# Prognostic values and clinical relationship of AHSA1 in hepatocellular carcinoma

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**Research Article**

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## Abstract

# Objective

To investigate the expression status of the activator of Hsp90 ATPase homolog 1-like protein (AHSA1)in hepatocellular carcinoma（HCC）, further analyzed the relationship between AHSA1 expression level and prognosis and possible mechanism of action .

# Methods

HCCDB, GEPIA and Oncomine databases were used to analyze the expression of AHSA1 mRNA in hepatocellular carcinoma and normal liver tissues. Download RNA-seq data and the clinical information of LIHC from the TCGA database, Univariate and multivariate Cox proportional hazards regression models and Kaplan–Meier plots were used to evaluate the prognostic value of AHSA1 in HCC. R software was used to construct the nomogram based on the expression level of AHSA1. The calibration curve was plotted to evaluate the consistency between the actual survival and the predicted survival. GO and KEGG gene set enrichment analysis revealed tumor-associated biological processes related to AHSA1. The TIMER2.0 and GFPIA database are used to evaluate the correlation between AHSAl and tumor immune infiltration. The correlation between AHSA1 and the expression of 8 glycolysis-related genes in HCC was studied through the GEPIA database. Screening of small molecule targeted drugs for AHSA1 through CMAP.

# Results

The expression level of AHSA1 in patients with HCC was much higher than that in normal tissues（P < 0.01). High expression of AHSA1 was associated with a worse prognosis of HCC compared low expression of AHSA1(P < 0.05). AHSA1 expression was an independent factor affecting overall survival（HR = 1.970, p < 0.001）. The association between AHSA1 gene expression and the risk of HCC was presented in a nomogram. The AUC of OS for 1、3 and 5 years is 0.721、0.711 and 0.725 respectively. The calibration chart shows that the predicted survival rate is in good agreement with the actual survival rate curve. GO and KEGG enrichment analysis showed that AHSAl can promote tumor progression by mediating neutrophil activation and participating in biological processes such as glycolysis and gluconeogenesis. The expression level of AHSA1 mRNA was positively associated with the degrees of immune infiltration by B cells, CD4 + T cells, regulatory T cells, macrophages, neutrophils and dendritic cells in HCC(P < 0.05). The expression of AHSA1 was significantly positively correlated with the expression of glycolysis-related genes. Etacrynic acid and Blebbistatin may be small molecule targeted drugs that can reverse the expression of AHSA1.

# Conclusions

AHSA1 mRNA may be a potential oncogene in hepatocellular carcinoma, and its up-regulated expression plays an important role in the development and progression of hepatocellular carcinoma. High levels of AHSA1 mRNA expression may promote the immune infiltration of HCC and indicates the poor prognosis of HCC patients. AHSA1 might be a prognostic molecule and therapeutic target of hepatocellular carcinoma.

# Introduction

Hepatocellular carcinoma (HCC) is among the most causes of cancer-related deaths worldwide. Most HCC patients are already in the advanced stage when they are first diagnosed. Despite improved diagnosis and treatment strategies for HCC, HCC patients still have a high mortality rate and poor prognosis[1–3]. The development of HCC is a complex multi-step process, which may be closely related to the abnormal expression of some genes. Therefore, the discovery and identification of biomarkers related to the occurrence, development and prognosis of HCC and their possible molecular mechanisms are very important.

AHSA1 (activator of heat shock 90 kDa protein ATPase homolog 1) is a general up-regulator of Hsp90 (heat shock protein 90) function and an essential molecular chaperone in eukaryotic cells[4, 5]. The N-terminal domain of the co-chaperone factor AHSA1 interacts with the middle domain of Hsp90 to induce the catalytic loop region of the Hsp90 middle domain to form a stable conformation, and then the C-terminal domain of AHSA1 recognizes and binds to the dimerized N-terminal domain of Hsp90, And finally up-regulate the ATP hydrolysis function of Hsp90[6, 7]. Therefore, the AHSAl chaperone molecule has a unique ability to strongly enhance the inherent low ATPase activity of human Hsp90. It creates and maintains the functional conformation of a subset of proteins called "client proteins", which is a key component of multiple signal networks that mediate the proliferation, survival and metastasis of cancer cells[8]. Related studies have found that AHSA1 is significantly related to the occurrence and development of breast cancer[9], osteosarcoma[10], endometrial cancer[11], multiple myeloma[12] and other malignant tumors. However, there is currently no systematic study on the expression and function of AHSA1 in liver cancer. To this end, this study comprehensively explored the expression of AHSA1 in hepatocellular carcinoma based on multiple cohorts, and assessed the prognostic value of AHSA1 in hepatocellular carcinoma and the potential biological mechanism.

# Results

1. Pan-cancer analysis of AHSA1 mRNA expression

The HPA database shows that among the 63 types of human tissues, the top three tissues and organs with high AHSA1mRNA expression are the fallopian tube (pTPM = 222.2), testis (pTPM = 207.8), and smooth muscle (pTPM = 145.0). In the liver (pTPM = 33.8) Only ranked 42nd (Fig. 1A), suggesting the low expression of AHSA1 in human normal liver tissue. Further analysis of the expression and location of AHSA1 in a variety of tumor cells, the results suggest that AHSA1 is expressed in the cytosol(Fig. 1B).

We further analyzed the expression of AHSA1 mRNA in human tumors using TIMER database. The results showed that the difference of AHSA1 in different tumor tissues and normal tissues. Compared with normal tissues, the expression level of AHSA1 was significantly increased in BLCA(bladder urothelial carcinoma), BRCA (breast invasive carcinoma), CHOL (cholangiocarcinoma), COAD (colon adenocarcinoma), ESCA (esophageal carcinoma), HNSC (head and neck squamous cell carcinoma), KIRC (kidney renal clear cell carcinoma), LIHC (liver hepatocellular carcinoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), PRAD (prostate adenocarcinoma), READ (rectum adenocarcinoma) and STAD (stomach adenocarcinoma), while it was significantly decreased in GBM (glioblastoma multiforme) (Fig. 2).

2. Expression levels of AHSA1 in HCC patients.

Nine HCC gene sets were analyzed in the HCCDB database: HCCDB1 (p = 0.000005880), HCCDB3 (p = 2.860e-9), HCCDB4 (p = 1.990e-76), HCCDB6 (p = 8.830e-40) ), HCCDB7 (p = 0.00001210), HCCDB11 (p = 0.07038), HCCDB12 (p = 0.04648), HCCDB13 (p = 7.240e-7), HCCDB15 (p = 3.840e-17), HCCDB16 (p = 0.00001120), HCCDB17 (p = 1.110e-17), HCCDB16 (p = 3.730e-32), this study found that AHSA1 mRNA is highly expressed in HCC compared with neighboring normal tissues (Fig. 3A).

The GEPIA analysis of the expression level of AHSA1 in a total of 369 liver cancer samples and 160 normal liver tissue samples in the TCGA database and GTEx database showed that the expression level of AHSA1 in liver cancer tissue was higher than that in normal liver tissue, and the difference was statistically significant (Log2FC Cutoff = 0.8, P < 0.001) (Fig. 3B). Further verified by the Oncomine database, there are 4 sub-studies in the database that meet the screening criteria. The 4 sub-studies, namely Roessler Liver2, and Wurmbach Liver2, contained 299 liver cancer tissue samples and 261 normal liver tissue samples. A meta-analysis on it showed that the expression level of AHSA1 in liver cancer tissue was higher than that in normal liver tissue (P = 0.005) (Fig. 3C).

3. Prognostic value of AHSA1 for HCC patients.

We next investigated the prognostic value of AHSA1 for HCC using the Kaplan-Meier plotter. High AHSA1 was related to worse prognosis in HCC (OS: HR = 1.51(1.07–2.31), P = 0.019; DSS: HR = 1.67（1.07–2.68）, P = 0.032) (Fig. 4). Moreover, the univariate showed that high AHSA1 expression is significantly associated with poor overall survival（HR = 2.115（1.498–2.988）, P（0.001）. Multivariate analysis showed that AHSA1 was an independent prognostic factor for OS of HCC using TCGA database（HR = 1.970（1.395–2.782）, P（0.001）(Fig. 5).

A nomogram based on AHSA1 was established to estimate the 1-, 3-, and 5-year survival by using clinicopathological factors and Gene expression. Herein, as showed in Fig. 4F, the actual 1-, 3-, and 5-year survival times were consistent with the predicted ones by calibration plots of the nomogram. In the ROC curve, the AUC used to predict 1-year, 3-year, and 5-year survival rates are 0.721, 0.711, and 0.725, respectively (Fig. 6).

4. Biological process and pathway enrichment analysis.

For a more comprehensive understanding to the functional characteristics of AHSA1, we applied GO and KEGG analysis to DEGs using the clusterProfiler package. GO CC analysis showed that these DEGs were significantly enriched in secretory granule lumen, cytoplasmic vesicle lumen, vesicle lumen, tertiary granule and microvillus. For GO BP analysis, it is mainly related to mediating the activation of neutrophils to participate in the immune response. In terms of GO MF, it is mainly related to monosaccharide binding, glucose binding, vitamin binding, etc (Fig. 7A). KEGG pathway indicated that these differentially expressed genes primarily took part in glycolysis/gluconeogenesis, central carbon metabolism in cancer, tyrosine metabolism, and phenylalanine metabolism pathways (Fig. 7B). KEGG signaling pathway analysis showed that the expression of AHSAl is involved in the signal transduction process related to tumor cell energy metabolism.

5. AHSA1 expression is correlated with immune infiltration in LIHC

TIMER2.0 was used to analyze the correlation between AHSA1 expression and hepatocellular carcinoma immune infiltration, and the results showed: AHSA1 expression and B cells ($r = 0.256$, $p = 1.47e-06$), CD4 + T cells ($r = 0.166$, $p = 1.95e-03$), regulatory T cells ($r = 0.152$, $p = 4.83e-03$), macrophages ($r = 0.171$, $p = 1.43e-03$), neutrophils ($r = 0.167$, $p = 1.87e-03$), the degree of immune infiltration of dendritic cells ($r = 0.405$, $p = 4.37e-15$) has a significant correlation. This suggests that AHSA1 may be involved in the immune infiltration process of liver cancer cells (Fig. 8).

To further study the relationship between different immune infiltrating cells and AHSA1, GEPIA database was analyzed to analyze the association between AHSA1 and immune marker genes of several immune cells. The results showed that compared with normal liver tissues, the expression level of AHSA1 was significantly correlated with the immune marker set of some immune cells in liver cancer tissues($cor > 0$, $p < 0.05$) (Table 1). These results further suggest that the expression of AHSA1 may be related to Part of the tumor's immune cell infiltration is related, and the tumor progression is further promoted through immune infiltration.

6. Correlations of AHSA1 Expression with Glycolysis in HCC

Glycolysis of tumor cells plays an important role in the progression of HCC. By GEPIA database to investigate the correlation between AHSA1 and the expression of 8 glycolysis related genes in HCC. The results showed that the expression of AHSA1 was significantly positively correlated with PAM, NUP155, KDELR3, NSDHL, ENO1, SRD5A3, GOT2, and PKM (Fig. 9).

7. AHSA1 Related Potential Drug in HCC.

To further explore AHSA1 as a potential therapeutic target for HCC, CMap assays were employed. Based on the rationale of CMap analysis, we loaded the genes with positive cor-relation to AHSA1 as upregulated genes and the genes with negative correlation as downregulated genes into the CMap database to obtain drugs with inhibitory effect on AHSA1. the two compounds with the highest negative

enrichment score (etacrynic acid and blebbistatin) were identified as potential therapeutic agents for HCC (Fig. 10).

# Discussion

Due to complex molecular mechanisms, hepatocellular carcinoma is still one of the most life-threatening malignancies in the world. Although surgical resection, tumor embolization and radiofrequency ablation can improve survival, the prognosis of liver cancer is still very poor. immune escape, invasion and metastasis further reducing the long-term survival of patients with liver cancer. Therefore, there is an urgent need for prognostic biomarkers to assess the prognosis of liver cancer patients. With the development of gene sequencing technology, some potential gene markers with predictive value for liver cancer patients have been discovered. However, the number of such biomarkers is still limited. In order to improve the prognosis of liver cancer patients, more biomarkers with higher predictive accuracy need to be discovered. This study explored the role of AHSA1 in hepatocellular carcinoma for the first time through the analysis of public bioinformatics data, and further understood the potential value of AHSA1 in hepatocellular carcinoma.

Relevant studies have shown that decreased expression of tumor suppressor gene p53 will lead to increased expression of AHSA1[13]; AHSA1 up-regulates Hsp90, thereby activating the downstream protein MMP2 of Hsp90, which plays an important role in cell migration, invasion and angiogenesis in cancer. The down-regulation of AHSA1 leads to the reduction of angiogenesis in the body. The regulation of Hsp90 activity by regulating AHSA1 will affect the eNOS activity and VEGF-mediated effects in primary vascular endothelial cells. It can be seen that AHSA1 is a potential target for regulating Hsp90 activity in vascular endothelial cells, and it is a means to affect NO release and VEGF to stimulate angiogenesis. It can be seen that AHSAl is a potential target for regulating Hsp90 activity in vascular endothelial cells, and it is a means to affect NO release and VEGF to stimulate angiogenesis. Therefore, the up-regulation of AHSA1 can promote tumor tissue angiogenesis, thereby enhancing tumor cell proliferation and metastasis[14]. AHSA1 is highly expressed in osteosarcoma cells, and knocking out AHSA1 can inhibit cell growth, migration and invasion. microRNA-338-3p inhibits the proliferation, migration, invasion and EMT of osteosarcoma cells through an activator targeting 90 kDa heat shock protein ATPase homolog 1[10, 15]. It shows that AHSA1 plays an important role in tumor cell growth, migration and invasion.

Through a series of bioinformatics analyses conducted on publicly accessible online databases, we studied the expression level of AHSA1 in liver cancer and corresponding normal tissues, and the effect of AHSA1 expression on the prognosis. This study found that the expression of AHSA1 mRNA in hepatocellular carcinoma tissues is increased, and it is significantly related to the prognosis of hepatocellular carcinoma patients. It is an independent risk factor for predicting the overall survival of hepatocellular carcinoma patients. Potential prognostic indicators. In addition, according to the above-mentioned prognostic factors, the multi-factor COX risk ratio model was incorporated and the nomogram was established and verified. The results further proved the prognostic value of AHSA1 in hepatocellular carcinoma.

Immune cell infiltration and tumor microenvironment have been confirmed to play a key role in the occurrence and development of cancer[16]. Many studies have shown that hepatocellular carcinoma is closely related to immune tolerance and suppression[17], therefore, immunotherapy may be a promising anti-liver cancer strategy. Recently, immunotherapy has been found to be effective against many types of human cancers, such as melanoma[18] and lung cancer[19], but has less benefit in liver cancer. Most liver cancers develop from hepatitis B or C, which is an inflammation-driven disease. Studies have shown that immune cell infiltration accelerates the progression of chronic hepatitis to liver cancer and is also related to the poor prognosis of patients with liver cancer[20, 21], Including tumor-associated macrophages (TAM) [22], CD4 + regulatory T cells[23] and bone marrow-derived suppressor cells (MDSCs)[24] and so on. In this case, regulating the immune cell infiltration in the tumor microenvironment may be a new effective strategy for the treatment of liver cancer. However, how liver cancer cells evade immune surveillance has not been fully elucidated. This study showed through multiple databases that the expression level of AHSA1 mRNA is associated with increased infiltration of B cells, CD4 + regulatory T cells, regulatory T cells (Tregs), macrophages, neutrophils, and dendritic cells, indicating that AHSA1 may pass Regulating the immune microenvironment has a negative impact on the prognosis of liver cancer. And the GO function enrichment analysis results suggest that the biological process of AHSA1 is mainly related to mediating the activation of neutrophils and participating in the immune response. It also proves that AHSA1 may increase the infiltration of immune cells in the tumor microenvironment, leading to poor prognosis. Tregs can limit the effective anti-tumor immune response by releasing inhibitory cytokines, such as transforming growth factor (TGF)-β, IL-10 and IL-35, thereby promoting the progression of liver cancer[25, 26]; Tumor-associated macrophage (TAM) infiltration can attract Tregs to cancer sites by expressing cytokines and chemokines, thereby inhibiting anti-tumor immunity and promoting the progression of liver cancer[27], while promoting the distant metastasis of liver cancer[28], which is related to the poor prognosis of liver cancer patients. CD8 + cytotoxic T lymphocytes (CTL) are the immune cells of choice for targeting cancer[29]. Effector CTL infiltrates into the core or infiltrated part of the tumor and plays an important role in killing cancer cells, but tumor cells can pass change the phenotype to avoid CTL cell recognition and killing, promote tumor cell invasion and inhibit tumor immunotherapy. And this effect is interfered by cells such as cancer-associated fibroblasts (CAF), regulatory T cells (Tregs) and macrophages M2[30]. Studies have shown that AHSA1 is one of the core genes of tumor immune escape[27], the results of this study suggest that the expression of AHSA1 is related to the infiltration of Tregs and macrophages, and indirectly indicates that the expression of AHSA1 may trigger the infiltration of Tregs and M2 macrophages to trigger CTL immune evasion, leading to further tumor deterioration. This research provides a reference for further exploration of new immune-based therapy for liver cancer.

Aerobic glycolysis is one of the most important characteristics of tumor, which provides survival advantage for tumor, also known as the “Warburg effect”[31]. It not only provides energy for the growth of tumor cells but also provides raw materials for their biosynthesis. The enhancement of glycolysis is strongly associated to the development of cancer and the poor prognosis. Targeting cancer glycolysis metabolism is a new strategy for cancer treatment[32]. This study found that the KEGG signaling pathway

analysis results showed that AHSA1 differentially expressed genes are mainly enriched in glycolysis/gluconeogenesis. Related studies have reported 8 glycolysis-related genes that are significantly related to the poor prognosis of liver cancer. They are: PAM, NUP155, KDELR3, NSDHL, ENO1, SRD5A3, GOT2 and PKM[33]. This study found that the expression of AHSA1 is significantly related to these 8 genes. We suggest that AHSA1 may enhance the glycolytic ability of HCC by promoting the expression of PAM, NUP155, KDELR3, NSDHL, ENO1, SRD5A3, GOT2 and PKM. and thus promote the occurrence and development of HCC.

At present, many relevant studies have emerged in the clinic, using inhibitors to destroy the Hsp90α/AHSA1 complex, causing Hsp90α and AHSA1 to redistribute in the cytoplasm and reduce cell migration[34, 35]. Studies have shown that flavonoid TL-2-8 can induce the death of breast cancer cells by reducing the formation of Hsp90-AHSA1 complex, and play an anti-cancer effect[9]. This study uses the CMAP database to discover small molecule drugs that may reverse liver cancer gene expression. This discovery will help develop new target drugs for liver cancer. We mainly screened two small-molecule targeted drugs from CMAP: etacrynic acid and blebbistatin. There have been many documents confirming the anti-cancer effect of etacrynic acid. Studies have shown that etacrynic acid is glutathione. Glycine S-transferase (GST) inhibitor, and the expression of GST-π in hepatocytes is related to precancerous lesions and tumorigenesis, and leads to the emergence of drug-resistant phenotypes, so etacrynic acid exerts anti-cancer effects by inhibiting GST[36]. Sorafenib is a targeted molecular drug commonly used in the treatment of liver cancer. Etacrynic acid and sorafenib can play a better anti-cancer effect[37]; The combination of etacrynic acid and ciclopirox (CPX) has a powerful synergistic anti-tumor effect on liver cancer cells[38]. Blebbistatin is a myosin inhibitor. The excessive activity of myosin II ATPase is related to tumor metastasis and invasion[39], The use of blebbistatin to specifically inhibit non-muscle myosin II confirmed the key role of this molecule in pancreatic adenocarcinoma, breast cancer cell invasion and extracellular matrix interaction[40, 41]. But there is no research on blebbistatin in liver cancer. In the future, more experimental evidence and long-term clinical trials are needed to verify the role of etacrynic acid and blebbistatin in the treatment of liver cancer.

In summary, this study provides multi-level clues about the importance of AHSA1 in tumorigenesis and development and its potential as a new indicator of hepatocellular carcinoma. Although big data analysis can quickly and comprehensively mine potential data and functional biomolecules, various false positive results are inevitable. Our work is mainly to provide a quick and easy method for the screening of functional genes, suggesting that AHSA1 may become a molecular marker and new therapeutic target for judging the prognosis of patients with hepatocellular carcinoma, confirming the important role of AHSA1 in hepatocellular carcinoma, And point out the direction for future research. However, accurate conclusions require further experimental analysis and clinical verification.

# Materials And Methods

1. Expression of AHSA1 in HCC

We used HPA (human protein atlas) online database and TIMER database(https://cistrome.shinyapps.io/timer/) to analyze the difference of AHSA1 expression in different tumors. The HCCDB database (http://lifeome.net/Database/hccdb/home.html) was used to detect the expression level of AHSA1 mRNA in hepatocellular carcinoma tissues on multiple liver cancer data sets. The GEPIA database (http://gepia.cancer-pku.cn/) and Oncomine database were used to search for the expression of AHSA1 mRNA in liver cancer tissues and adjacent tissues. Finally, we verified the differential expression of AHSA1 in HCC and normal samples by (Immunohistochemistry) IHC staining.

2. Survival analysis

The prognostic value of AHSA1 for HCC in the TCGA database were appraised by the Kaplan-Meier plotter database(http://kmplot.com/analysis/), comprising overall survival (OS), relapse-free survival (RFS), and disease-specific survival (DSS).and then construct univariate and multivariate Cox regression models based on the patient information from the TCGA database using R software (version 3.5.2).

Nomograms are widely used in cancer prognosis to estimate the probability of a single event, such as death or recurrence, tailored to the characteristics of a single patient. In this study, the parameters and risk scores of the patients were combined to create a nomogram that was used to assess the prognosis. The nomogram was created for R software via the rms package. The bootstrap method was utilized in conjunction with a calibration curve (1,000 replicates) to visualize the difference between predicted and actual prob- abilities. The forecasting precision of a nomogram was measured using the concordance index (C-index).

3. Identification of DEGs、Biological process and pathway enrichment analysis.

The "wilcox.test" function of the R software was used to screen the differentially expressed genes (DEGs) between different AHSA1 expression groups in the tumor tissues of liver cancer patients in the TCGA database. To decrease the false positive rate, p values were adjusted in accordance with the Benjamini-Hochberg false discovery rate (FDR) method. And FDR < 0.05 and |log2 fold change|≥1 were set as the criteria to screen out DEGs.

GO analysis is a predominant bioinformatics tool for annotations of genes and their products, including three categories: cellular components (CC), molecular function (MF), and biological pathways (BP). KEGG is an aggregation of databases which consist of information about genomes, biological pathways, diseases, and chemicals. The clusterProfiler package (version: 3.18.0)[42] was employed to perform GO functional enrichment analysis and KEGG pathway analysis for DEGs in R studio version 1.1.456. The adjusted P < 0.05 was regarded as statistically significantly different.

4. Correlation Between AHSA1 and Tumor Immune Infiltrating Cells

To further explore the potential immunomodulatory mechanism of AHSA1 in the regulation of tumor-infiltrating immune cells, we used the TIMER2.0 database (www.cistrome. shinyapps.io/timer) to evaluate the correlation between AHSA1 expression in TCGA HCC samples and immune infiltrating cells. Immune infiltrating cells include B cells, neutrophils, CD4 + T cells, macrophages, CD8 + T cells and dendritic cells.

5. Correlations of AHSA1 Expression With Glycolysis in HCC

To further analyze the correlation between AHSA1 expression and HCC glycolysis, GEPIA database was used to analyze the correlation between expression of AHSA1 and glycolysis related genes including PAM, NUP155, KDELR3, NSDHL, ENO1, SRD5A3, GOT2, and PKM.

6. Small molecule targeted drugs screening of AHSA1 in HCC

Connectivity Map (CMap, https://portals.broadinstitute.org/ cmap/) is an online drug analysis tool that discovers and pre- dicts potential therapeutic drugs for certain diseases based on genome expression profiles. The different probe components commonly between HCC and normal samples were screened out with CMap data- base and divided into the up- and down-regulated groups. An enrichment score representing similarity was calculated. The positive score illustrated that the drug could induce cancer in human; the negative score illustrated the drug function oppositely and had potential therapeutic value.

# Declarations

### Ethics approval and consent to participate

The study was approved by the ethics committee of the Affiliated Huaian Hospital of Xuzhou Medical University Clinical and the Laboratory Research Ethical Council.

### Consent for publication

Not applicable.


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### Competing interests

The authors declare that they have no conflicts of interest with the contents of this article.


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None.


### Author contributions

QQ wrote the main manuscript text . All authors reviewed the manuscript.

### Availability of data and materials

The datasets used and/or analyzed during the current studies are available from the first author on reasonable request.

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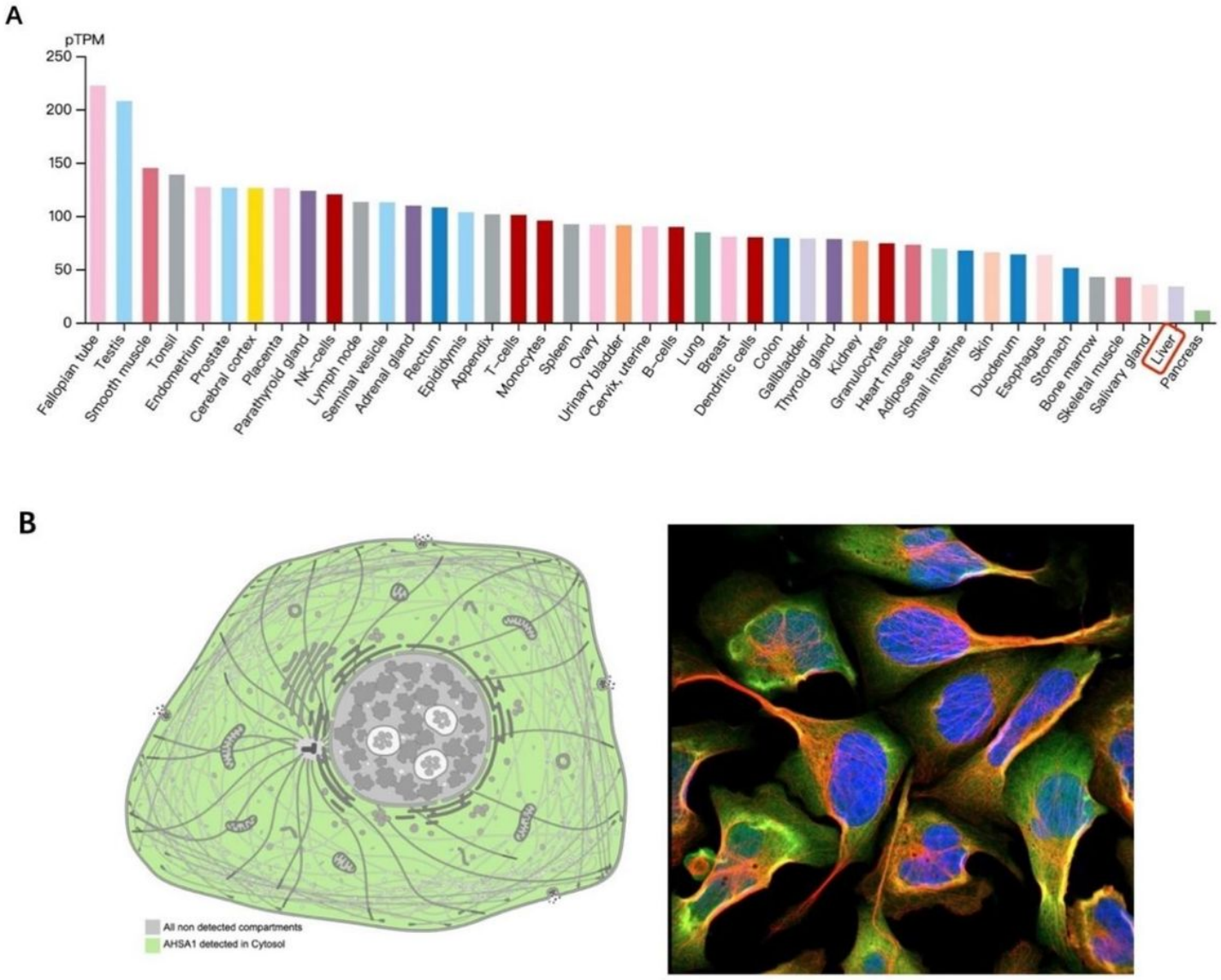
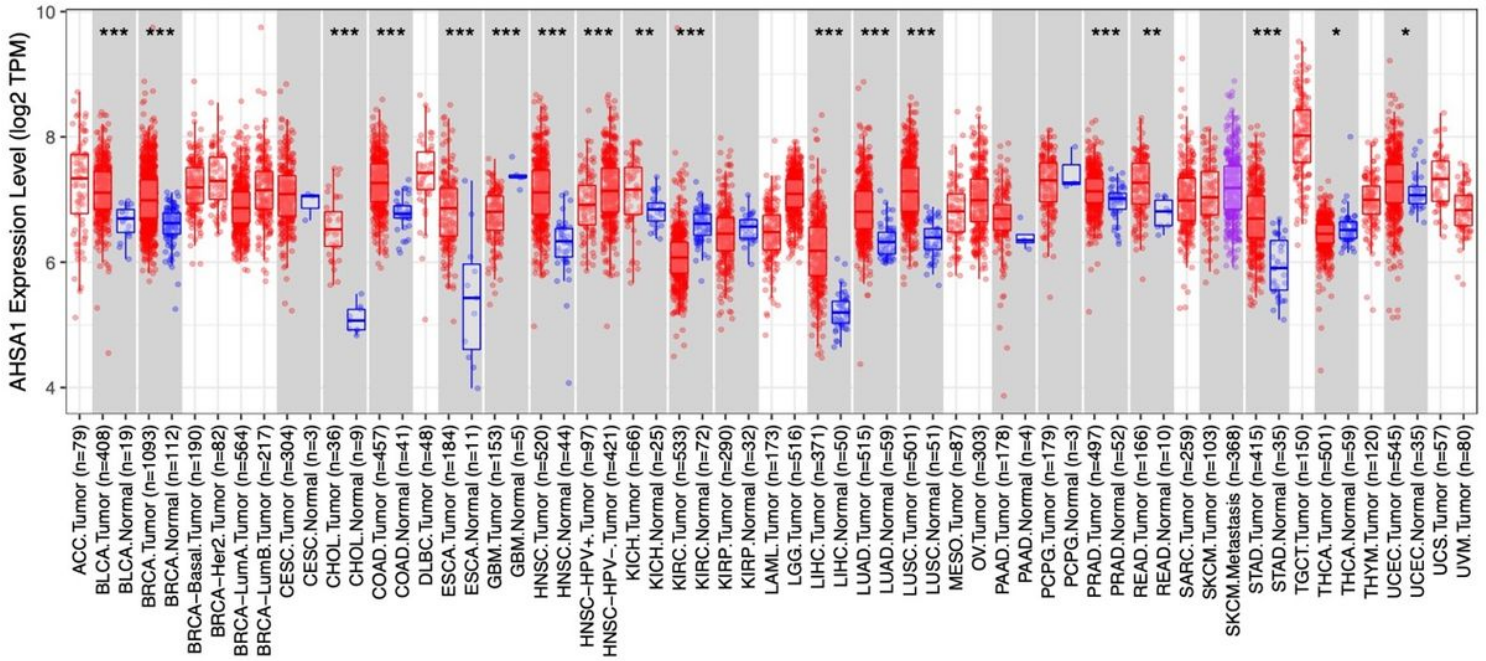
# Figures



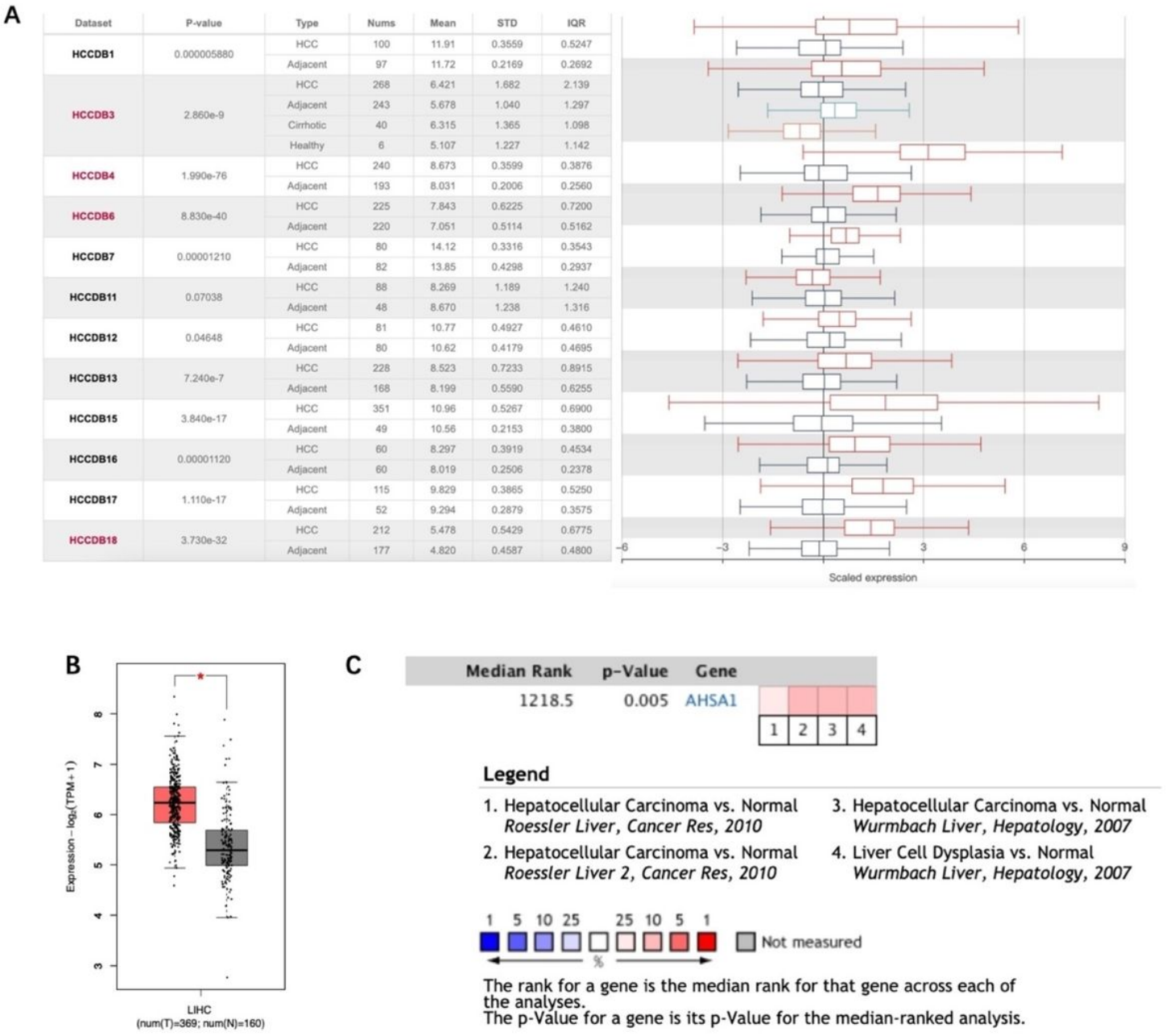

## Figure 1

(A Expression of AHSA1 in human normal tissues and organs. B Expression and localization of ahsa1 in tumor cells.



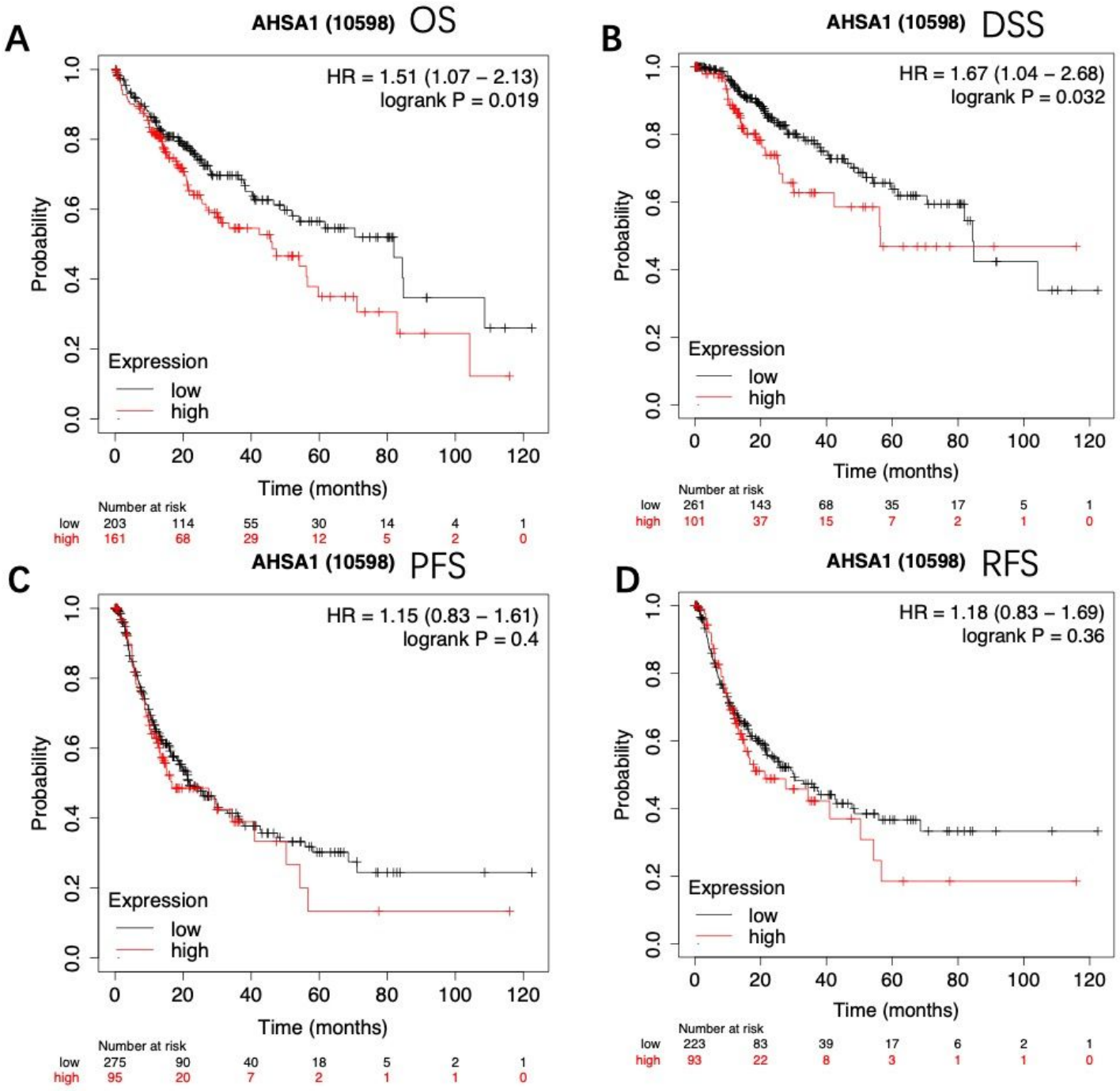

## Figure 2

The expression level of AHSA1 in different tumor types in TCGA database was determined by TIMER.



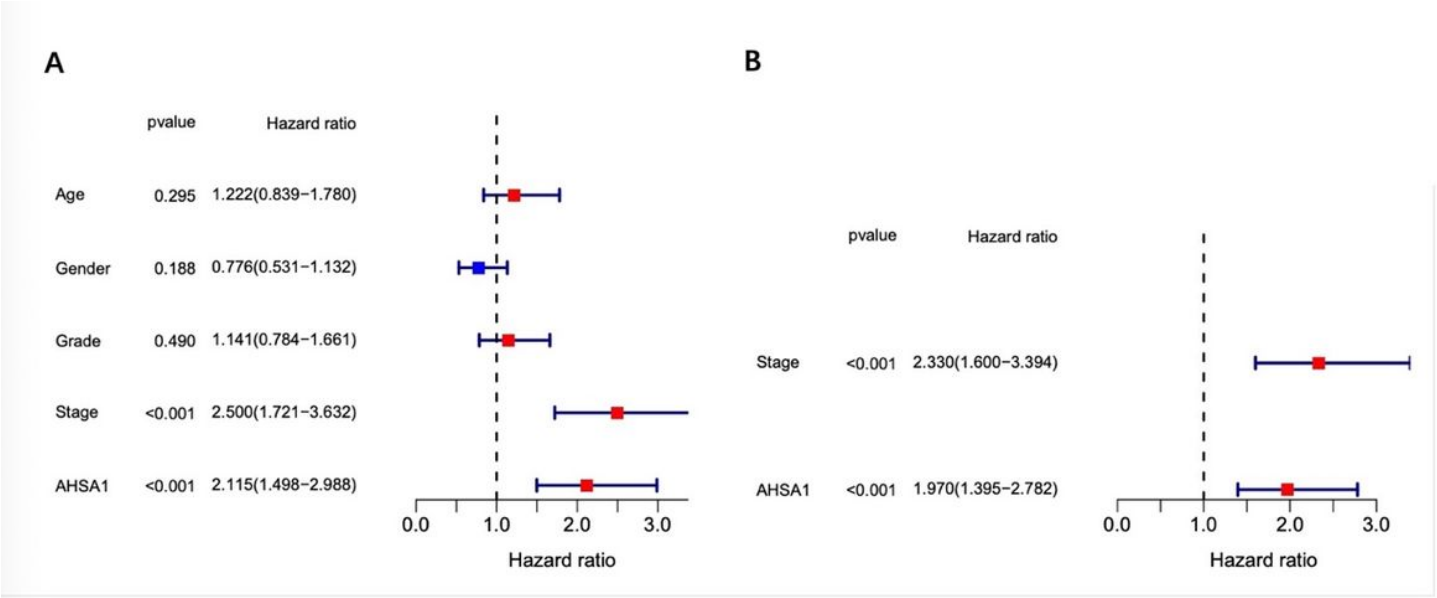

## Figure 3

High expression of AHSA1 mRNA in hepatocellular carcinoma （A）The differential expression of AHSA11 mRNA in different gene sets of HCC and adjacent normal tissues.（B）GEPIA was used to analyze the expression of ahsa1 in 369 liver cancer samples and 160 normal liver tissues collected from TCGA database and GTEX database.（C）The Oncomine database was used to conduct a meta-analysis of four sub studies.



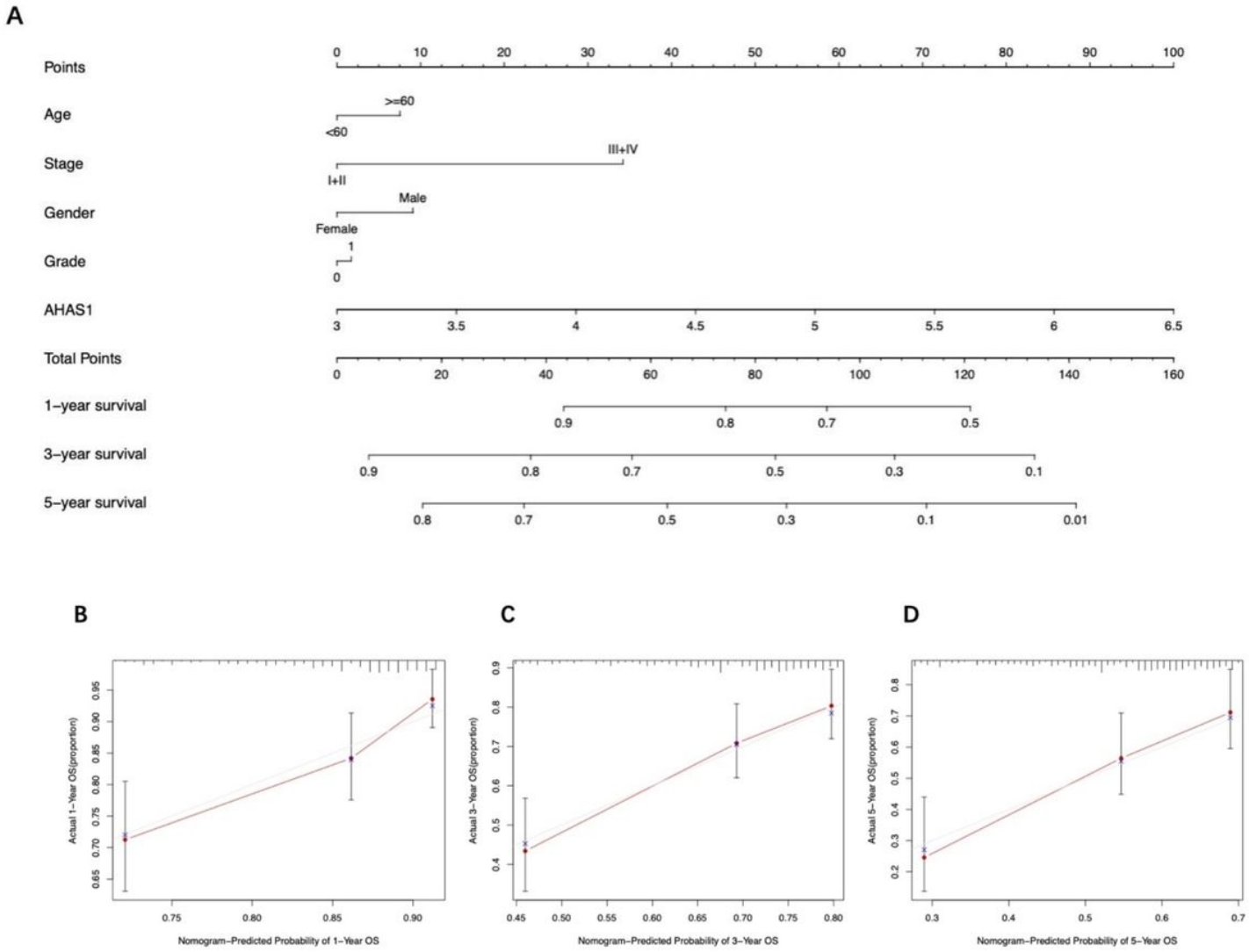

## Figure 4

Relationship between AHSA1 overexpression and prognosis of hepatocellular carcinoma (A) OS (overall survival) (HR=1.51 (1.07-2.31) P=0.019). (B) DSS (disease-free survival) (HR=1.67 (1.07-2.68) P=0.032). (C) PFS (progression-free survival) (HR=1.15 (0.83-1.61) P=0.4). (D) RFS (relapse-free survival) (HR=1.18 (0.83-1.69) P=0.36).



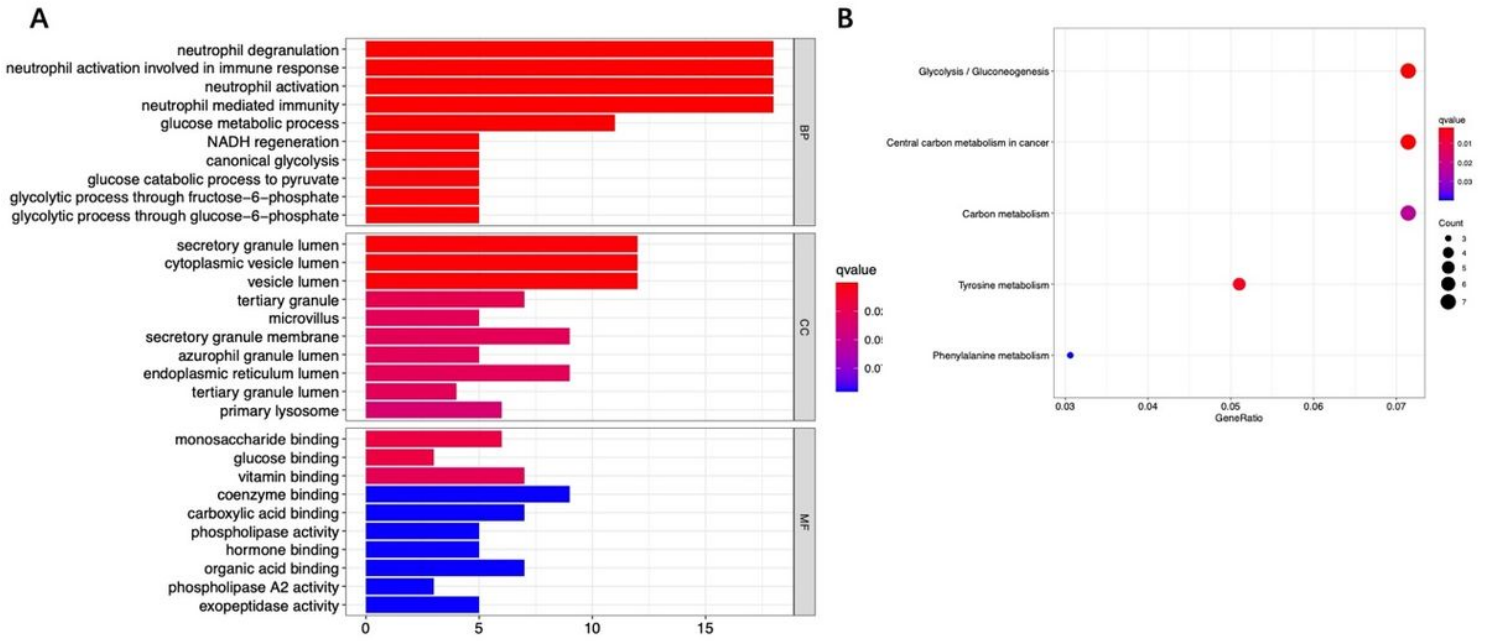

## Figure 5

Univariate Cox regression analysis (A) and multivariate Cox regression analysis (B) were used to analyze the clinical features associated with overall survival.



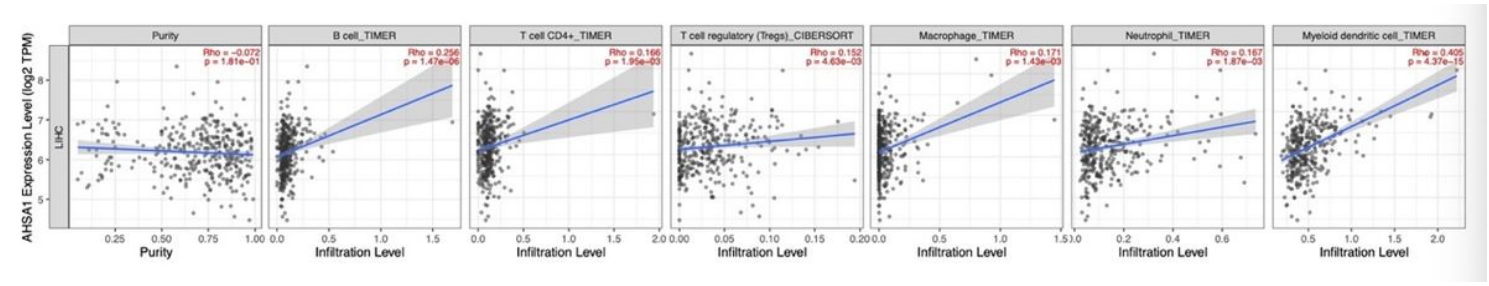

## Figure 6

(A Nomogram between ahsa1 and clinical parameters in TCGA HCC cohort. B-D Nomogram calibration curve for predicting 1 -, 3 -, and 5-year survival in TCGA cohort. E-G Based on the time-dependent ROC curve of TCGA cohort and AUC (area under the curve) of 1 -, 3 -, and 5-year overall survival.



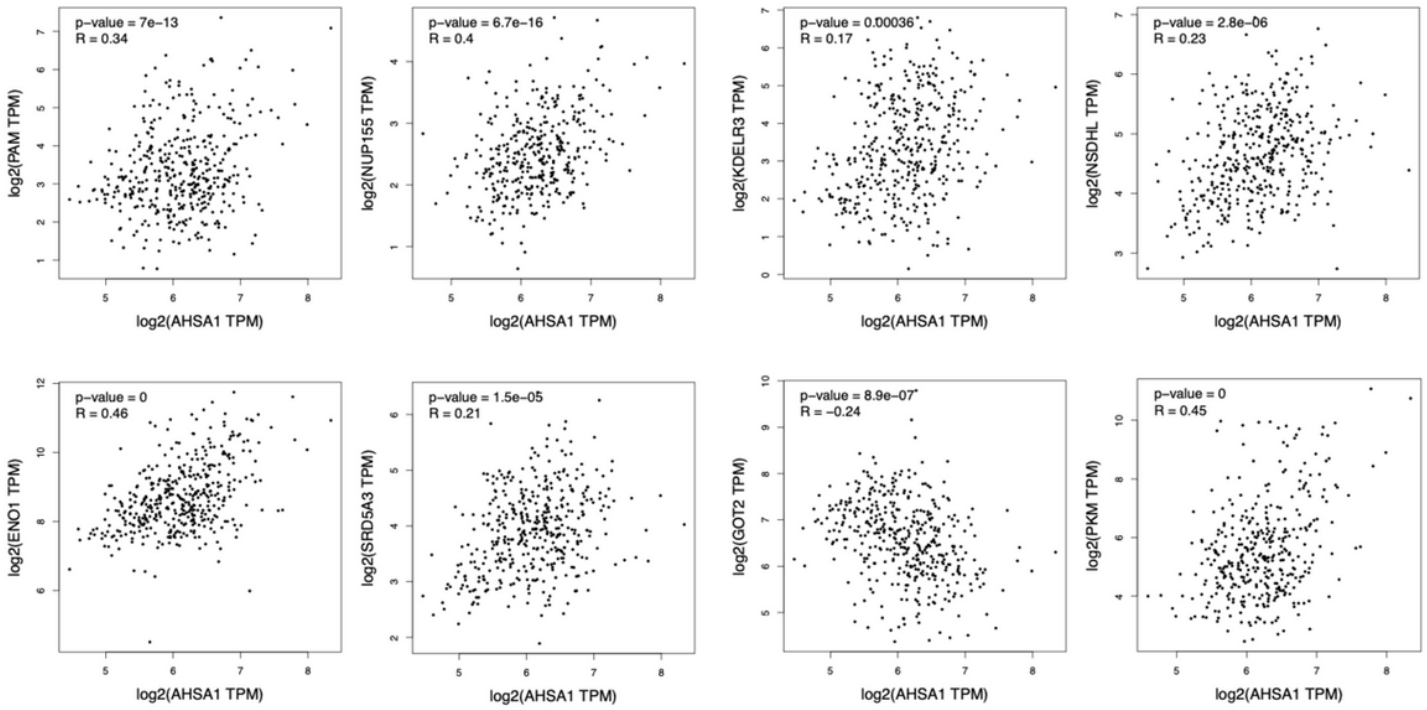

## Figure 7

(A) Go enrichment analysis of AHSA1 expression in hepatocellular carcinoma. (B)KEGG pathway analysis of AHSA1 expression in hepatocellular carcinoma.




## Figure 8

The relationship between AHSA1 expression and immune cells.




## Figure 9

Correlations of AHSA1 expression with glycolysis related genes in HCC, include PAM, NUP155, KDELR3, NSDHL, ENO1, SRD5A3, GOT2, and PKM ($P < 0.05$).

## Figure 10

The 13 most important potential small molecule drugs screened by CMAP that can reverse the expression of AHSA1 in liver cancer cells ($p<0.01$).