Prognostic Values of Stabilin-2 in Hepatocellular Carcinoma

Zhicheng Du, Pengfei Zhu, Long Yu, Kunlun Chen, Janwen Ye, Wenlong Zhai

Zhicheng Du
Zhengzhou University First Affiliated Hospital
ORCiD: https://orcid.org/0000-0002-1257-6619

Pengfei Zhu
Zhengzhou University First Affiliated Hospital

Long Yu
Zhengzhou University First Affiliated Hospital

Kunlun Chen
Zhengzhou University First Affiliated Hospital

Janwen Ye
Zhengzhou University First Affiliated Hospital

Wenlong Zhai
fcczhaiwl@zzu.edu.cn Corresponding Author
ORCiD: https://orcid.org/0000-0003-2559-2105

Prescreen

10.21203/rs.3.rs-27280/v1

Subject Areas
Keywords

*Hepatocellular carcinoma, Stabilin-2, biomarker, prognostic*
Abstract

Background: Hepatocellular carcinoma (HCC) is the primary malignancy of the liver. However, biomarkers for early HCC diagnosis are not available. Stabilin (STAB) proteins are scavenger receptors involved in apoptosis and clearance of hyaluronic acid. The role of STAB in HCC has not been previously explored; therefore, the aim of this study was to assess whether STAB gene expression can be used as a novel HCC biomarker.

Materials and Methods: Data on 370 HCC patients in the Cancer Genome Atlas database and 221 patients in the Gene Expression Comprehensive Database were retrieved and analyzed. Kaplan–Meier analysis and Cox regression model were used to calculate median survival time using hazard ratio (HR) and 95% confidence interval (CI).

Results: The Gene Expression Omnibus dataset showed that high Stabilin-2STAB2 expression implies longer overall survival (HR after correction = 0.541; 95% CI, 0.339–0.865; p = 0.0182, after correction p = 0.010) and longer recurrence-free survival time (adjusted HR = 0.554; 95% CI, 0.376-0.816; p = 0.0085, adjusted p = 0.003).

Conclusions: STAB2 is a potential biomarker for the diagnosis and prognosis of HCC.

Background

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor worldwide, and the third leading cause of cancer-related deaths accounting for 8.2% of all cancer deaths. China contributes to about half of the new liver cancer cases and deaths recorded worldwide every year[1, 2]. Hepatocellular carcinoma accounts for about 90% of liver tumors and causes more than 700,000 deaths worldwide each year due to poor prognosis [3, 4]. Cirrhosis is a main hepatocellular carcinoma risk factor and about 70-90% of hepatocellular carcinoma patients have a cirrhosis history[5]. Chronic hepatitis B virus (HBV) infection is one of the high risk factors for human HCC, accounting for 50% to 80% of the HCC cases worldwide[6]. In addition, alcoholism, aflatoxin B1 intake, non-alcoholic fatty liver and hepatitis C virus are also risk factors for hepatocellular carcinoma[7-9]. Although surgical resection and liver transplantation can effectively treat HCC, the 5-year overall survival rate remains at 7% [10]. Early screening for liver cancer relies on liver ultrasound and alpha-fetoprotein (AFP) [11]. However, ultrasound monitoring is limited by low detection sensitivity, which often leads to misdiagnosis of malignant nodules[12]. AFP is also reported to have low sensitivity and specificity [13]. Recent studies have reported potential HCC diagnostic markers such as protein induced by vitamin K absence or antagonist-II (PIVKA-II), Lens culinaris-agglutinin-reactive fraction of AFP (AFP-L3), MicroRNA-4651 (miR-4651) and MicroRNA-125b (miR-125b) [14-17]. HCC diagnosis using these markers requires invasive procedures, therefore, there is need to identify non-invasive biomarkers.

Stabilin-1(STAB1) and Stabilin-2(STAB2) also known as FEEL-1and FEEL-2, are structurally highly conserved type I transmembrane proteins and members of the scavenger receptor family [18]. Previous studies report that STAB is implicated in the proliferation and distant metastasis of melanoma cells, lymph node metastasis of prostate cancer and tongue cancer[19-21]. However, few studies have explored STAB expression in HCC. Therefore, our study utilized multiple datasets to explore the relationship between STAB expression levels and HCC.

Materials And Methods

Patient information

We retrieved STAB1 and STAB2 mRNA expression levels data for HCC patients from The Cancer Genome Atlas (TCGA) database. We normalized and transformed the raw data using R software (RStudio 1.2.5001 Inc., Boston, MA, USA). The expression levels were classified into high and low groups using a 50% cutoff value. Processed
data for 370 patients including race, gender, age, TNM stage, body mass index (BMI), family history, survival status and time were also retrieved.

Further, the GSE14520 dataset consisting of [HT_HG-U133A] Affymetrix HT human genome U133A array (445 samples) and [HT_HG-U133A_2] Affymetrix HT human genome U133A genes was downloaded from the Gene Expression Synthesis (GEO) database. The expression data consisted of a 2.0 array (57 samples) [22, 23]. To avoid batch effects, we obtained mRNA expression data from 221 HCC patients selected from the TCGA dataset. The mRNA expression levels in 221 HCC patients were also divided into two groups using a 50% cutoff value.

GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, USA) for Windows was used to assess differential gene expression between primary liver tumors and normal liver tissues. Mutations in the STAB gene family were verified using cBioPortal webserver for Cancer Genomics.

**Survival analysis**

We evaluated the prognosis of HCC patients from the TCGA database using median survival time (MST) and we adjusted for race, gender, age, BMI, TNM staging, and family history using the Cox regression model. We assessed the prognosis of HCC patients in the GEO database using the overall survival (OS) and recurrence-free survival (RFS). Further, we adjusted for gender, age, hepatitis B virus (HBV) infection status, alanine aminotransferase (ALT) status, main tumor size, multinodule status, cirrhosis, alphafetoprotein (AFP) level, and Barcelona Clinic Liver Cancer (BCLC) stage using the Cox proportional hazards regression model in the GEO database.

**Stratified chi-square test**

We stratified the data and calculated the MST, hazard ratio (HR) at 95% confidence interval (CI). These data revealed differences in the different layers.

**Functional enrichment analysis**

We performed a gene ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using Metascape (a gene annotation and analysis tool) and the Database for Annotation, Visualization, and Integrated Discovery (DAVID) version 6.8[24-26]. In GO analysis, we assessed biological processes, cellular components, and molecular functions. Further, we used the STRING (Search Interaction Gene / Protein Search Tool) database to predict protein-protein interactions between STAB gene family members and other proteins [27].

**Statistical Analysis**

We used GraphPad Prism version 7.0 for Windows to generate survival curves and forest plots. All other statistical analyses were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA). P<0.05 was considered statistically significant.

**Result**

**Gene expression in tumors and normal tissues**

Presentation of STAB1 and STAB2 expression data from the TCGA dataset on a scatter plot showed that these two factors have different expressions levels (Fig. 1a, b). However, STAB1 and STAB2 were not differentially expressed (Fig. 1c, d) in tumor tissues and normal tissues of the GEO dataset. In addition, 7% of cancer samples showed STAB1 mutations, including missense mutation, truncating mutation, amplification, and deep deletion. Further, 5% of cancer samples showed mutations in the STAB2 gene including missense mutation, truncating mutation, amplification (Fig. 1e).

**Basic patient information**
Detailed information on the 370 HCC patients in TCGA database is shown in Table 1. BMI and TNM staging showed a significant correlation with MST ($p = 0.021$ and $<0.001$, respectively). Information on patients evaluated in the GEO database is shown in Table 2. Major tumor size, TNM stage, cirrhosis, BCLC stage, and alpha-fetoprotein levels were significantly correlated with OS ($p = 0.003$, 0.019, $<0.001$, $<0.001$, $<0.001$, 0.032, 0.019, and 0.026, respectively). Further, Gender, TNM stage, and BCLC stage were significantly correlated with RFS ($p = 0.016$, $<0.001$, and $<0.001$, respectively).

Table 1 Basic characteristics of HCC patients in the TCGA database

| Variables         | Patients (n=370) | No. of events (%) | MST (months) | HR (95%CI) | Log-rank P |
|-------------------|-----------------|------------------|--------------|------------|------------|
| Race              |                 |                  |              |            | 0.127      |
| Asian             | 157             | 44(28.0%)        | 82           | Ref        |            |
| White+others      | 203             | 81(40.1%)        | 68           | 1.42(0.98-2.05) |            |
| Missing           | 10              |                  |              |            |            |
| Gender            |                 |                  |              |            | 0.488      |
| Male              | 249             | 79(31.7%)        | 57           | Ref        |            |
| Female            | 121             | 51(42.1%)        | 59           | 1.07(0.89-1.28) |            |
| Age               |                 |                  |              |            | 0.248      |
| ≤60               | 177             | 55(31.1%)        | 71           | Ref        |            |
| >60               | 193             | 75(38.9%)        | 52           | 1.23(0.87-1.75) |            |
| BMI               |                 |                  |              |            | 0.021      |
| ≤25               | 177             | 61(34.5%)        | 68           | Ref        |            |
| >25               | 157             | 51(32.5%)        | 66           | 0.82(0.64-1.04) |            |
| Missing           | 36              |                  |              |            |            |
| TNM staging       |                 |                  |              |            | <0.001     |
| I+II              | 274             | 76(27.7%)        | 84           | Ref        |            |
| III+IV            | 94              | 54(57.4%)        | 26           | 2.40(1.69-3.41) |            |
| Missing           | 2               |                  |              |            |            |
| Family history    |                 |                  |              |            | 0.192      |
| Yes               | 112             | 49(44.1%)        | 40           | Ref        |            |
| No                | 207             | 69(33.3%)        | 62           | 0.78(0.54-1.13) |            |
| Missing           | 51              |                  |              |            |            |

MST, median survival time; HR, hazard ratio; 95%CI, 95% confidence interval; Ref, reference; BMI, body mass index; TNM stage, tumor, node and metastasis stage.

Survival analysis
The TCGA data were analyzed using a multivariate Cox regression model with adjustment for race, gender, age, BMI, TNM stage, and family history, which showed no significant correlation between STAB1 and STAB2 with prognosis (adjusted $p=0.300$ and 0.865 respectively) (Fig. 2a, b, g). GEO data were evaluated by a multiple Cox regression model, with adjustment for race, age, HBV, ALT, major tumor size, multiple nodule status, liver cirrhosis, TNM staging, BCLC staging, and AFP, showing that STAB2 was significantly correlated with OS (HR = 0.541; 95% CI, 0.339-0.865; adjusted $p = 0.010$) and RFS (HR = 0.554; 95% CI, 0.376-0.816; adjusted $p = 0.003$) after adjustment (Fig. 2c, d, e, f, h, i).

**Stratified chi-square test**

We performed a hierarchical chi-square test on all clinical features. The results showed that high expression of STAB2 implied a more favorable prognosis in male, HBV (CC + NO), ALT $> 50$u / L, non-multinodular, cirrhosis, early TNM(I and II), early BCLC (0 and A) and AFP $<=300$ng/ml ($p=0.013$, 0.008, 0.039, 0.040, 0.024, 0.018, 0.010 and 0.038 respectively). (Table 3)

**Functional enrichment analysis**

GO analysis showed STAB2 gene assembly in Hyaluronan catabolic process, hyaluronan metabolic process, regulation of calcineurin−NFAT signaling cascade, aminoglycan catabolic process, glycosaminoglycan catabolic process, mucopolysaccharide metabolic process, aminoglycan metabolic process, glycosaminoglycan metabolic process, carbohydrate derivative catabolic process, glycosaminoglycan binding, organonitrogen compound catabolic process cellular response to fibroblast growth factor stimulus, regulation of cell–substrate adhesion, regulation of apoptotic signaling pathway and cell migration. KEGG pathway enrichment showed that members of the STAB gene family is implicated in glycosaminoglycan degradation vitamin digestion and absorption, ECM−receptor interaction shigellosis bile secretion gastric acid secretion cardiac muscle contraction, hematopoietic cell lineages salivary secretion pancreatic secretion proteoglycans in cancer thyroid hormone signaling pathway adrenergic signaling in cardiomyocytes cAMP signaling pathway Epstein−Barr virus infection and regulation of actin cytoskeleton. Details of the enrichment analysis are shown in Fig. 3b and Fig. 3b.

Analysis of the protein-protein interaction network using the STRING database shows that STAB1 and STAB2 are directly or indirectly related to some hub genes in HCC (such as CD44 and APOB) (Fig. 4).

| Table 2. Basic characteristics of HCC patients in the GEO database |
### Table 3. Stratified chi-square test of basic characteristics of HCC patients in the GEO database

| Variables          | Patients (n=221) | No. of events (%) | MST months | OS HR (95%CI) | Log-rank P |
|--------------------|-----------------|-------------------|------------|---------------|------------|
| **Gender**         |                 |                   |            |               |            |
| Female             | 30              | 13                | 56         | 1.70 (0.82-3.52) | 0.149      |
| Male               | 191             | 97                | 54         | 1.280 (0.31-5.37) | 0.013      |
| Age                |                 |                   |            |               |            |
| ≤60                | 181             | 13                | 56         | 1.88 (1.22-2.89) | 0.055      |
| >60                | 40              | 97                | 54         | 0.65 (0.33-1.30) | <0.001     |
| HBV status         |                 |                   |            |               |            |
| AVR-CC             | 56              | 13                | 56         | 0.91 (0.12-6.74) | 0.449      |
| CC+NO              | 162             | 97                | 54         | 1.22 (0.61-2.44) | 0.411      |
| Missing            | 3               | 3                 |            |               | 0.734      |
| ALT ≤50u/L         | 130             | 13                | 56         | 1.08 (0.70-1.66) | 0.003      |
| >50u/L             | 91              | 3                 |            | 1.25 (0.87-1.78) | 0.229      |
| Main tumor size    |                 |                   |            |               |            |
| ≤5cm               | 140             | 13                | 56         | 1.88 (1.22-2.89) | 0.055      |
| >5cm               | 81              | 97                | 54         | 0.65 (0.33-1.30) | <0.001     |
| Multinodular       |                 |                   |            |               |            |
| Yes                | 45              | 13                | 56         | 0.91 (0.12-6.74) | 0.055      |
| No                 | 176             | 3                 |            | 1.22 (0.61-2.44) | 0.427      |
| Cirrhosis          |                 |                   |            |               |            |
| Yes                | 203             | 13                | 56         | 0.91 (0.12-6.74) | 0.055      |
| No                 | 18              | 97                | 54         | 1.22 (0.61-2.44) | 0.055      |
| TNM staging        |                 |                   |            |               |            |
| I+II               | 169             | 13                | 56         | 0.91 (0.12-6.74) | 0.055      |
| III                | 49              | 3                 |            | 1.22 (0.61-2.44) | 0.055      |
| Missing            | 3               | 3                 |            |               | 0.734      |
| BCLC staging       |                 |                   |            |               |            |
| 0+A                | 168             | 13                | 56         | 0.91 (0.12-6.74) | 0.055      |
| B+C                | 51              | 97                | 54         | 1.22 (0.61-2.44) | 0.055      |
| Missing            | 2               | 2                 |            |               | 0.734      |
| AFP ≤300ng/ml      | 118             | 13                | 56         | 0.91 (0.12-6.74) | 0.055      |
| >300ng/ml          | 100             | 2                 |            | 1.22 (0.61-2.44) | 0.055      |
| Missing            | 3               | 3                 |            |               | 0.734      |

OS, overall survival; RFS, recurrence-free survival; HBV status, hepatitis B virus status; AVR-CC, active viral replication chronic carrier; CC, chronic carrier; ALT, alanine aminotransferase; AFP, alpha fetoprotein; TNM stage, tumor, node and metastasis stage; BCLC staging, Barcelona Clinic Liver Cancer.
### HBV status

|                | low expression | high expression | Ref     | p-value |
|----------------|----------------|----------------|---------|---------|
| AVR-CC         |                |                |         |         |
| low expression | 31 1445.2%     | 46 1144.0%     | Ref     | 0.865   |
| high expression| 25 1144.0%     | 45 1.070.49-2.36|         |         |
| CC+NO          |                |                |         |         |
| low expression | 76 3647.4%     | 45 Ref         |         | 0.008   |
| high expression| 86 2326.7%     | 55 0.500.29-0.84|         |         |

### ALT

|                | low expression | high expression | Ref     | p-value |
|----------------|----------------|----------------|---------|---------|
| ≤50u/L         |                |                |         |         |
| low expression | 63 2742.9%     | 46 Ref         |         | 0.191   |
| high expression| 67 2131.3%     | 51 0.680.39-1.21|         |         |
| >50u/L         |                |                |         |         |
| low expression | 47 2451.1%     | 43 Ref         |         | 0.039   |
| high expression| 44 1329.5%     | 54 0.490.25-0.96|         |         |

### Multinodular

|                | low expression | high expression | Ref     | p-value |
|----------------|----------------|----------------|---------|---------|
| Yes            |                |                |         |         |
| low expression | 26 1557.7%     | 41 Ref         |         | 0.413   |
| high expression| 19 842.1%      | 47 0.700.30-1.65|         |         |
| No             |                |                |         |         |
| low expression | 84 3642.9%     | 46 Ref         |         | 0.04    |
| high expression| 92 2628.3%     | 54 0.590.36-0.98|         |         |

### Cirrhosis

|                | low expression | high expression | Ref     | p-value |
|----------------|----------------|----------------|---------|---------|
| Yes            |                |                |         |         |
| low expression | 102 5049.0%    | 44 Ref         |         | 0.024   |
| high expression| 101 3332.7%    | 51 0.600.39-0.94|         |         |
| No             |                |                |         |         |
| low expression | 18 112.5%      | 56 Ref         |         | 0.892   |
| high expression| 10 110.0%      | 63 0.830.05-13.21|         |         |

### TNM staging

|                | low expression | high expression | Ref     | p-value |
|----------------|----------------|----------------|---------|---------|
| I+II           |                |                |         |         |
| low expression | 83 3339.8%     | 50 Ref         |         | 0.018   |
| high expression| 86 2023.3%     | 58 0.510.29-0.89|         |         |
| III            |                |                |         |         |
| low expression | 24 1770.8%     | 28 Ref         |         | 0.422   |
| high expression| 25 1456.0%     | 32 0.750.37-1.52|         |         |
| Missing        |                |                |         |         |
BCLC staging

|       | low expression | high expression | p     |
|-------|----------------|-----------------|-------|
| 0+A   | 83             | 1821.2%         | 50    | Ref          | 0.01 |
|       | 85             | 3339.8%         | 58    | 0.470.26-0.83|
| B+C   | 25             | 1768.0%         | 28    | Ref          | 0.756|
|       | 26             | 1661.5%         | 31    | 0.900.45-1.78|

AFP

|       | low expression | high expression | p     |
|-------|----------------|-----------------|-------|
| ≤300ng/ml | 49             | 2244.9%         | 49    | Ref          | 0.038|
| >300ng/ml | 69             | 1724.6%         | 56    | 0.510.27-0.96|
|       | 59             | 2949.2%         | 41    | Ref          | 0.378|
|       | 41             | 1741.5%         | 47    | 0.760.42-1.39|

OS, overall survival; RFS, recurrence-free survival; HBV status, hepatitis B virus status; AVR–CC, active viral replication chronic carrier; CC, chronic carrier; ALT, alanine aminotransferase; AFP, alpha fetoprotein; TNM stage, tumor, node and metastasis stage; BCLC staging, Barcelona Clinic Liver Cancer.

**Discussion**

In this study, we investigated the association between Stabilin family genes and HCC. The findings show that the changes in mRNA expression level of Stabilin-2 are effective in HCC prognosis. Stabilin-1 is expressed in non-continuous sinusoidal endothelium of liver, spleen, adrenal cortex, lymph node and sinusoidal macrophages[21]. Stabilin-1 is a multifunctional transmembrane protein involved in scavenging, cell adhesion, lymphocyte transmigration and angiogenesis[28]. A previous study reports that high levels of Stabilin-1 peritumoral macrophages are positively correlated with survival (p =0.04) in colorectal cancer. However, in more advanced stages (Stage IV), patients with a high number of peritumoral or intratumoral Stabilin-1 macrophages showed a shorter disease-specific survival (p=0.05, and p=0.008, respectively) [28]. In addition, previous studies have reported that Stabilin-1 is associated with the prognosis of bladder urothelial carcinoma and oral squamous cell carcinoma[29, 30]. Riabov V demonstrated that Stabilin-1 is expressed on tumor-associated macrophages (TAM) in human breast cancer, and with higher expression levels recorded in stage I [31]. In addition, higher Stabilin-1 expression levels are observed at inflammatory sites with increased leucocyte recruitment and vessels supplying HCCs [32]. In our study, Stabilin-1 mRNA expression in liver cancer and normal tissues was significantly different, however, Stabilin-1 levels showed no significant correlation with HCC prognosis.

Stabilin-2 is expressed in low levels in several human tissues, but highly expressed in non-continuous sinusoidal endothelium of liver, lymph node, spleen and bone marrow tissues[33]. This protein serves as a hyaluronan receptor in endocytosis, as a scavenger receptor that binds to bacteria, and as a protein that endocytoses modified low-density lipoprotein and the end-products of glycation [19]. A previous study reports that Stabilin-2 can be used as a diagnostic biomarker for detecting prostate cancer in seminal plasma [34]. Further, a systemic block of Stabilin-2 was found to inhibit lymph node (LN) metastasis in an orthotopic prostate cancer model [20]. Therefore, Stabilin-2 may be associated with lymph node metastasis of tumors. Notably, a previous study
reported that Stabilin-2 was significantly associated with lymph node metastasis in tongue, lung, stomach, and colon cancer, and was implicated in tongue cancer prognosis [19]. Stabilin-2 modulates LN metastasis through Stabilin-2-mediated homotypic interaction between tumor cells and LN. Hyaluronic acid (HA) is a biopolymer composed of repeating units of disaccharides, which consisting of D-glucuronic acid and N-acetylglucosamine molecules linked by β- (1-4) and β- (1-3)glycosides[35]. HA has been shown to regulate proliferation, invasion, cell movement, multidrug resistance, and epithelial-mesenchymal transition in many in vivo and in vitro tumor cell lines[36]. Stabilin-2 is a key HA scavenger receptor, which mediates macrophage binding and engulfs bacteria or apoptotic cells [37]. HA levels are used as non-invasive biomarker to assess liver fibrosis [38]. Cirrhosis, the highest stage of liver fibrosis, is the main risk factor for HCC. HCC cells treated with HA show aggressive proliferation, migration and energy metabolism properties in vitro[39]. Preoperative high serum HA levels predict poor prognosis for HCC patients after liver resection [40]. In our study, STAB2 expression level was higher in normal liver tissue compared with liver cancer tissue. High expression of STAB2 implies a better prognosis in HCC tissues. Therefore, we presume that STAB2 may be a protective factor for HCC, which may be related to the clearance of HA. However, another study reported that absence of STAB2 in tissues surrounding tumors is associated with longer survival [41]. Notably, inhibition of Stabilin-2 increases circulating hyaluronic acid levels and prevents tumor metastasis [18]. However, the findings from this study were not consistent with previous findings, therefore, the relationship between STAB2 and HCC should be explored with larger samples and molecular methods.

STAB2 expression was shown to improve the symptoms of patients with certain characteristics such as male, HBV (CC + NO), high level of ALT(ALT> 50u / L), non-multinodular, cirrhosis, early TNM(I and II), early BCLC (0 and A), and AFP <= 300ng / ml. (p=0.012, 0.007, 0.035, 0.038,0.022,0.016,0.008 and 0.035 respectively). These effects may have amalgamative effect on STAB2 thus improving the protective role on HCC. Previous studies have shown that low level of AFP, early stages of TNM and BCLC are positively correlated with the HCC prognosis [42, 43].

In GO and KEGG functional enrichment analyses, we found that STAB2 plays a role in the metabolism of mucopolysaccharide metabolic process, glycosaminoglycan metabolic process, glycosaminoglycan binding, fat digestion and absorption, regulation of cell–substrate adhesion and regulation of apoptotic signaling pathway. In a previous study, stichopus japonicus acid mucopolysaccharide (SJAMP) effectively inhibited the growth of HCC through the stimulation of immune organs and tissue proliferation[44]. In addition, inhibitors of glycosaminoglycan biosynthesis may affect liver metastasis potential of tumor cells[45]. The proteoglycan composed of protein core and glycosaminoglycan chain plays a vital role in the development of HCC, and is been explored as a potential HCC biomarker and therapeutic target[46]. Glycosaminoglycans are involved in HA synthesis, and Stabilin-2 as the main scavenger receptor for systemic HA[47], is involved in regulating HA metabolic pathways as reported previously. Reduction of CD44 may lead to reduced cell-substrate adhesion of fibroblasts, resulting in cell migration and invasion[48]. Non-alcoholic steatohepatitis (NASH) is a common cause of cirrhosis and hepatocellular carcinoma (HCC). Studies have shown that a high-fat diet promotes serum cholesterol levels in mice liver, subsequently increases vascular endothelial-derived growth factor (VEGF) levels, and ultimately leads to high proliferation of liver tumor cells[49]. STAB2 acts as a scavenger receptor and is implicated in cell apoptosis[50]. Defective or inadequate apoptosis may increase metastasis, tumor progression, and tumor cell radiotherapy[51]. Apoptosis also plays an important role in the development of liver cancer.

Based on the protein-protein interaction results, STABs interacts with some hub genes in HCC, including CD44 and APOB. A recent study showed that the initiation of HCC requires the inhibition of p53 by CD44-enhanced growth factor signaling[52]. In another study, APOB inactivation was shown to be associated with poor outcome in patients with HCC, therefore, APOB may play a role in regulating multiple genes involved in HCC development [53].

However, our research has limitations. First, our sample size is small. Second, we studied single genes, therefore they should be analyzed in conjunction with other genes. Finally, more clinical data should be collected to evaluate the relationship between STAB gene family and HCC. This clinical information may include smoking, drinking, Child-Pugh score, arterial chemoembolization, antitumor status, number of tumors, tumor envelope status, intrahepatic metastasis and vascular invasion.
Conclusion

In conclusion, our research reports that the expression levels of STAB2 are higher in HCC tissue than in normal tissue and STAB2 is significantly correlated with OS and RFS. It may be a potential HCC prognostic marker.

Abbreviations

HCC: Hepatocellular carcinoma; STAB: Stabilin; HR: hazard ratio; CI: confidence interval; HBV: hepatitis B virus; AFP: alpha-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; miR: microRNA; TCGA: The Cancer Genome Atlas; GEO: Gene Expression Synthesis; MST: median survival time; OS: overall survival; RFS: recurrence-free survival; ALT: alanine aminotransferase; BCLC: Barcelona Clinic Liver Cancer; GO: gene ontology; TAM: tumor-associated macrophages; LN: lymph node; HA: hyaluronic acid; SJAMP: stichopus japonicus acid mucopolysaccharide; NASH: non-alcoholic steatohepatitis; DAVID: Database for Annotation, Visualization, and Integrated Discovery; KEGG: Kyoto Encyclopedia of Genes and Genomes; AVR-CC, active viral replication chronic carrier; CC, chronic carrier; TNM, tumor, node and metastasis; BMI: body mass index; VEGF: vascular endothelial-derived growth factor.

Declarations

Acknowledgment

Not applicable

Authors' contributions

ZD and WZ designed the study; ZD and PZ analyzed the data; ZD wrote the manuscript; LY, KC, JY and WZ revised the manuscript. All authors read and approved the final.

Funding

This research was supported by the National Natural Science Foundation of China (81702863), Medical Science and Technology Project of Henan Province (SBGJ2018021).

Availability of data and materials

The datasets generated and analyzed during the current study are available in the TCGA and GEO repository.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1 Department of Hepatobiliary and Pancreatic Surgery, The First Affiliated Hospital of Zhengzhou University, No.1 Jianshe East Road, Zhengzhou 450052, Henan Province, China.
1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: Global cancer statistics, 2012. CA: a cancer journal for clinicians 2015, 65(2):87-108.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 2018, 68(6):394-424.
3. Wang J, Zhao H, Yu J, Xu X, Liu W, Jing H, Li N, Tang Y, Li Y, Cai J et al: MiR-92b targets p57kip2 to modulate the resistance of hepatocellular carcinoma (HCC) to ionizing radiation (IR) -based radiotherapy. Biomed Pharmacother 2019, 110:646-655.
4. Cadier B, Bulsei J, Nahon P, Seror O, Laurent A, Rosa I, Layese R, Costentin C, Cagnot C, Durand-Zaleski I et al: Early detection and curative treatment of hepatocellular carcinoma: A cost-effectiveness analysis in France and in the United States. Hepatology 2017, 65(4):1237-1248.
5. Forner A, Llovet JM, Bruix J: Hepatocellular carcinoma. The Lancet 2012, 379(9822):1245-1255.
6. Chen Y, Tian Z: HBV-Induced Immune Imbalance in the Development of HCC. Front Immunol 2019, 10:2048.
7. Liu Q, Li F, Zhuang Y, Xu J, Wang J, Mao X, Zhang Y, Liu X: Alteration in gut microbiota associated with hepatitis B and non-hepatitis virus related hepatocellular carcinoma. Gut Pathog 2019, 11:1.
8. Febbraio MA, Reibe S, Shalapour S, Ooi GJ, Watt MJ, Karin M: Preclinical Models for Studying NASH-Driven HCC: How Useful Are They? Cell Metab 2019, 29(1):18-26.
9. Ninio L, Nissani A, Meirson T, Domovitz T, Genna A, Twafra S, Srikanth KD, Dabour R, Avraham E, Davidovich A et al: Hepatitis C Virus Enhances the Invasiveness of Hepatocellular Carcinoma via EGFR-Mediated Invadopodia Formation and Activation. Cells 2019, 8(11).
10. Ilikhan SU, Bilici M, Sahin H, Akca AS, Can M, Oz, Il, Guven B, Buyukususal MC, Ustundag Y: Assessment of the correlation between serum prolidase and alpha-fetoprotein levels in patients with hepatocellular carcinoma. World J Gastroenterol 2015, 21(22):6999-7007.
11. Hagag NA, Ali YBM, Elsharawy AA, Talaat RM: Clinical Impact of Circulated mir-1291 in Plasma of Patients with Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC): Implication on Glypican-3 Expression. J Gastrointest Cancer 2019.
12. Li J, Cheng ZJ, Liu Y, Yan ZL, Wang K, Wu D, Wan XY, Xia Y, Lau WY, Wu MC et al: Serum thioredoxin is a diagnostic marker for hepatocellular carcinoma. Oncotarget 2015, 6(11):9551-9563.
13. Luo P, Wu S, Yu Y, Ming X, Li S, Zuo X, Tu J: Current Status and Perspective Biomarkers in AFP Negative HCC: Towards Screening for and Diagnosing Hepatocellular Carcinoma at an Earlier Stage. Pathol Oncol Res 2019.
14. Yu R, Tan Z, Xiang X, Dan Y, Deng G: Effectiveness of PIVKA-II in the detection of hepatocellular carcinoma based on real-world clinical data. BMC Cancer 2017, 17(1):608.
15. Oka H, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, Osaki Y, Seki T, Kudo M, Tanaka M: Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. Journal of gastroenterology and hepatology 2001, 16(12):1378-1383.
16. Wu XM, Xi ZF, Liao P, Huang HD, Huang XY, Wang C, Ma Y, Xia Q, Yao JG, Long XD: Diagnostic and prognostic potential of serum microRNA-4651 for patients with hepatocellular carcinoma related to aflatoxin B1. Oncotarget 2017, 8(46):81235-81249.
17. Chen S, Chen H, Gao S, Qiu S, Zhou H, Yu M, Tu J: Differential expression of plasma microRNA-125b in hepatitis B virus-related liver diseases and diagnostic potential for hepatitis B virus-induced hepatocellular carcinoma. Hepatol Res 2017, 47(4):312-320.
18. Hirose Y, Saijou E, Sugano Y, Takeshita F, Nishimura S, Nonaka H, Chen YR, Sekine K, Kido T, Nakamura T et al: Inhibition of Stabilin-2 elevates circulating hyaluronic acid levels and prevents tumor metastasis. Proc Natl Acad Sci U S A 2012, 109(11):4263-4268.
19. Han MW, Lee J, Park SY, Kim YM, Cho KJ, Kim SW, Lee M, Nam SY, Kim IS, Kim SY: Homotypic Interaction of Stabilin-2 Plays a Critical Role in Lymph Node Metastasis of Tongue Cancer.
Anticancer Res 2016, 36(12):6611-6618.

20. Simpson MA, Weigel JA, Weigel PH: Systemic blockade of the hyaluronan receptor for endocytosis prevents lymph node metastasis of prostate cancer. Int J Cancer 2012, 131(5):E836-840.

21. Kzhyshkowska J: Stabilin-1, a homeostatic scavenger receptor with multiple functions. Journal of Cellular and Molecular Medicine 2006, 10(3).

22. Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, Thorgeirsson SS, Sun Z, Tang ZY, Qin LX et al: A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. Cancer research 2010, 70(24):10202-10212.

23. Roessler S, Long EL, Budhu A, Chen Y, Zhao X, Ji J, Walker R, Jia HL, Ye QH, Qin LX et al: Integrative genomic identification of genes on 8p associated with hepatocellular carcinoma progression and patient survival. Gastroenterology 2012, 142(4):957-966.e912.

24. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK: Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nature communications 2019, 10(1):1523.

25. Huang da W, Sherman BT, Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols 2009, 4(1):44-57.

26. Kanehisa M, Goto S, Hattori M, Aoki-Kinoshita KF, Itoh M, Kawashima S, Katayama T, Araki M, Hirakawa M: From genomics to chemical genomics: new developments in KEGG. Nucleic acids research 2006, 34(Database issue):D354-D357.

27. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P et al: STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic acids research 2019, 47(D1):D607-d613.

28. Algars A, Ijrala H, Vaittinen S, Huhtinen H, Sundstrom J, Salmi M, Ristamaki R, Jalkanen S: Type and location of tumor-infiltrating macrophages and lymphatic vessels predict survival of colorectal cancer patients. Int J Cancer 2012, 131(4):864-873.

29. Wang B, Huang H, Yang M, Yang W, Liu Z, Hou W, Zeng H, He Z, Lin T, Huang J: Microlocalization and clinical significance of stabilin-1(+ ) macrophages in treatment-naive patients with urothelial carcinoma of the bladder. World J Urol 2019.

30. Kwon M, Yeo SC, Lee JS, Park JI: Not CD68 but stabilin-1 expression is associated with the risk of recurrence in patients with oral cavity squamous cell carcinoma. Head Neck 2019, 41(7):2058-2064.

31. Riabov V, Yin S, Song B, Avdic A, Schledzewski K, Osviy I, Gratchev A, Llopis Verdiell M, Sticht C, Schmuttermaier C et al: Stabilin-1 is expressed in human breast cancer and supports tumor growth in mammary adenocarcinoma mouse model. Oncotarget 2016, 7(21):31097-31110.

32. Patten DA, Shetty S: The Role of Stabilin-1 in Lymphocyte Trafficking and Macrophage Scavenging in the Liver Microenvironment. Biomolecules 2019, 9(7).

33. Miller CM, Donner AJ, Blank EE, Egger AW, Kellar BM, Ostergaard ME, Seth PP, Harris EN: Stabilin-1 and Stabilin-2 are specific receptors for the cellular internalization of phosphorothioate-modified antisense oligonucleotides (ASOs) in the liver. Nucleic Acids Res 2016, 44(6):2782-2794.

34. Neuhaus J, Schiffer E, von Wilcke P, Bauer HW, Leung H, Siwy J, Ulrici W, Paasch U, Horn LC, Stolzenburg JU: Seminal plasma as a source of prostate cancer peptide biomarker candidates for detection of indolent and advanced disease. PLoS One 2013, 8(6):e67514.

35. Salwowska NM, Bebenek KA, Żądło DA, Wcisło-Dziadecka DL: Physicochemical properties and application of hyaluronic acid: a systematic review. Journal of cosmetic dermatology 2016, 15(4):520-526.

36. Sironen RK, Tammi M, Tammi R, Auvinen PK, Anttila M, Kosma VM: Hyaluronan in human malignancies. Exp Cell Res 2011, 317(4):383-391.

37. Weigel PH: Discovery of the Liver Hyaluronan Receptor for Endocytosis (HARE) and Its Progressive Emergence as the Multi-Ligand Scavenger Receptor Stabilin-2. Biomolecules 2019, 9(9).

38. Neuman MG, Cohen LB, Nanau RM: Hyaluronic acid as a non-invasive biomarker of liver fibrosis. Clin Biochem 2016, 49(3):302-315.

39. Li JH, Wang YC, Qin CD, Yao RR, Zhang R, Wang Y, Xie XY, Zhang L, Wang YH, Ren ZG: Over expression
of hyaluronan promotes progression of HCC via CD44-mediated pyruvate kinase M2 nuclear translocation. American journal of cancer research 2016, 6(2):509-521.

40. Mima K, Beppu T, Ishiko T, Chikamoto A, Nakagawa S, Hayashi H, Watanabe M, Sakamaki K, Baba H: Preoperative serum hyaluronic acid level as a prognostic factor in patients undergoing hepatic resection for hepatocellular carcinoma. Br J Surg 2014, 101(3):269-276.

41. Geraud C, Mogler C, Runge A, Evdokimov K, Lu S, Schledzewski K, Arnold B, Hammerling G, Koch PS, Breuhahn K et al: Endothelial transdifferentiation in hepatocellular carcinoma: loss of Stabilin-2 expression in peri-tumourous liver correlates with increased survival. Liver Int 2013, 33(9):1428-1440.

42. Ma WJ, Wang HY, Teng LS: Correlation analysis of preoperative serum alpha-fetoprotein (AFP) level and prognosis of hepatocellular carcinoma (HCC) after hepatectomy. World journal of surgical oncology 2013, 11:212.

43. Hu L, Xue F, Li Y, Shao M, Sun Y, Wei G: A long-term follow-up and comprehensive observation of risk and prognosis factors of recurrence and survival after resection of hepatocellular carcinoma. Cell Biochem Biophys 2014, 69(3):421-431.

44. Song Y, Jin SJ, Cui LH, Ji XJ, Yang FG: Immunomodulatory effect of Stichopus japonicus acid mucopolysaccharide on experimental hepatocellular carcinoma in rats. Molecules 2013, 18(6):7179-7193.

45. Wei H, Wang J, Li W, Ma R, Xu Z, Luo Z, Lu Y, Zhang X, Long X, Pu J et al: The underlying pathophysiology association between the Type 2-diabetic and hepatocellular carcinoma. J Cell Physiol 2019, 234(7):10835-10841.

46. Tanaka Y, Tateishi R, Koike K: Proteoglycans Are Attractive Biomarkers and Therapeutic Targets in Hepatocellular Carcinoma. Int J Mol Sci 2018, 19(10).

47. Harris EN, Cabral F: Ligand Binding and Signaling of HARE/Stabilin-2. Biomolecules 2019, 9(7).

48. Tsuneki M, Madri JA: CD44 Influences Fibroblast Behaviors Via Modulation of Cell-Cell and Cell-Matrix Interactions, Affecting Survivin and Hippo Pathways. J Cell Physiol 2016, 231(3):731-743.

49. Miura K, Ohnishi H, Morimoto N, Minami S, Ishioka M, Watanabe S, Tsukui M, Takaoka Y, Nomoto H, Isoda N et al: Ezetimibe suppresses development of liver tumors by inhibiting angiogenesis in mice fed a high-fat diet. Cancer Sci 2019, 110(2):771-783.

50. Penberthy KK, Ravichandran KS: Apoptotic cell recognition receptors and scavenger receptors. ImmunoL Rev 2016, 269(1):44-59.

51. Li L, Hong H-H, Chen S-P, Ma C-Q, Liu H-Y, Yao Y-C: Activation of AMPK/MnSOD signaling mediates anti-apoptotic effect of hepatitis B virus in hepatoma cells. World journal of gastroenterology 2016, 22(17):4345-4353.

52. Dhar D, Antonucci L, Nakagawa H, Kim JY, Glitzner E, Caruso S, Shalapour S, Yang L, Valasek MA, Lee S et al: Liver Cancer Initiation Requires p53 Inhibition by CD44-Enhanced Growth Factor Signaling. Cancer Cell 2018, 33(6):1061-1077 e1066.

53. Lee G, Jeong YS, Kim DW, Kwak MJ, Koh J, Joo EW, Lee JS, Kah S, Sim YE, Yim SY: Clinical significance of APOB inactivation in hepatocellular carcinoma. Exp Mol Med 2018, 50(11):147.
STAB1 and STAB2 gene expression levels between tumor and normal tissue in the TCGA (A, B). STAB1 and STAB2 gene expression levels between tumor and normal tissue in the GEO dataset (C, D). Genetic alteration information of the STAB gene family (E). ***: p<0.001, ****: p<0.0001, ns: no significant difference.
Figure 2

Kaplan-Meier overall survival curves and forest plots of the STAB gene family in TCGA (A, B, G). Overall survival curves and forest plots of the STAB gene family (C, D, H), as well as recurrence-free survival and forest plots (E, F, I) in the GEO dataset.
Regulation of apoptotic signaling pathway

Cell migration

Gene ra

Gene rat

Description

- Glycosaminoglycan degradation
- Vitamin digestion and absorption
- Fat digestion and absorption
- ECM–receptor interaction
- Shigellosis
- Bile secretion
- Gastric acid secretion
- Cardiac muscle contraction
- Hematopoietic cell lineage
- Salivary secretion
- Pancreatic secretion
- Proteoglycans in cancer
- Thyroid hormone signaling pathway
- Adrenergic signaling in cardiomyocytes
- cAMP signaling pathway
- Epstein–Barr virus infection
- Regulation of actin cytoskeleton
Figure 3
GO (A) and KEGG (B) enrichment analysis of the two STAB genes.
Figure 4

Protein-protein interaction networks among the Stabilin-1, Stabilin-2 and other proteins.