High Expression of TTYH3 Predicts Poor Outcome in Hepatocellular Carcinoma

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Research Article

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Abstract

Objective Analyze the expression level of TTYH3 gene in liver cancer, promoter methylation, gene mutation, co-expression, Immune infiltration, protein interaction and overall survival by database mining.

Methods We utilized the UALCAN, GEPIA, cBioPortal, Metascape, STRING, TIMER, TISIDB, and MEXPRESS databases to investigate the transcription level, genetic alteration, methylation, and biological function of TTYH3 in HCC patients, and its association with the occurrence, progress, prognosis, and immune cell infiltration in patients with HCC.

Results Multiple databases have confirmed that the mRNA level of TTYH3 in liver cancer tissues is significantly higher than that in normal tissues. UALCAN and GEPIA confirmed that the overexpression of TTYH3 mRNA was associated with clinical stage, and the expression of TTYH3 mRNA increased with the increase of stage ($P<0.05$). Multiple databases confirmed that the overexpression of TTYH3 gene was associated with low survival rate ($P<0.05$). cBioPortal database analysis showed that TTYH3 gene mutation was associated with low survival rate and poor prognosis ($P<0.05$). GEPIA search for genes co-expressed with TTYH3. Timer database showed that TTYH3 was involved in inflammatory response and immune cell infiltration. Methylation data from MEXPRESS indicate significant probe level variation of CpG island methylation status of the gene TTYH3, which was associated with low survival rate ($P<0.05$), Moreover, we also found that the expression of TTYH3 was significantly correlated with tumor-infiltrating lymphocytes and immunomodulators, The STRING protein analysis network showed that TTYH3 may interact with BEST1, BEST2, BEST3, BEST4, ZnF467, CLCC1, GLRB, ANO1, IQCE, ANO2 and other proteins.

Conclusion Through a variety of databases, it was found that TTYH3 was highly expressed in liver cancer, and the expression level was related to the survival and prognosis of liver cancer patients. Found TTYH3 involved in inflammation and immune cell infiltration and influence the outcomes in patients with liver cancer clinical, TTYH3 promoter methylation of meaningful expression differences in liver cancer, the co-expressed genes, proteins interaction can be in-depth study, as well as TTYH3 gene and the relationship between the liver cancer, for the prognosis of liver cancer biomarkers and therapeutic targets.

Introduction

The incidence of liver cancer is increasing and is an important source of cancer-related mortality worldwide (1,2), Hepatocellular carcinoma (HCC) is a rare heterogeneous malignant disease that most often occurs in the context of chronic liver inflammation and fibrosis, and progresses to tumor in individual patients through multiple processes (3), Liver cancer is very deadly, with death rates rising faster than any other cancer, but a significant proportion of deaths could be prevented with targeted prevention, early detection and treatment (4), The Tweety gene family (TTYHs) is reported to be a chloride channel response gene that plays a role in a variety of cellular processes including cell adhesion, cell division, tumogenesis and regulation of calcium activity (5), TTYH3 is a member of TTYH family and has

chloride channel activity (6), TTYH3 is also known as a Ca2 + conductor-activated chloride channel. Human TTYH3 gene is mainly expressed in excitable tissues, including brain, heart and skeletal muscle (7), However, there is little research on TTYH3 in liver cancer, despite progress in identifying risk factors, the incidence of HCC is increasing. Moreover, therapeutic options are limited and survival is poor (8,9). Therefore, alternative and innovative therapeutic strategies are urgently required.

Methods

UALCAN

UALCAN (http://ualcan.path.uab.edu) is an interactive web resource based on level 3 RNA sequences and clinical data from 31 cancers in the TCGA database. It is mainly used to analyze the relative transcriptional expression of a gene between tumor and normal samples, and the correlation between transcriptional expression and related clinicopathological parameters [10]. In this study, UALCAN was used to analyze the mRNA expressions of TTYH3 in HCC tissues and their association with clinicopathologic parameters and individual cancer stages. the cutoff of p-value was set as 0.01 in the student's t-test.

GEPIA

As an online database, Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer pku.cn/) is a database that involves tumor and normal samples from TCGA and GTEx [11]. In our study, we looked for the top 30 genes that are similar to the TTYH3 in HCC by using the similar gene detection module. The cutoff of p-value was set as 0.05 in Student's t-test.

cBioPortal

cBioPortal (http://www.cbioportal.org) has been used as an online access database to explore cancer genomic data from multiple perspectives [12]. Our current study obtained gene mutation and survival data from 366 HCC samples in the TCGA database in cBioPortal. We set ± 1.8 as the z-score threshold of mRNA Expression (RNASeq V2 RSEM) were also applied to explore the relationship among the genetic alterations in TTYH3 and the overall survival (OS) of HCC patients. p value as 0.05 was accepted.

Metascape

Metascape (http://metascape.org/gp/index.html#/main/ step1) is a database that uses data from more than 40 independent knowledge bases combined with rich features, interaction analysis, gene annotation, and member search. In addition, it facilitates the comparative analysis of multiple independent and orthogonal experiments across data sets by the portal [13]. The GO module can analyze the functional roles of genes related to TTYH3 in biological processes (BP), cellular components (CC), and molecular functions (MF). And KEGG pathways of the PTTG family members.

STRING

STRING (http://string-db.org) is a database that collects, aggregates, and scores publicly available data to explore potential protein interaction networks. TTYH3 was generated PPI network by STRING (14).

TIMER

TIMER is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types (https://cistrome. shinyapps.io/timer/) (15). TIMER applies a deconvolution previously published statistical method (16) to infer the abundance of tumor-infiltrating immune cells (TIICs) from gene expression profiles. The TIMER database includes 10,897 samples across 32 cancer types from The Cancer Genome Atlas (TCGA) to estimate the abundance of immune infiltrates. We analyzed TTYH3 expression in liver cancer and the correlation of TTYH3 expression with the abundance of immune infiltrates, including B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells, via gene modules.

TISIDB

To reveal the immune infiltration of TTYH3 in cancer, we used the TISIDB (tumor-immune system interactions and drugbank, http://cis.hku.hk/TISIDB/index.php) database to infer the relations between abundance of tumor-infiltrating lymphocytes (TILs) and expression of TTYH3. The immune-related signatures of 28 TIL types from Charoentong's study, the relative abundance of TILs was inferred by using genomic variation analysis based on gene expression profiles (17).

MEXPRESS

MEXPRESS (https://mexpress.be/) database was also applied to explore the association between DNA methylation and expression levels of the TTYH3. The adjusted p-value (Benjamini-Hochberg) and the Pearson correlation coefficient value (R) of each probe were provided (18).

Statistical analysis

Survival curves were generated by Kaplan-Meier plots. The results generated in GEPIA□UALCAN were displayed with HR and P or Cox P-values from a log-rank test. The correlation of gene expression was evaluated by Spearman's correlation and statistical significance, P-values < 0.05 were considered statistically significant.

Results

TTYH3 expression and Prognostic values in HCC

The mRNA levels of TTYH3 in HCC were obtained from UALCAN database. As shown in Fig. 1A, mRNA expression of TTYH3 was found to be significantly up-regulated in HCC tissues compared to normal samples ($p < 0.01$), Then We used UALCAN to analyze the prognostic values of the mRNA expression of TTYH3 in HCC patients. As was shown in Fig. 1B, mRNA expression in members of the TTYH3 was significantly associated with prognosis in patients with HCC disease. We found that higher mRNA expression of TTYH3 was significantly associated with shorter OS of HCC patients, then we used GEPIA

to analyze the prognostic value of the expression of TTYH3 in HCC patients, The results showed that the expression level of TTYH3 was significantly correlated with prognosis in HCC with poor OS and DFS, These results indicate that TTYH3 mRNA was significantly associated with prognosis in patients with HCC and can be used as a biomarker to predict survival in patients with HCC.

Relationship between TTYH3 and clinical pathological in HCC patients

we analyzed the relationship between TTYH3 with clinicopathological parameters of HCC patients by UALCAN and GEPIA, As was shown in Fig. 2, The TTYH3 was correlated with individual cancer stage, indicating that patients with more advanced cancer stages tended to have higher TTYH3 expression. the results above suggested that TTYH3 was significantly associated with clinicopathological parameters in HCC patients.

Genetic mutation and Prognostic values of TTYH3 in HCC patients

We used cBioPortal to analyze the genetic mutation of TTYH3 in HCC patients. Based on Fig. 3, the mutation rate of TTTYH3 was 6% in 366 samples. What's more, the association between genetic mutation and the prognosis of HCC patients was explored. And a statistically significant correlation was found between genetic mutation of TTYH3 and OS ($p = 0.0353$) in HCC patients, and DS was also statistically significant ($p = 0.0128$), However, there was no statistical difference with DF.

Methylation analysis of TTYH3 in HCC

In order to find the main causes of aberrant expression of TTYH3 in HCC, we used UACLAN database as well as MEXPRESS database. As was shown in Fig. 4, We found that TTYH3 is hypomethylation in HCC by UACLAN database ($p < 0.01$), Then, the methylation level of TTYH3 detected by MEXPRESS database. The result revealed that the promoter region of TTYH3 showed significantly lower methylation levels ($p < 0.05$) in HCC compared to normal tissues, the result showed the negative correlation between TTYH3 expression and promoter methylation.

TTYH3 positively correlates with immune cell infiltration in HCC

the correlation of TTYH3 expression level with immune cell infiltration level was evaluated. As presented in Figs. 5A, TTYH3 expression was significantly positively associated with all analyzed immune cells, including B cell, CD8 + T cell, CD4 + T cell, macrophage, neutrophil, and dendritic cell in HCC, As were shown in Fig. 5B-C and Fig. 6, the TISIDB database was used to infer the relations between abundance of TILs and expression of TTYH3. The relations between abundance of 28 TIL types and expression of TTYH3 was weakly to moderately correlated. Specifically, TTYH3 expression was positively closely related with infiltrating levels of Act CD4 ($r = 0.222$, $P = 1.6e-05$) and Act DC ($r = 0.257$, $P = 5.58e-07$), then we used GEPIA to analyze the correlation between TTYH3 and monocytes, TAM and macrophage-related genes and markers (Fig. 7). Moreover, the correlation between TTYH3 expression and the marker genes of immune cells implicate the role of TTYH3 in regulating tumor immunology. First, gene markers of M1 macrophages such as PTGS2 and IRF5 showed positive correlations with TTYH3 expression, M2

macrophage markers such as CD163, VSIG4, and MS4A4A also showed moderate and strong correlations.

Networks Analyses and Functional Enrichment Analysis of TTYH3 and their Neighboring Genes in HCC patients

similar genes of the TTYH3 (30 in total) obtained from GEPIA and Metascape were used for GO enrichment to explore the interaction between similar genes. Based on 30 adjacent genes, the online tools of Metascape and STRING were used for functional and pathway enrichment analysis, and a PPI interaction network was established to explore the biological classification of TTYH3. The functions of TTYH3 neighboring genes were predicted by analyzing GO and KEGG in Metascape. The GO enrichment items were classified into four functional groups,KEGG pathway, biological process group, molecular function group, and cellular component group (Figs. 8). The TTYH3 and similar genes was Enriched in the following information, bone morphogenesis, regulation of proteolysis, regulation of anatomical structure size, carbohydrate metabolic process, immune response-activating cell surface receptor signaling pathway, actin cytoskeleton organization, angiogenesis (BP); and focal adhesion, polymeric cytoskeletal fiber (CC); and glycosaminoglycan biosynthesis, Tuberculosis in KEGG pathway. The PPI network interaction of TTYH3 was conducted by String to seek possible downstream targets and mechanism research, and it was found that BEST1、BEST2、BEST3、BEST4、ZNF467、CLCC1、GLRB、ANO1、IQCE、ANO2 and other genes could be used as the target genes for further research and analysis.

Discussion

Hepatocellular carcinoma (HCC) is one of the most common fatal malignancies. Most patients have advanced HCC when they are found, leading to poor prognosis. More than 90% of all liver cancer are HCC (19), It is a malignant tumor with high recurrence rate and chemotherapy resistance (20), Most patients are asymptomatic and advanced HCC is no longer suitable for surgical treatment (21), Despite many advances in detection and treatment of liver cancer, the incidence of the disease is still on the rise (22), and most patients are prone to relapse (23), So there is an urgent need to develop new screening genes or therapeutic targets. TTYH gene is a chloride channel family with three members, and increased TTYH2 expression has been implicated in the progression of multiple cancers (24), The TTYH gene family encodes conductive chloride channels and is involved in a variety of cellular processes, including cell division, cell adhesion, regulation of calcium activity, and tumgenesis (25), Ttyh1 targeting has been reported to effectively inhibit glioma cell formation and growth, which has potential significance for future targeting strategies (26), TTYH2, a calcium-activated inward rectifying anionic channel, has been implicated in kidney and colon cancers (27), Some studies have predicted that TTYH3 may be a potential prognostic marker for gastric cancer patients (28), TTYH3 has a potential role in regulating cancer microenvironment and is an important protein in various cancers (29), but about TTYH3 promoter methylation and immunity infiltration, there has never been reported in liver cancer, so we predict through a variety of database TTYH3 played a role in development of liver cancer, and help more about TTYH3

mechanism in liver cancer research, help liver cancer early detection, early intervention and discover new treatments and therapeutic targets.

Conclusion

TTYH3 involved in inflammation and immune cell infiltration and influence the outcomes in patients with liver cancer clinical, TTYH3 promoter methylation of meaningful expression differences in liver cancer, the co-expressed genes, proteins interaction can be in-depth study, as well as TTYH3 gene and the relationship between the liver cancer, for the prognosis of liver cancer biomarkers and therapeutic targets.

Uniqueness and Limitations

Although the role of TTYH3 in the occurrence and progression of liver cancer has hardly been reported, there are some limitations need to be recognized in the current study. Firstly, our data came from online public database, so we did not confirm the potential diagnostic and therapeutic values of TTYH3 in HCC patients because there are no lab results, and our results should be confirmed by further studies.

Abbreviations

HCC
hepatocellular carcinoma

Declarations

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Authors' contributions

Yonghui Gui is responsible for writing and submitting the papers; Yuanhong Xu is responsible for data analysis and collation; Yonghui Gui is responsible for the production of pictures; Peng Yang is responsible for the manuscript fees and ideas guidance. The authors have read and approved the final manuscript.

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Availability of data and materials

The data used to support the findings of this study are included within the article.

Ethics approval and consent to participate

There were no cell, tissue, or animal studies. No ethical requirements are involved.

Consent for publication

All authors agree to publish the paper.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

mRNA expression of TTYH3 in HCC and adjacent normal tissues (UALCAN)(A), Prognostic value of mRNA expression of TTYH3 in HCC patients (UALCAN)(B), Prognostic value of mRNA expression of TTYH3 in HCC patients (GEPIA)(C,D).

Figure 2

Association of mRNA expression of TTYH3 with individual cancer stage of HCC patients. mRNA expressions of TTYH3 was significantly related to individual cancer stage, and as stage increased, the mRNA expressions of TTYH3 tended to be higher .(A-B)

Figure 3

Genetic mutation rates of TTYH3 in HCC patients (cBioPortal). mutation rate (22%) of TTYH3 was observed in HCC patients,(A). Genetic alterations in TTYH3 was associated with shorter OS and DS of HCC patients (B-C), Genetic alterations in TTYH3 was not associated with shorter DF of HCC patients (D).

Figure 4

Differential methylation of TTYH3 promoter in HCC (UALCAN) (A), MEXPRESS view of the TCGA data for TTYH3 in HCC (UALCAN) (B).

Figure 5

Correlation of TTYH3 expression with immune infiltration level in HCC (TIMER) (A), the landscape of relationship between TTYH3 expression and TILs in HCC (TISIDB) (B-C)

Figure 6

the landscape of relationship between TTYH3 expression and TILs in HCC (TISIDB)

Figure 7

TTYH3 expression correlated with macrophage polarization in HCC. Markers include CD86 and CSF1R of monocytes; CCL2, CD68, and IL10 of TAMs (tumor-associated macrophages); NOS2, IRF5, and PTGS2 of M1 macrophages; and CD163, VSIG4, and MS4A4A of M2 macrophages.

Figure 8

GO functional enrichment analysis predicted four main functions of TTYH3 Functionally similar gene including biological process, cellular components, molecular functions and KEGG pathway analysis (A, C, D), PPI network was generated from TTYH3 (STRING)(B)