

Data mining of the expression and regulatory role of BCAT1 in hepatocellular carcinoma

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Abstract. Branched chain amino acid transaminase 1 (BCAT1) catalyzes the production of glutamates and branched-chain α -ketoacids from branched chain amino acids, and a normal *BCAT1* expression is associated with tumorigenesis. Sequencing data from public databases, including The Cancer Genome Atlas, was used to analyze *BCAT1* expression and regulation networks for hepatocellular carcinoma (HCC). Expression and methylation were assessed using UALCAN analysis, and data from multiple datasets concerning the *BCAT1* expression level and associated survival rates were further analyzed using HCCDB; interaction networks of biological function were constructed using GeneMANIA. LinkedOmics was used to indicate correlations between *BCAT1* and any identified differentially expressed genes. Gene enrichment analysis of *BCAT1*-associated genes was conducted using the Web-based Gene Set Analysis Toolkit. The expression levels of *BCAT1* were increased in patients with HCC and in most cases, the level of *BCAT1* promoter methylation was reduced. Interaction network analysis suggested that *BCAT1* was involved in 'metabolism', 'carcinogenesis' and the 'immune response' via numerous cancer-associated pathways. The present study revealed the expression patterns and potential function networks of *BCAT1* in HCC, providing insights for future research into the role of *BCAT1* in hepatocarcinogenesis. In addition, the study provided researchers with a way to analyze the genes of interest so they can continue their research in the right direction.

Introduction

With the sixth highest incidence and fourth highest mortality rates, liver cancer is one of the most important causes of cancer-associated mortality worldwide. In 2018, ~840,000 cases of liver cancer were diagnosed in addition to 782,000 mortalities, of which $\geq 85\%$ were associated with hepatocellular carcinoma (HCC) (1,2). Although the development of predictive and treatment methods has increased the survival rates of patients with HCC, the early diagnosis and 5-year survival rates remain poor (2). Clinically, HCC can be diagnosed or prognosed using ultrasound, computerized tomography and the detection of serum α -fetoprotein (AFP) levels (3). However, the sensitivity and specificity of these methods is unsatisfactory. The complexity of the molecular mechanisms of HCC has prevented the elucidation of its development and therefore, the means with which to successfully treat the disease. Thus, it is necessary to identify novel therapeutics and drug targets by screening HCC-associated gene networks.

Branched chain amino acid transaminase 1 (BCAT1) is able to catalyze the synthesis of α -ketoglutarates from branched chain amino acids (BCAAs) and the subsequent production of glutamates and branched-chain α -ketoacids. BCAT1 is reportedly involved in the progression of various malignancies, including breast (4), ovarian (5), gastric (6) and pancreatic cancer (7), as well as HCC (8,9). The overexpression of *BCAT1* promoted cellular proliferation, migration and invasion in various types of tumor via multiple signaling pathways (10,11). In addition, previous studies have revealed that *BCAT1* is involved in DNA methylation and associated with inflammatory diseases (12,13). However, one study has suggested that *BCAT1* expression is not associated with the overall survival of patients with HCC (14), thus its exact role and mechanism in hepatocarcinogenesis remain controversial.

In a previous study, *BCAT1* expression levels were upregulated in the corresponding HCC tissues. Thus, to further investigate the role of *BCAT1* in HCC, patient data from The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) datasets (<https://www.ncbi.nlm.nih.gov/gds>) was used to assess the expression levels of *BCAT1* in patients with HCC. Functional networks of *BCAT1* in HCC were also analyzed. The results revealed that *BCAT1*

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may represent a novel therapeutic and drug target for HCC, which may provide insights for future research and the development of treatment strategies.

Materials and methods

UALCAN analysis. UALCAN (<http://ualcan.path.uab.edu>) is a publicly available interactive online portal used to perform in-depth analyses of TCGA gene expression data (15). UALCAN can be used to determine methylation and relative expression levels, as well as the survival of a target gene across several clinicopathological features. Expression levels were measured as transcripts per million. Promoter DNA methylation information was from the TCGA Infinium Human Methylation 450K BeadChip arrays. The *t* test was performed using a PERL script with Comprehensive Perl Archive Network module (<http://search.cpan.org/~yunfang/Statistics-TTest-1.1.0/TTest.pm>) to compare the statistical significance between two independent groups.

GeneMANIA analysis. GeneMANIA (<http://genemania.org/>) is a web interface that uses large sets of functional association data to identify single genes related to a set of input genes (16). Association data include protein and genetic interaction pathways, co-expression, co-localization and protein domain homology. GeneMANIA was used to construct the *BCAT1* biological network.

LinkedOmics analysis. The LinkedOmics database (<http://www.linkedomics.org/login.php>) contains the multi-omics and clinical data of 32 cancer types, and a total of 11,158 patients from TCGA project (17). LinkFinder, one of three modules, was used to identify differentially expressed genes (DEGs) in TCGA LIHC cohort whose expression levels correlated with those of *BCAT1*. The searching and targeting datasets were both of 'RNAseq', and the results were statistically analyzed using Pearson's correlation coefficient. LinkInterpreter, a module that recruited Web-based Gene Set Analysis Toolkit, was used to determine the pathways and networks in which the identified DEGs were involved (18).

HCCDB analysis. The HCCDB database (<http://lifeome.net/database/hccdb/home.html>) comprises ~4,000 clinical samples from 15 public HCC expression datasets (13 GEO microarray datasets and two RNA-Seq datasets, TCGA-LIHC and ICGC LIRI-JP) and serves as a one-stop online resource for the investigation of HCC gene expression (19). All GEO datasets are available from the GEO repository (<https://www.ncbi.nlm.nih.gov/gds>). The accession numbers are: GSE25097 (20-23), GSE22058 (24,25), GSE36376 (26), GSE14520 (27-30), GSE10143 (31), GSE9843 (32-35), GSE19977 (33,34), GSE46444 (36), GSE54236 (37,38), GSE63898 (39), GSE43619 (40), GSE64041 (41) and GSE76427 (42). ICGC LIRI-JP is available on the ICGC Data Portal (<https://dcc.icgc.org/projects/LIRI-JP>) and TCGA-LIHC is available on TCGA (<https://portal.gdc.cancer.gov/projects/TCGA-LIHC>). HCCDB was used to analyze the *BCAT1* co-expression networks of HCC and the corresponding adjacent tissues. The expression levels of *BCAT1* in different datasets were also detected. Samples

were classified into high-/low-expression groups by median expression value of *BCAT1*.

Results

Expression and promoter methylation levels of *BCAT1* in HCC tissues. The expression levels of *BCAT1* in HCC tissues were investigated using a number of datasets from TCGA. The data revealed that the mRNA expression levels of *BCAT1* were significantly increased in HCC tissues compared with normal tissues ($P < 0.001$; Fig. 1). Combined with the authors' previous results (43), *BCAT1* may serve as a novel diagnostic marker of HCC. Secondly, the level of *BCAT1* promoter methylation in HCC tissues was also assessed. The data suggested the same trend as that of *BCAT1* mRNA expression (Fig. 2); the higher the mRNA expression level, the lower the methylation level. Subsequently, data from 12 additional GEO datasets were analyzed (12 of the 15 datasets in the HCCDB database; three were not included because analysis results were not returned by HCCDB tools), which revealed contradictions between the results of different studies (Fig. 3). Studies including dataset 4, 6 and 8 revealed no significant differences in the mRNA expression levels of *BCAT1* between HCC and adjacent normal tissues.

Effects of *BCAT1* on the survival of patients with HCC. In tumors, the abnormal expression of specific genes has long been associated with overall patient survival. In the present study, two datasets from HCCDB were used to analyze the overall survival of HCC patients with high and low *BCAT1* expression levels. This revealed that where the expression level of *BCAT1* was not significantly different between the HCC and adjacent tissues, the overall survival of patients in the high- and low-*BCAT1* expression cohorts did not differ significantly (Fig. 4A). By contrast, if the expression level of *BCAT1* in HCC tissues was significantly increased compared within the adjacent tissues, patients with high *BCAT1* expression levels possessed a lower overall survival probability than those with low expression levels (Fig. 4B).

Interaction network of *BCAT1*. A *BCAT1* interaction network was constructed to identify potential interactions between *BCAT1* and other cancer-associated proteins. The results showed that *BCAT1* could be co-expressed with phosphoserine aminotransferase, was predicted to interact with 13 proteins, shared protein domains with its isoform *BCAT2* and directly interacted with six proteins (Fig. 5). These proteins are involved in 'metabolic pathway', 'amino acids biosynthesis and degradation', 'myc pathway', 'p53 signaling pathway' and 'other cancer-related pathways or biological processes'.

LinkedOmics online tools were then used to investigate the genes that were co-expressed with *BCAT1* in HCC. A volcano plot revealed that the expression of 2,970 genes negatively correlated with that of *BCAT1* [green spot; false discovery rate (FDR) < 0.05], while the expression of 9,077 genes positively correlated with *BCAT1* (red spot; FDR < 0.05 ; Fig. 6A). The top 50 negatively and positively correlated genes are displayed in Fig. 6B and C. These results indicate that *BCAT1* serves a critical role in HCC development. Pearson's correlation coefficient analysis revealed a strong positive association between

the expression levels of *BCAT1* and *plasminogen activator, urokinase receptor* (Pearson's correlation coefficient=0.7142; $P=3.89 \times 10^{-59}$; Fig. 7A), *RELT* (Pearson's correlation coefficient=0.6708; $P=7.628 \times 10^{-50}$; Fig. 7B), *Fc fragment of IgG receptor Ic* (Pearson's correlation coefficient=0.6651; $P=9.94 \times 10^{-49}$; Fig. 7C), and a negative association with *MLX interacting protein like (MLXIPL)* (Pearson's correlation coefficient=-0.5685; $P=3.787 \times 10^{-33}$; Fig. 7D), *RAR related orphan receptor C (RORC)* (Pearson's correlation coefficient=-0.5677; $P=4.868 \times 10^{-33}$; Fig. 7E) and *pyruvate dehydrogenase kinase 2 (PDK2)* (Pearson's correlation coefficient=-0.5421; $P=1.001 \times 10^{-29}$; Fig. 7F), which are involved in 'extracellular matrix degradation', 'NF- κ B pathway and immune response', 'IgG component', 'triglyceride synthesis', 'lymphoid organogenesis and thymopoiesis' and 'pyruvate dehydrogenation', respectively.

Biological process and molecular function analyses were conducted using gene set enrichment analysis, which showed that *BCAT1*-associated DEGs were involved in a number of biological processes and molecular functions, including 'protein activation cascade', 'respiratory burst', 'T cell activation', 'sphingolipid binding', 'MHC protein binding' and 'cytokine receptor activity' (Fig. 8). These data indicate that *BCAT1* serves an important role in immune system activation, cellular responses to stimulation, metabolism and a number of other processes.

Discussion

The proliferation of cancer cells depends on extra nutrients acquired from the tumor microenvironment (44,45). BCAAs are essential for tumor growth and the progression of multiple biological pathways (46). A number of studies have revealed that the enzymes catalyzing BCAAs into their corresponding nutrients are predominantly overexpressed in cancer (7,13,47) and that *BCAT1* is one of the most important enzymes involved in BCAA catabolism. The authors' previous study indicated that *BCAT1* was significantly upregulated in HCC and that this was associated with poor patient prognosis (43). To further investigate the function networks of *BCAT1* in HCC, bioinformatics analyses were performed using a selection of public databases to provide insights for future research into the metabolic regulation of HCC.

With a lack of appropriate markers of early stage HCC, 70-80% of patients are diagnosed at the advanced stage of disease and lose the opportunity to undergo surgery. Clinically, AFP has long been regarded as the standard for liver cancer diagnosis; however, a large number of HCC patients are AFP-negative (2), thus additional biomarkers for the early detection of HCC are required. A previous study explored the use of serum methylated *BCAT1* and *IKAROS family zinc finger 1 (IKZF1)* for the detection of colorectal neoplasia; this revealed that for the diagnosis of colorectal cancer, the detection of *BCAT1/IKZF1* DNA in the serum had a comparable sensitivity, but a higher specificity than the traditional fecal immunochemical test (48). The present study demonstrated that *BCAT1* mRNA expression levels in HCC tissues were increased compared with those in normal tissues. In addition, the level of *BCAT1* promoter methylation was decreased in HCC tissues compared with

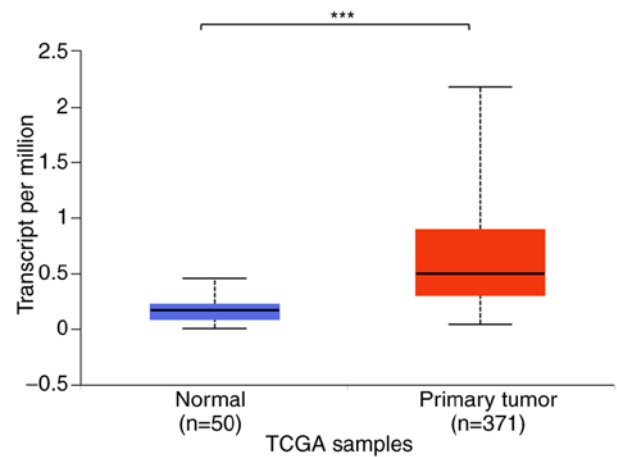


Figure 1. *BCAT1* expression level in HCC and different classifications. Relative expression of *BCAT1* in normal and HCC tissues. Data are presented as the mean \pm standard error. *** $P<0.001$. Transcripts per million was used to measure the expression. TCGA, the Cancer Genome Atlas; *BCAT1*, branched chain amino acid transaminase 1; HCC, hepatocellular carcinoma.

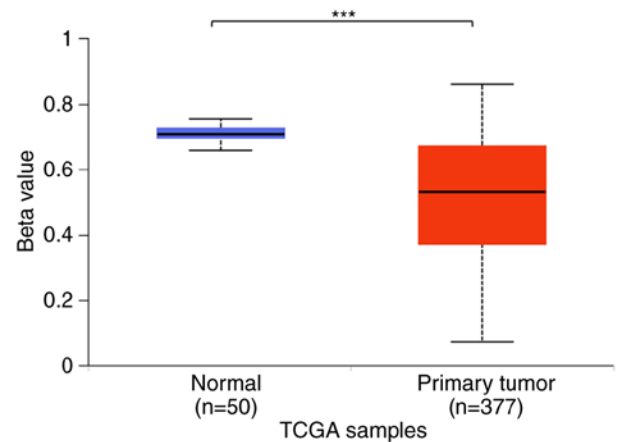


Figure 2. Promoter methylation level of *BCAT1* in HCC. Methylation level of *BCAT1* in normal and HCC tissues. Data are presented as the mean \pm standard error. *** $P<0.001$. *BCAT1*, branched chain amino acid transaminase 1; HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas.

normal tissues as that indicated for mRNA expression levels. However, analysis of 12 datasets from different regions indicated that in some cases, the expression level of *BCAT1* was not significantly different between the HCC and adjacent normal tissues. These results suggested that while *BCAT1* may serve as a marker for the detection of HCC, regional variations may apply. For this reason, further large-scale, multicenter studies are required to confirm the role of *BCAT1* in the prediction and diagnosis of HCC. Nonetheless, the analysis of *BCAT1* expression and promoter methylation levels in patients demonstrated its potential use as a reliable biomarker of HCC.

The abnormal expression of oncogenes is closely associated with patient survival (49,50). Overexpression of *MTHFD1* predicted poorer overall survival in patients with HCC (51). Also, the expression levels of *SIRT1* were significantly increased in HCC and associated with poor patient survival (52). In this study, although the

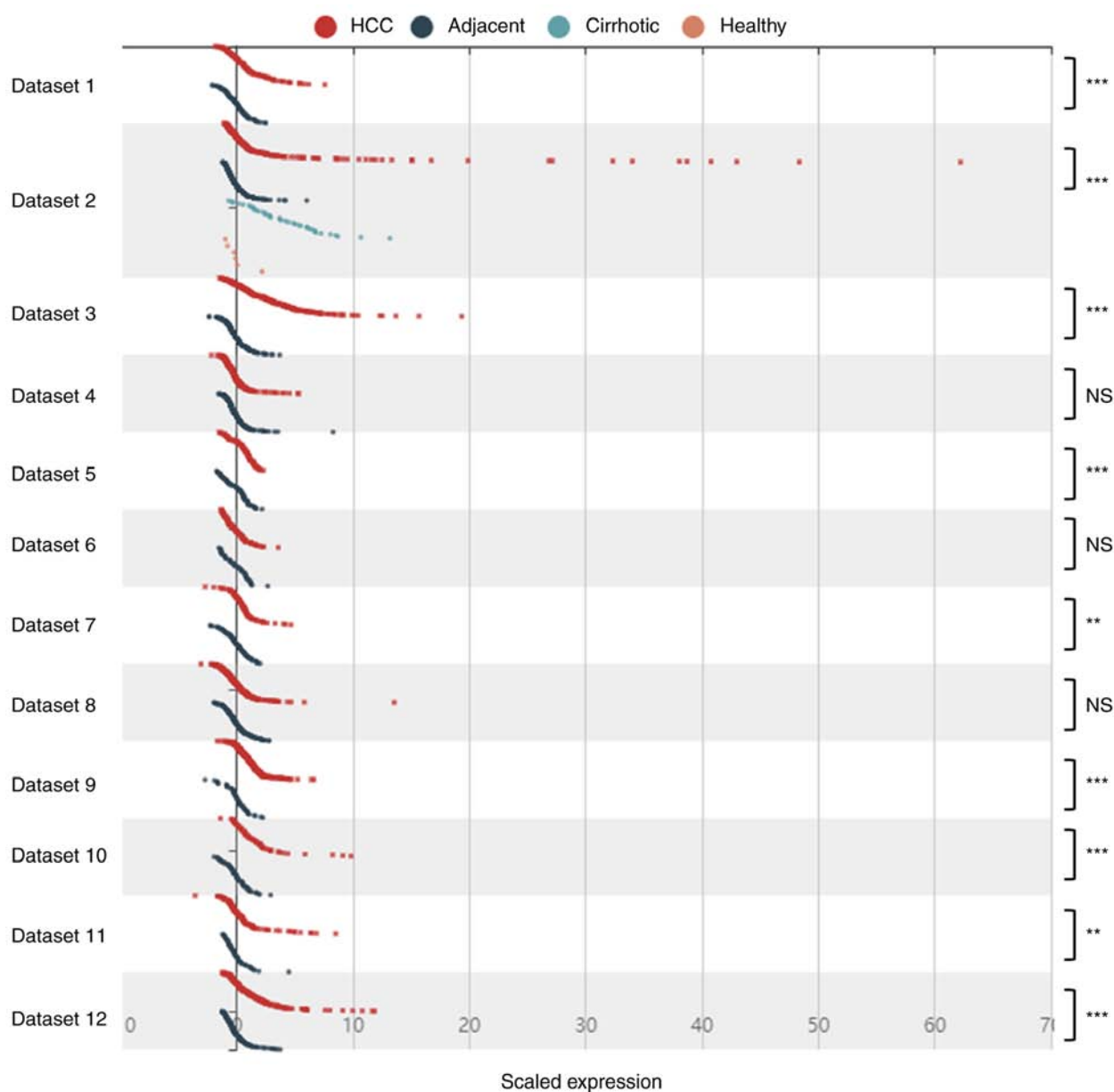


Figure 3. Expression levels of *BCAT1* in different datasets. A total of 9 of 12 datasets showed that expression levels of *BCAT1* in HCC tissues are significantly increased compared with in adjacent tissues, but 3 of 12 datasets showed no significant difference between the expression levels of *BCAT1* in HCC tissues and in adjacent tissues. ** $P < 0.01$ and *** $P < 0.001$. *BCAT1*, branched chain amino acid transaminase 1; HCC, hepatocellular carcinoma; NS, not significant.

majority of studies illustrated an upregulation in *BCAT1* expression levels in HCC tissues (compared with adjacent tissues), selected cases presented an opposing result. Notably, further analyses within the present study indicated that patients with no significant differences in the level of *BCAT1* expression between tumor and paracancerous tissues possessed a *BCAT1* expression-independent survival rate. Meanwhile, patients with higher *BCAT1* expression levels in HCC tissues exhibited a *BCAT1*-dependent survival probability. These results suggest that additional methods may be required to demonstrate the role of *BCAT1* in HCC; this may include not only focusing on mRNA and protein expression, but also assessing the products of *BCAT1* enzymatic reactions. *BCAT1* is one of the key enzymes involved in amino acid metabolism, such that a repeatable method such as metabolomics analysis may further clarify the role of

BCAT1. However, the results ultimately revealed that *BCAT1* serves an important role in the survival of patients with HCC.

Tumorigenesis is a complex process with abnormal changes in protein-protein interactions and signaling pathways (53). *BCAT1* was identified to be involved in the proliferation (4,54), apoptosis (55), invasion and metastasis (56) of cancer cells, and to be associated with poor prognosis in patients with various types of cancer (57,58). In addition, *BCAT1* was reported to regulate mTOR signaling (59), as well as the Wnt (60) and FoxO signaling pathways (61). However, the effects of *BCAT1* on other pathways and cellular functions remain unclear.

In the present study, the expression of *BCAT1*-associated genes was investigated and *BCAT1* was found to be involved in various other signaling pathways and biological processes; to the best of our knowledge, the relationship between

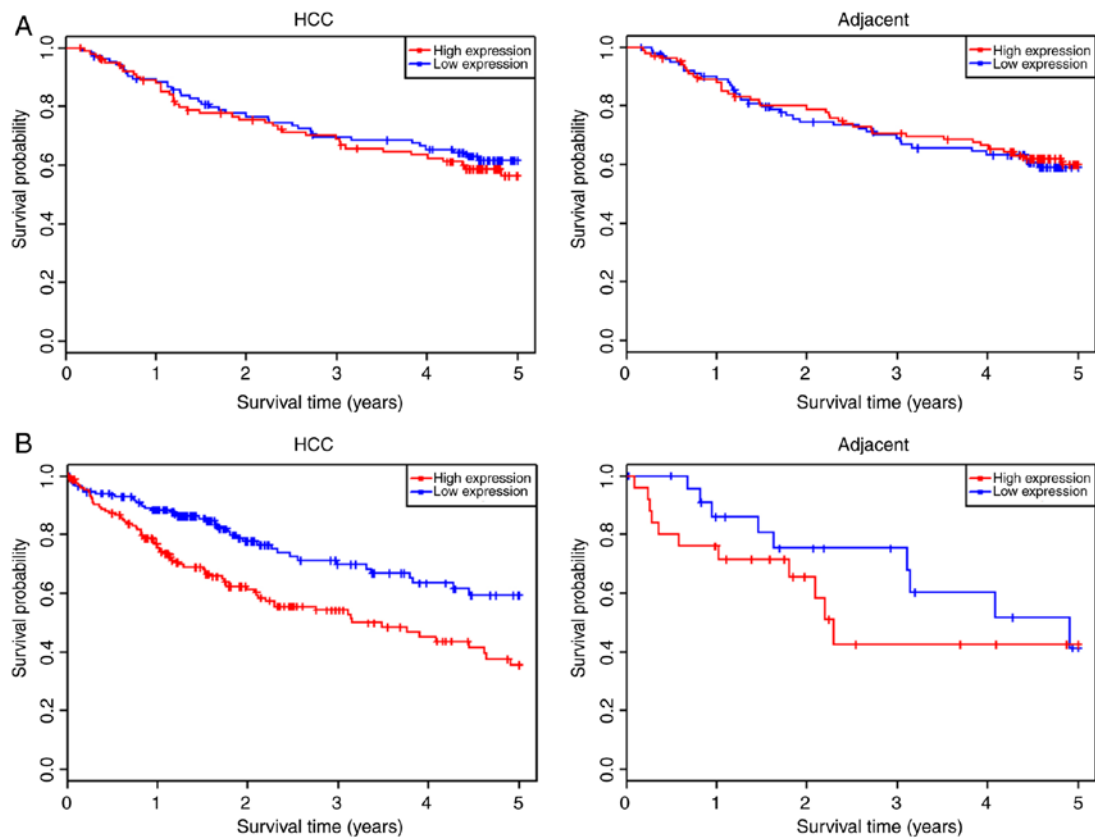


Figure 4. Effect of *BCAT1* in survival of HCC. (A) Survival of patients with no significant difference of *BCAT1* expression between HCC tissues and adjacent tissues. (B) Survival of patients with significant difference of *BCAT1* expression between HCC tissues and adjacent tissues. *BCAT1*, branched chain amino acid transaminase 1; HCC, hepatocellular carcinoma.

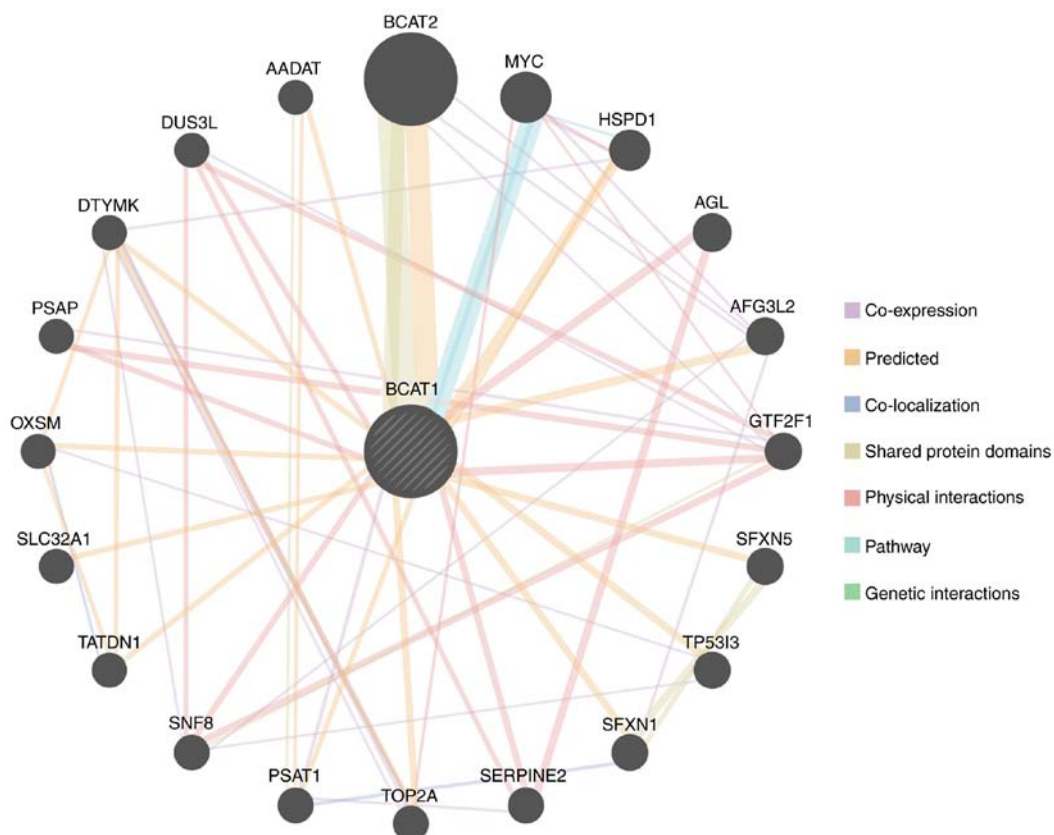


Figure 5. Biological interaction network of *BCAT1*. Different colors represent diverse bioinformatics methods. *BCAT1*, branched chain amino acid transaminase 1.

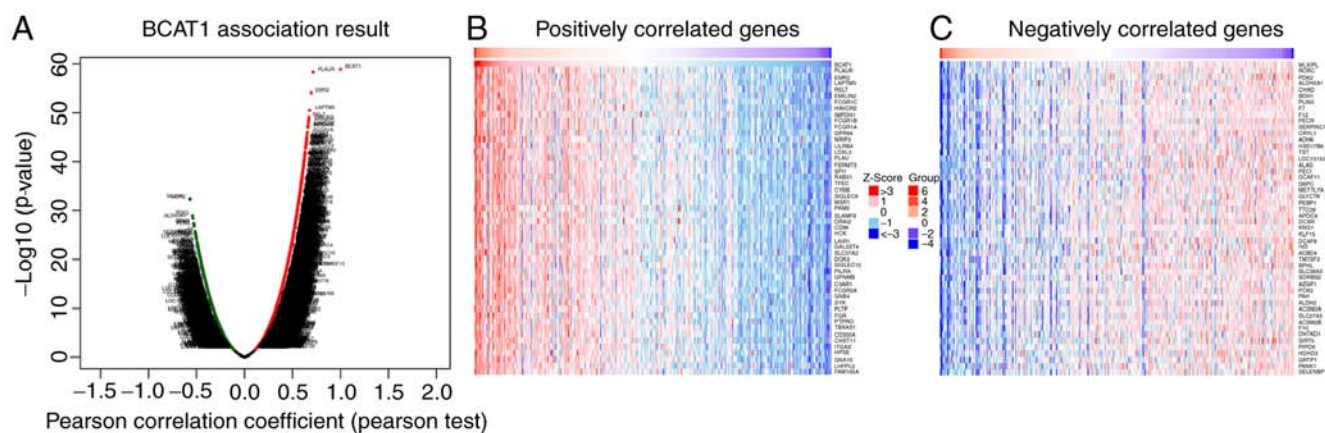


Figure 6. Differentially expressed genes in correlation with *BCAT1*. (A) Correlations between *BCAT1* and differentially express of genes in HCC were analyzed by Pearson test. (B) Heat map of positively correlated genes with *BCAT1* in HCC. (C) Heat map of negatively correlated genes with *BCAT1* in HCC. Red indicates positive and blue indicates negative. *BCAT1*, branched chain amino acid transaminase 1; HCC, hepatocellular carcinoma.

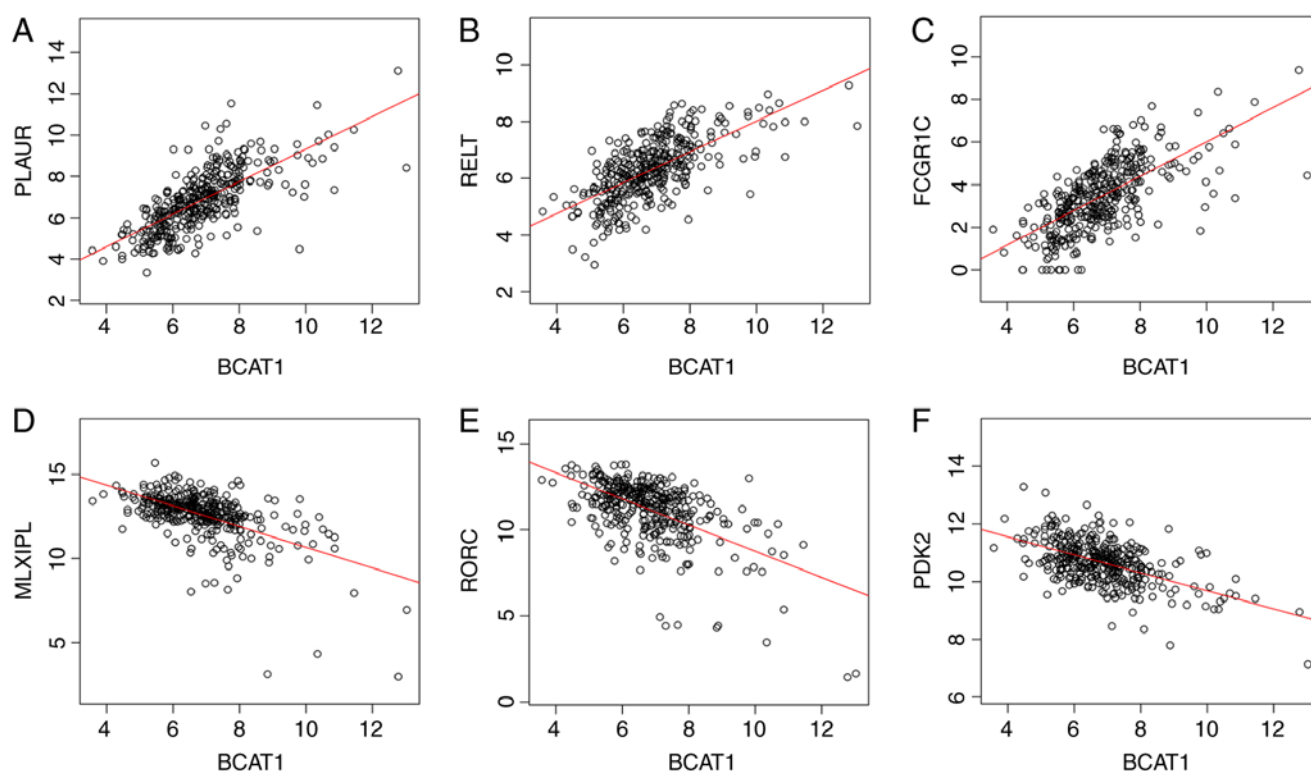


Figure 7. Gene expression correlation analysis of *BCAT1* and other genes. Pearson correlation of *BCAT1* expression with (A) *PLAUR* expression, (B) *RELT* expression, (C) *FCGR1C* expression, (D) *MLXIPL* expression, (E) *RORC* expression and (F) *PDK2* expression. *BCAT1*, branched chain amino acid transaminase 1; *FCGR1C*, Fc fragment of IgG receptor 1c; *PDK2*, pyruvate dehydrogenase kinase 2; *PLAUR*, plasminogen activator, urokinase receptor; *RORC*, RAR related orphan receptor C; *MLXIPL*, MLX interacting protein like.

BCAT1 and TP53 was previously unacknowledged. TP53 is induced by the tumor suppressor p53 and is involved in p53-mediated apoptosis (62). The present study predicted that *BCAT1* may be involved in p53 signaling via its interaction with TP53I3, suggesting that *BCAT1* serves a more important role in HCC than was originally hypothesized. In addition to TP53I3, *BCAT1* was predicted to interact with AADAT, TOP2A and DTYMK, which are involved in tryptophan and lysine metabolism, DNA topology state regulation and pyrimidine metabolism, respectively. These predictions indicated that *BCAT1* may participate in a large

number of biological functions in HCC via its potential interaction with (or regulation of) critical factors. In HCC, *BCAT1* was predicted to associate with a number of critical genes including *RELT*, *PDK2* and *RORC*, which regulate T-cell responses, apoptosis, the tricarboxylic acid cycle and inflammation (63-65).

However, there were limitations to the present study. First, only 371 tumor cases and 50 normal controls were employed for analysis, and a larger number of patients is required to verify the present findings. In addition, few patients at cancer stage 4, between 81 and 100 years of age and with extreme

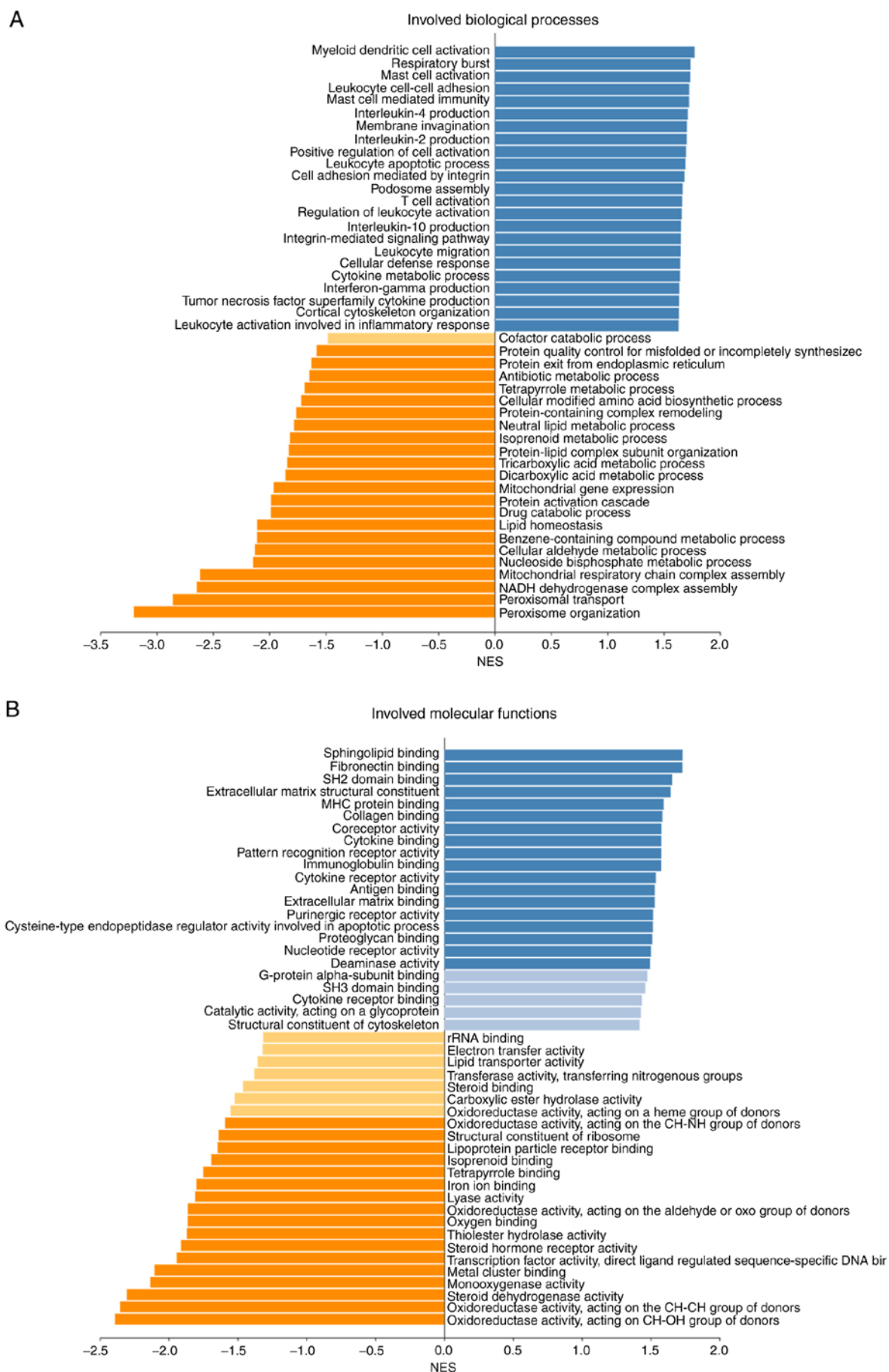


Figure 8. Enriched Gene Ontology annotations of *BCAT1* correlated genes in hepatocellular carcinoma. (A) Biological process analysis. (B) Molecular function analysis. Dark blue and orange indicate $FDR \leq 0.05$, light blue and orange indicate $FDR > 0.05$. *BCAT1*, branched chain amino acid transaminase 1; FDR, false discovery rate.

obesity were included, which may suggest that a large number of HCC patients die prior to diagnosis. Secondly, only bioinformatics analysis results were displayed in this study. Further verification experiments, such as PCR experiments, western blot assays and co-immunoprecipitation, are necessary to verify the present results. Another limitation is that transcriptome sequencing cannot directly provide information on protein activity, which can only be verified by follow-up studies.

In conclusion, the present study provides bioinformatics evidence of the significance of *BCAT1* in hepatocarcinogenesis and its potential as an early detection marker. The results indicated that the function and effects of *BCAT1* in HCC are multifaceted, and that *BCAT1* may be associated with immune activation, metabolic pathways and the cell cycle. Furthermore, a *BCAT1* miRNA-target network was analyzed using TargetScan, demonstrating that thousands of miRNAs were predicted to interact with *BCAT1* (data not shown) and providing a clear direction for future studies. More importantly, analysis results of the present study of this well-known gene indicated that the interactions between *BCAT1* and numerous other critical proteins were uncharted. This will help us to determine the future research direction and draw a complete molecular network of *BCAT1* participating in HCC. Also, the present study provided researchers, especially young researchers who lack experimental and bioinformatics analysis platforms, with guidance on finding directions for functional genome research.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the TCGA repository, <https://portal.gdc.cancer.gov/>; the GEO repository (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE25097>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE22058>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36376>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14520>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10143>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9843>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19977>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46444>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54236>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63898>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43619>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64041>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76427>); and the ICGC repository, <https://dcc.icgc.org/projects/LIRI-JP>.

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43619>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64041>, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76427>); and the ICGC repository, <https://dcc.icgc.org/projects/LIRI-JP>.

Authors' contribution

RZY and WJL conceived, designed the research and drafted the manuscript; HFZ and WTX were responsible for the acquisition and analysis of data; HFZ and MJL analyzed and interpreted the data; RZY revised the manuscript for important intellectual content. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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.

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