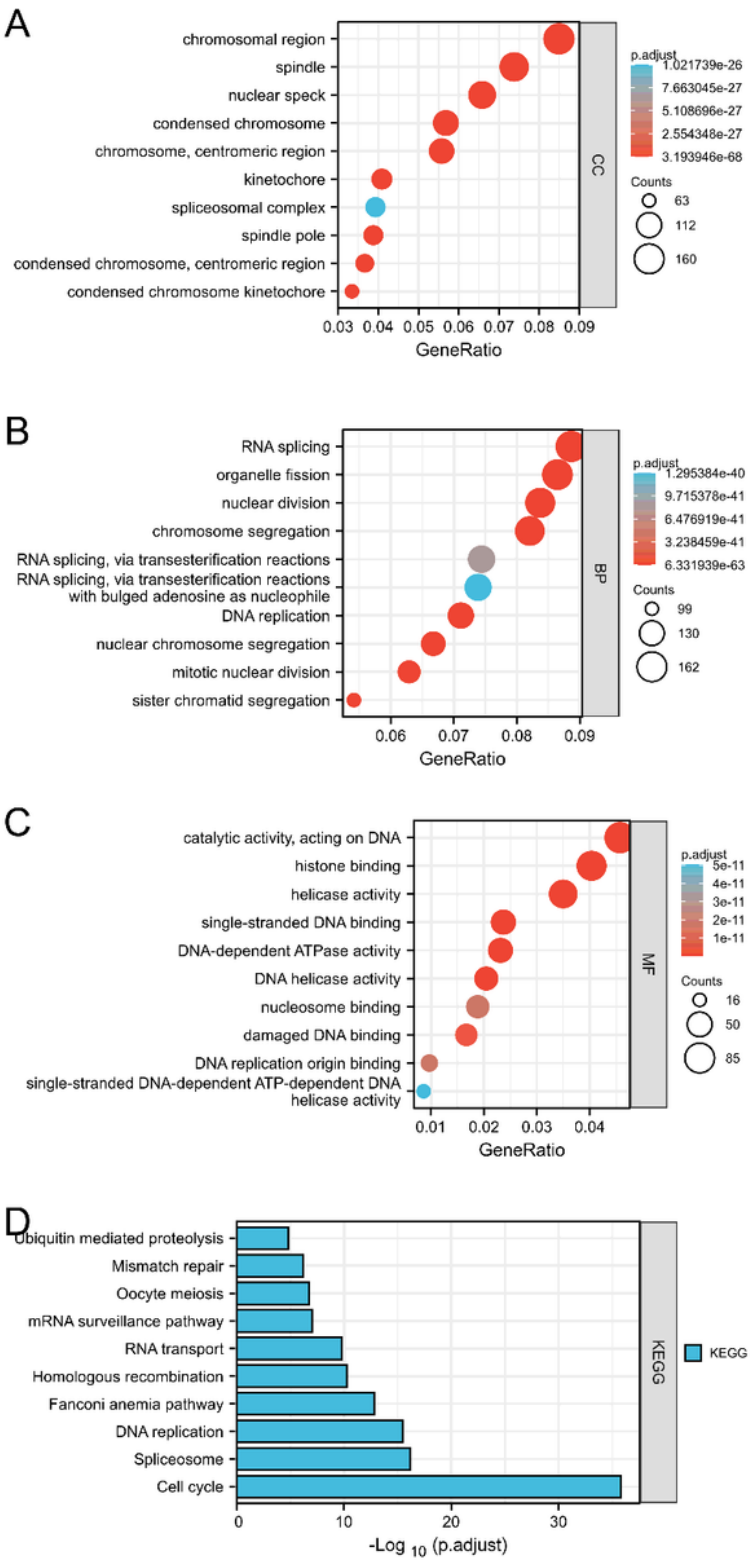

Figure 4

GO annotation and KEGG pathway enrichment analysis of co-expression genes. The top 10 enriched GO (A)BP, (B) CC and (C) MF terms as well (D) KEGG pathways.

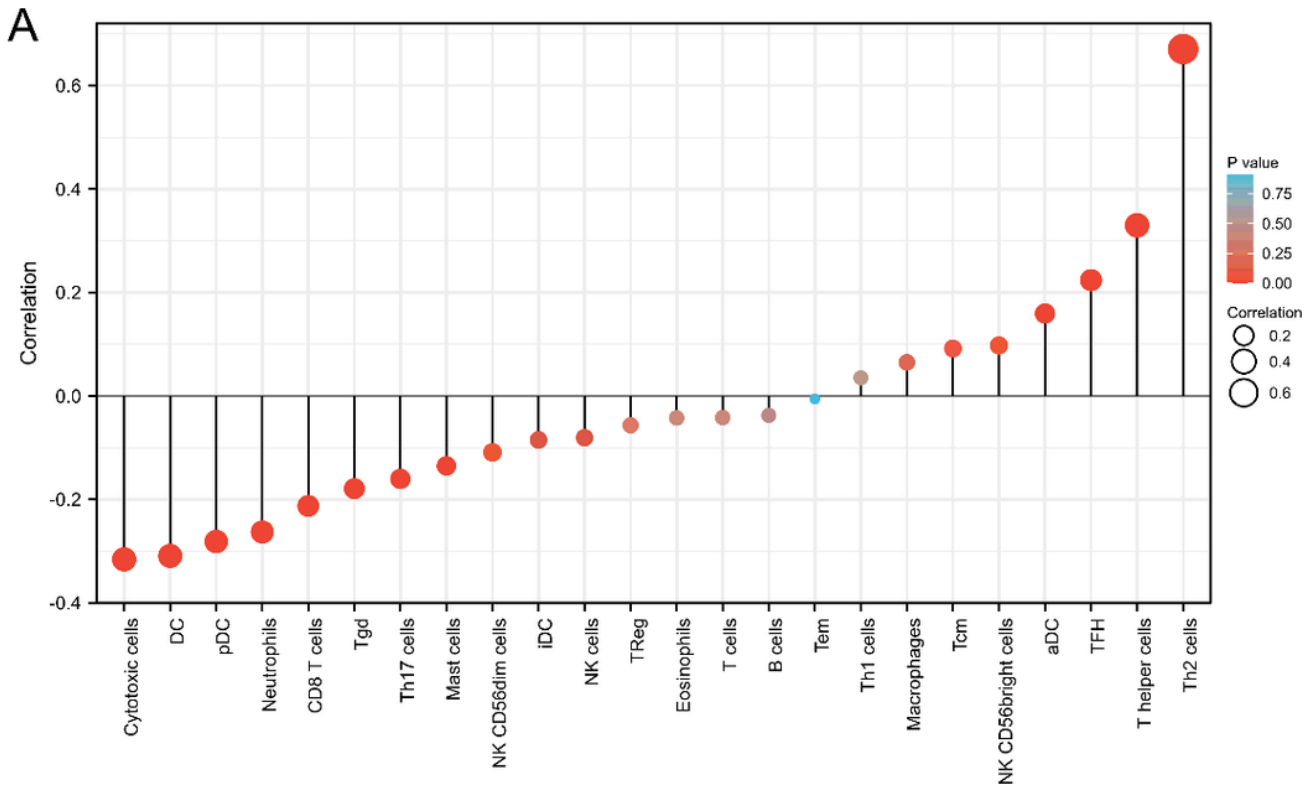

Figure 5

Correlations between the relative abundance of 24 immune cells and STIL expression levels. The size of the dots represents the absolute Spearman's correlation coefficient values.

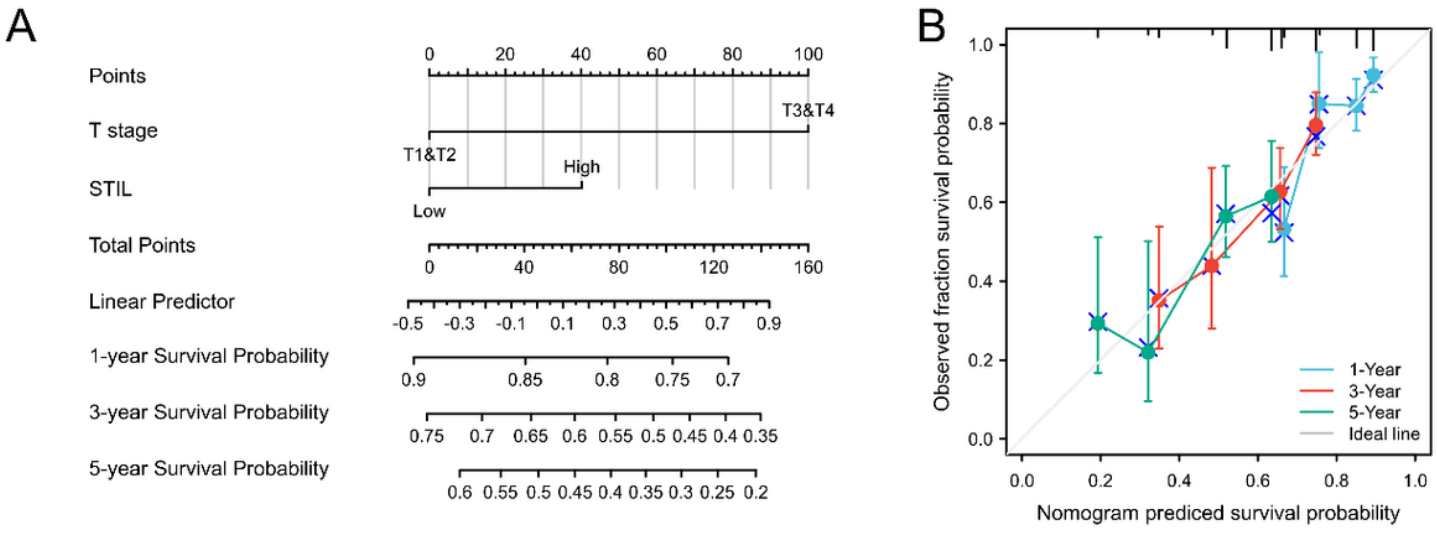

Figure 6

Relationship between STIL and T stage with overall survival (OS). (A) Nomogram for predicting the probability of 1-, 3-, and 5-year OS for HCC patients. (B) Calibration plot of the nomogram for predicting the OS likelihood

Supplementary Files

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

- SupplementaryTable1.xlsx
- SupplementaryTable2.xlsx