

# Bioinformatics Analysis of Prognostic Significance of VPS72 and Correlations with Immune Infiltrates in Hepatocellular Carcinoma

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## Research Article

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# Abstract

## Background

VPS72 is part of the EP400 and Snf2-related CBP-activator protein (SRCAP) chromatin remodeling complexes, and the prognostic value of VPS72 in hepatocellular carcinoma has not been reported.

## Methods

we used the Oncomine, UALCAN, GEPIA, String and Timer databases to study the expression of VPS72 in hepatocellular carcinoma.

## Results

We found the overexpression of VPS72 was markedly correlated with clinical stages and pathological grades in LIHC, higher mRNA expression of VPS72 was significantly related with shorter overall survival. Moreover, we found that VPS72 promoter hyper-methylation in liver cancer is different and significant. Furthermore, we identified significant correlations among the expression of VPS72 and infiltration of B cells, CD4 + T cells, CD8 + T cells, macrophages, neutrophils, and dendritic cells in LIHC. Then we searched the protein interaction network of VPS72 by string.

## Conclusion

VPS72 may be used as prognostic biomarkers and immunotherapeutic targets LIHC patients.

## Introduction

Globally, liver cancer is one of the most common fatal malignancies, and patients are often diagnosed with liver cancer in advanced stages, leading to a poor prognosis. More than 90% of all liver cancer cases are hepatocellular carcinomas [1]. Despite advances in identifying risk factors and in the detection and treatment of liver cancer, the incidence, mortality and recurrence rates of LIHC disease continue to rise [2, 3]. Therefore, there is an urgent need to identify novel potential prognostic and therapeutic targets that are related with tumor formation and progression in patients with LIHC.

VPS72 is part of the EP400 and Snf2-related CBP-activator protein (SRCAP) chromatin remodeling complexes. In these complexes, VPS72 functions as a chaperon for H2A to H2A.Z exchange and as a reader for H2A.Z in the ATP-dependent SRCAP or EP400 complexes [4, 5] However, the relationship between vps72 and liver cancer has never been reported, Therefore, in this study, we evaluated the significance of VPS72 gene expression in hepatocellular carcinomas by using comprehensive bioinformatics analysis of the clinical indicators and survival data in several large online databases.

## Materials And Methods

### ONCOMINE

We analyzed The VPS72 mRNA expression level in hepatocellular carcinomas and matched normal tissues based on the Oncomine (<https://www.oncomine.org>) [6]. In the current study, we obtained the expression data for the VPS72 in hepatocellular carcinomas using the following filters: Gene, VPS72; differential analysis, cancer vs. normal analysis; cancer type, liver cancer; and data type, mRNA. In this study, we selected 1.5fold change, p-value = 0.01, and top 10% gene rank as threshold. Besides, we obtained co-expressed genes with VPS72 in the co-expression module. All statistical methods and statistical values were obtained directly from the corresponding database.

### UALCAN

UALCAN (<http://ualcan.path.uab.edu/analysis.html>), a comprehensive online accessible database, providing the analysis of tumor gene expression and survival difference of prognosis based on The Cancer Genome Atlas (TCGA) and the clinical prognosis outcomes [7]. We abstracted the expression data between LIHC and normal tissues and further analyzed the survival difference in various tumor stage in patients in LIHC. Then we analyzed the differences in promoter hypermethylation in LIHC. Using UALCAN online analyzed resource, the cutoff of p-value was set as 0.05 in the Student's t-test.

### GEPIA

GEPIA (<http://gepia.cancer-pku.cn/index.htm>), which was developed by peaking university, is a newly online analysis tool to obtain the RNA-seq expression data of more than 9,736 tumors and 8,587 normal tissue samples [8]. In our study, we analyzed differential gene expression of VPS72 among the tumor and normal tissues, different survival status, and the various pathological stage. The cutoff of p-value was set as 0.05 in Student's t-test.

### TIMER

TIMER (<https://cistrome.shinyapps.io/timer/>) is a reliable database for estimating immune cell infiltration using data from TCGA, including 10,897 samples from 32 types of cancer [9]. In this study, we examined the correlation between the expression of vps72 and immune cell infiltrates, including B cells, CD8 + T cells, CD4 + T cells, macrophages, DCs, and neutrophils. The generated scatterplots suggest statistical significance.

### STRING

STRING (<http://string-db.org>) is an available protein-protein interaction (PPI) database designed for collecting, integrating, and scoring publicly available data to explore the potential protein interaction network [10]. In our study, we searched the protein interaction network of VPS72 by string.

## Results

# Expression levels and prognosis of VPS72 in LIHC

To explore prognostic and potential therapeutic value of *vps72* in LIHC patients, mRNA expression were analyzed by ONCOMINE database and UALCAN. According to online analysis database Oncomine, As shown in Fig. 1A, mRNA expression of *vps72* in 20 types of cancers were first measured and compared to normal tissues by ONCOMINE database, and in Fig. 1D-G Significant changes in *vps72* transcriptional levels between LIHC and normal tissues were observed in different datasets, In Roessler Liver 2 Statistics, *vps72* over-expression was found in Hepatocellular Carcinoma compared with normal tissues with a fold change of 2.365 ( $p = 2.50E-81$ ), and in Roessler Liver Statistics, they found 2.605 fold change in *vps72*mRNA expression in LIHC tissues ( $p = 8.51E-11$ ). In Chen Liver Statistics, they found 2.342fold change in *vps72* mRNA expression in LIHC tissues ( $p = 4.54E-20$ ) when in Wurmbach Liver Statistics, 2.006fold change ( $p = 3.79E-8$ ) has been found. We further examined the mRNA expression profile of *vps72* using UALCAN and GEPIA, mRNA expression of *vps72* were found to be significantly up-regulated in LIHC tissues compared to normal samples (all  $p < 0.05$ ), We then analyzed the prognostic value of *vps72* gene, The UNCLAN and GEPIA both revealed that lower level of *vps72* correlated with preferable overall survival. Taken together, our results showed that transcriptional expression of *vps72* were over-expressed in patients with LIHC. ( $p < 0.001$  for all).

## ***Correlation of mRNA expression levels of vps72 and the tumor progression related clinicopathological parameters in LIHC patients and differential expression of VPS72 promoter hypermethylation***

After overexpression of VPS72 was observed in LIHC patients, we used GEPIA and ULACN databases to investigate individual clinicopathological parameters for cancer stage and tumor grade. As we found in Fig. 2A-C, there are remarkably correlations between mRNA expression of *vps72* and patient' pathological stages in Gepia and unclan. However, The decrease of the expression level of phase 4 May be related to the lack of data, which was only available in 6 and 12 patients. ( $p < 0.01$  for all). As we found in Fig. 2D, we found that VPS72 promoter hyper-methylation in liver cancer is different and significant by unclan.

## Immune cell infiltration of *vps72* in LIHC

Tumor-infiltrating lymphocytes can be used as independent predictors for sentinel lymph node status and survival in cancer (37). Therefore, we investigated the correlations between differentially expressed *vps72* and the infiltration of immune cells using the TIMER database. As shown in Fig. 3, there was a positive relationship between the expression of *vps72* and the infiltration of B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and DCs. *vps72* expression was positively associated with the infiltration of CD8 + T cells (correlation: 0.182,  $p = 7.33e - 04$ ), macrophages (correlation: 0.333,  $p = 3.01e - 10$ ), neutrophils (correlation: 0.258,  $p = 1.19e - 06$ ), CD4 + T cells (correlation: 0.322,  $p = 1.03e - 09$ ), and DCs (correlation: 0.306,  $p = 8.39e - 09$ ). These results strongly suggested that, in patients with liver cancer, the *vps72* may act a specific role for immune cell infiltration, including B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and DCs.

## VPS72 and KRTCAP2 are co-expressed in LIHC

In order to further study the potential mechanism of VPS72 in LIHC, we used the co-expression database to mine the co-expression data of VPS72. (Figs. 4). Further analysis using TIMER revealed the correlation between vps72 and KRTCAP2 (Fig. 4B), The data above indicated that vps72 could be associated with the KRTCAP2 signaling pathways in LIHC.

## The validation and survival analysis of KRTCAP2 in LIHC

The expression of KRTCAP2 were validated in GEPIA and unclan database. We unearthed that KRTCAP2 were significantly upregulated in LIHC (Fig. 5A-B). The survival analysis in GEPIA and unclan database confirmed that the upregulation of KRTCAP2 were correlated with shorter overall survival of LIHC patients respectively (Fig. 5C-D).

## Functional interaction network of VPS72 protein in LIHC patients

The PPI network interaction of vps72 was conducted by String to seek possible downstream targets and mechanism research, and it was found that TRRAP, RUVBL1, MORF4L2, MRGBP, YEATS4, KAT5, MORF4L1, SRCAP, DMAP1 and RUVBL2 could be used as the target genes for further research and analysis(Fig. 6).

## Discussion

Hepatocellular carcinoma (HCC) is one of the major cancers worldwide and its incidence is increasing every year [11]. In recent years, many traditional Chinese medicines have been found to inhibit angiogenesis, reduce microvessel density, and delay or prevent the development of liver cancer. [12] Liver cancer stem cells (CSCs) have unlimited ability of self-renewal, differentiation and tumor regeneration, and the maintenance of stem cells may be the reason for the refractory treatment of liver cancer [13]. The high mortality rate of liver cancer indicates that most patients are diagnosed at a late stage, and strategies for early detection are needed to reduce the burden of liver cancer [14]. VPS72 is an important factor in functional nuclear recombination after mitosis and prolongs telophase of HeLa cells [15]. Human VPS72, known as YL1, YL-1, or SWC2, was initially identified as a nuclear, DNA-binding protein that suppressed anchorage-independent growth suppressor activity in Kirsten sarcoma virus-transformed NIH3T3 cells [16, 17]. However, the relationship between vps72 and liver cancer has never been reported, This is the first study to identify vps72 as a potential predictive biomarker for prognosis of LIHC.

In our study, we analyzed the expression profile of vps72 by Oncomine database. Vps72 was higher expressed in 15 types of tumors, including but not limited to LIHC. and Significant changes in vps72 transcriptional levels between LIHC and normal tissues were observed in different datasets, Then we used UNCLAN and GEPIA to verify the high expression of VPS72 in LIHC, We further investigated the prognostic value of vps72 in LIHC using the UNCLAN and GEPIA databases, Patients with increased vps72 showed worse overall survival. These findings collectively elucidated that the expression of vps72 might be a predictive biomarker for prognosis of LIHC.

Besides, in our study, significantly higher mRNA expression of *vps72* were found in LIHC tissues, and mRNA expression of *vps72* was remarkably correlated with patients' individual cancer stages and tumor grades by UNCLAN and GEPIA databases, The difference increased with the increase of individual cancer stages and tumor grades,

However, The decrease of the expression level of phase 4 May be related to the lack of data, which was only available in 6 and 12 patients. ( $p < 0.01$  for all). we also found that VPS72 promoter hyper-methylation in liver cancer is different and significant by UNCLAN, the *vps72* may act a specific role for immune cell infiltration, including B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and DCs, thereby affecting clinical outcomes in patients with LIHC, and our study indicated that *vps72* could be associated with the KRTCAP2 signaling pathways in LIHC, The expression of KRTCAP2 in LIHC is different and the difference is significant, which is significant for survival and then VPS72 was found to interact with TRRAP, RUVBL1, MORF4L2, MRGBP, Yeats4, Kat5, MORF4L1, SRCAP, DMAP1 and RUVBL2 proteins.

## Conclusion

Vps72 was found to be significantly positive correlated with clinical cancer stages and tumor grades in patients with liver cancer. In addition, high mRNA expression of *vps72* was significantly related with poor OS. Increased expression levels of *vps72* are correlated with increased infiltration levels of immune cells, including B cells, CD8 + T cells, CD4 + T cells, neutrophils, macrophages, and DCs in LIHC and can be used as potential prognostic biomarkers for patients with LIHC,

## Abbreviations

LIHC  
Liver hepatocellular carcinoma

## Declarations

### Acknowledgements

Not applicable

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### Availability of data and materials

All data are included in the article.

### Ethics approval and consent to participate

Not applicable.

### **Consent for publication**

All authors read the final manuscript and agreed to publish it.

### **Competing interests**

The author reports no conflicts of interest in this work.

### **Authors' contributions**

YHG and XNL designed study; CW researched literature; YHG and PY analyzed experimental results; YHG wrote the manuscript.

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## Figures

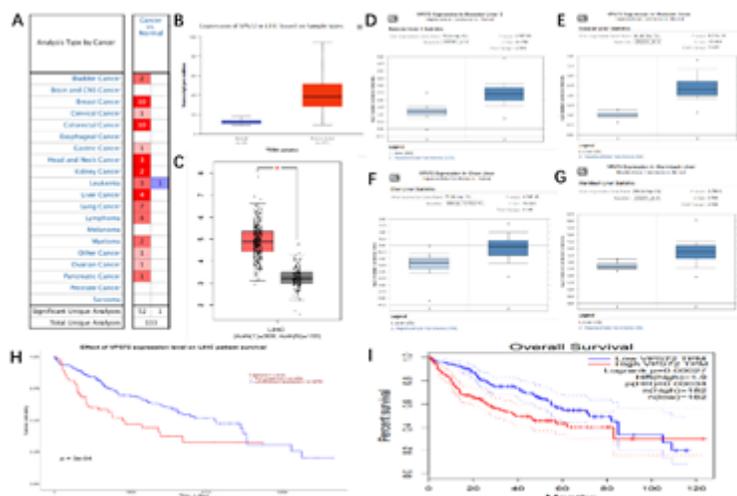


Figure 1

(A) Transcriptional expression of vps72 in 20 different types of cancer (D-G) Differences in transcriptional expression were compared using Student's t-test. The cutoff criteria were as follows:  $p=0.01$ , fold change=1.5, gene rank=10%, and data type of mRNA (Oncomine). (B) mRNA expression of vps72 in LIHC samples and adjacent normal liver samples (UALCAN). (C) mRNA expression of vps72 in LIHC samples and adjacent normal liver samples (GEPIA). (H) Prognostic value of mRNA expression of vps72 in LIHC patients (UALCAN) (I) Prognostic value of mRNA expression of vps72 in LIHC patients (GEPIA)

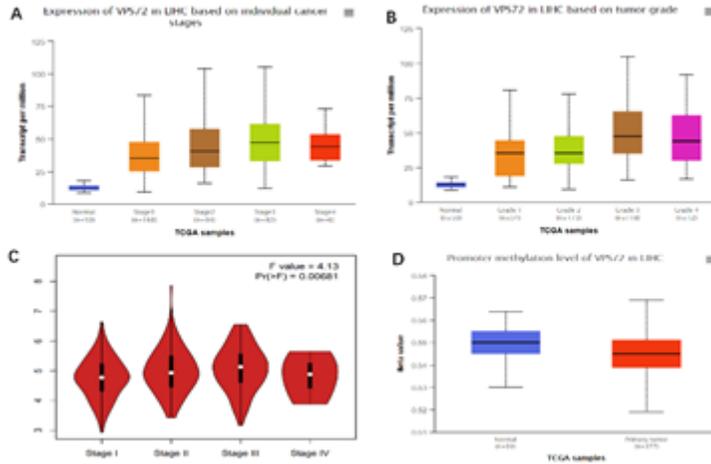


Figure 2

(A-C) Relationship between the mRNA expression of VPS72 and individual cancer stages in LIHC patients. The mRNA expression of VPS72 was strongly related with the cancer stages of individual patients.  $P < 0.05$  (UNCLAN, GEPIA) (D) Differential hypermethylation of VPS72 promoter

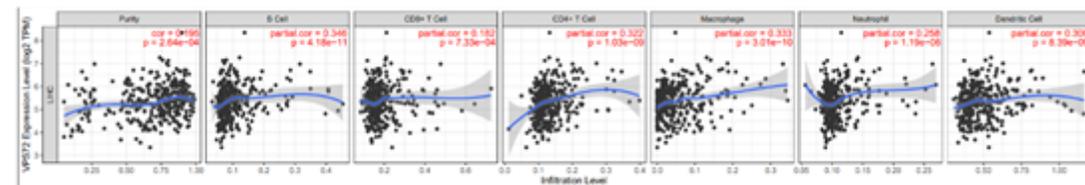
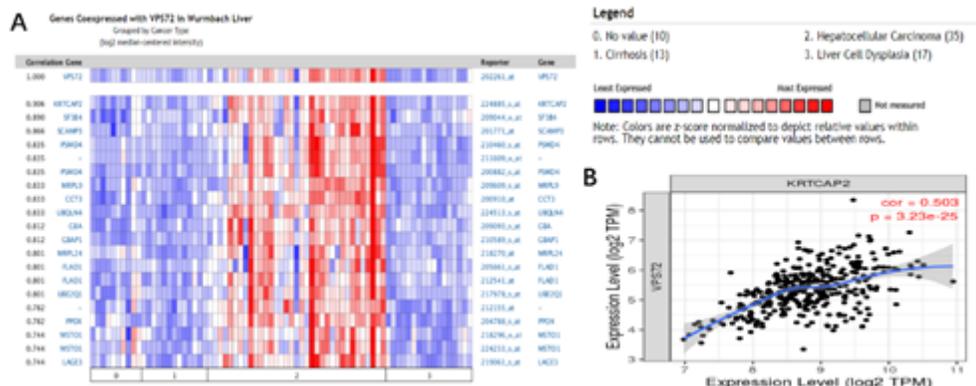


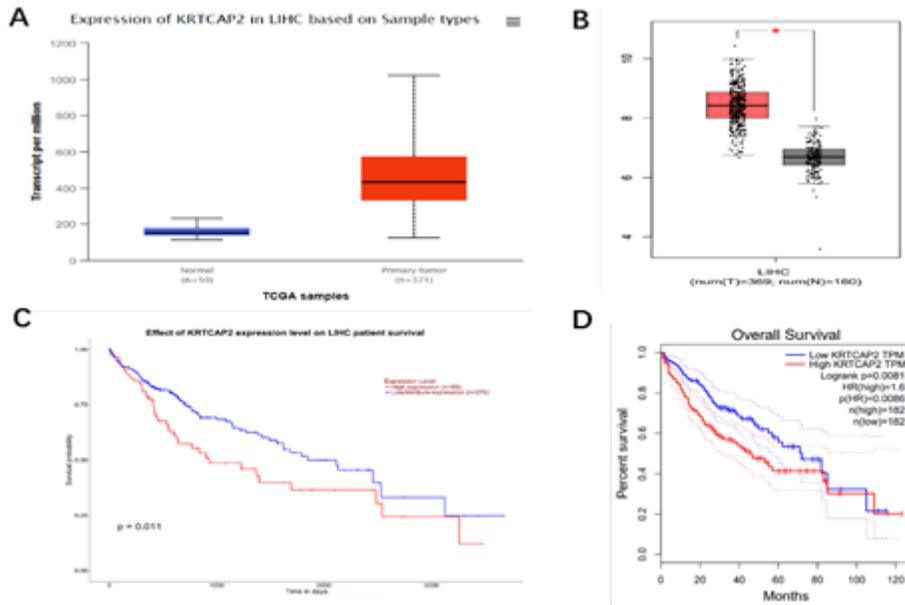
Figure 3

Correlation between vps72 and immune cell infiltration (TIMER)



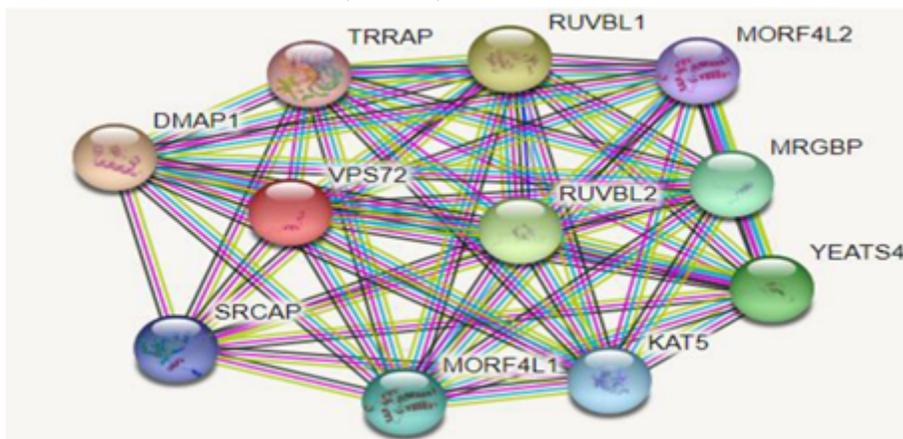
**Figure 4**

(A) Co-expression profile of vps72 identified (Oncomine) (B) the correlation between vps72 and KRTCAP2 expression in LIHC(GEPIA)



**Figure 5**

(A) mRNA expression of KRTCAP2 in LIHC samples and adjacent normal liver samples (UNCLAN) (B) mRNA expression of KRTCAP2 in LIHC samples and adjacent normal liver samples (GEPIA). (C) The overall survival status for the expression of KRTCAP2(UNCLAN) (D) The overall survival status for the expression of KRTCAP2(GEPIA).



**Figure 6**

6 vps72 generated PPI network (STRING)