TEAD2 as a novel prognostic factor for hepatocellular carcinoma

JONG SEOK JOO[1,2*], SANG YEON CHO[1*], WOO SUN ROU[1,2], JU SEOK KIM[1,2], SUN HYUNG KANG[1,2], EAUM SEOK LEE[1,2], HEE SEOK MOON[1,2], SEOK HYUN KIM[1,2], JAE KYU SUNG[1,2], IN SUN KWON[3], HYUK SOO EUN[1,2] and BYUNG SEOK LEE[1,2]

[1]Department of Internal Medicine, Chungnam National University School of Medicine; [2]Department of Internal Medicine; [3]Clinical Trial Center, Chungnam National University Hospital, Jung-gu, Daejeon 35015, Republic of Korea

Received August 21, 2019; Accepted February 14, 2020

DOI: 10.3892/or.2020.7578

Abstract. TEA Domain Transcription Factors (*TEADs*) are important in development and serve essential roles in tumorigenesis; however, the role of *TEAD2* expression in hepatocellular carcinoma (HCC) has not been widely examined. The present study was conducted to investigate the expression status of *TEAD2* in HCC and to evaluate whether the expression of *TEAD2* is associated with the prognosis of patients with HCC. mRNA expression data was retrieved for Hippo pathway genes of 50 normal control and 377 HCC samples from The Cancer Genome Atlas data portal. Gene set enrichment, GeneNeighbors, ClassNeighbors and survival analyses were then performed based on the gene expression levels. The mRNA expression of *TEAD2* and *VGLL4* was significantly higher in HCC compared with the normal control samples, and the mRNA expression of *TEAD2* was higher in advanced stages than in early stages. Specifically, survival analysis revealed that higher mRNA expression of *TEAD2* was significantly associated with a less favorable overall survival rate (P=0.0067) and there was a trend towards significance between higher mRNA expression of *VGLL4* and poor overall survival rate (P=0.051). According to the gene set enrichment analysis, patients with higher mRNA expression of *TEAD2* and *VGLL4* had strongly enhanced epithelial-mesenchymal transition and angiogenesis, which are associated with tumor progression. In conclusion, increased mRNA expression of *TEAD2* is associated with a poor prognosis in patients with HCC. *TEAD2* may serve as a prognostic factor for HCC and a novel therapeutic target.

Correspondence to: Professor Byung Seok Lee or Professor Hyuk Soo Eun, Department of Internal Medicine, Chungnam National University School of Medicine, 266 Munhwa-ro, Jung-gu, Daejeon 35015, Republic of Korea
E-mail: gie001@cnuh.co.kr
E-mail: liver@kaist.ac.kr

*Contributed equally

Key words: *TEAD2*, hepatocellular carcinoma, The Cancer Genome Atlas data, prognosis, survival analysis

Introduction

Hepatocellular carcinoma (HCC) was the sixth most common cancer type and fourth most common cause of cancer-associated mortalities worldwide in 2018 (1). Although the various therapeutic modalities for HCC have improved significantly in recent years, patients with HCC exhibit a poor survival rate, with a five-year survival rate of ~30% (2). Two leading causes of the unfavorable prognosis are delays in the diagnosis of HCC and a lack of appropriate treatment for advanced HCC (3). Although researchers have evaluated the treatment of advanced HCC with immunotherapy or molecular-targeted therapy, the survival rate of patients with HCC has not significantly improved. The occurrence of HCC is thought to be associated with disturbances in the relationships between various genes (4). Therefore, identifying the genes and proteins that regulate HCC development is important for developing novel therapeutic targets (4).

The Hippo signaling pathway antagonizes the oncogenic transcriptional co-activators yes-associated protein (*YAP*) and Tafazzin (*TAZ*) and serves an important role in limiting organ size during developmental processes (5,6). Without the activation of Hippo signaling, non-phosphorylated *YAP* and *TAZ* enter the nucleus and activate the expression of antiapoptotic and proliferative genes with TEA domain transcription factors (*TEADs*) (5,6). Mammals harbor four *TEAD* genes (*TEAD1-4*). *VGLL* is a hippo-independent coactivator that regulates gene expression via interaction with *TEAD*, and there are four *VGLL* genes (*VGLL1-4*). Notably, *VGLL4* inhibits tumor growth by binding to *TEAD* and *VGLL1* inhibits *YAP*/*TAZ* target genes by competitively binding to *TEAD* (7-10). Several previous studies revealed that *TEAD*s are involved in human cancer (11-16). However, these studies were primarily focused on *TEAD1* and *TEAD4*, while no clinical studies have examined the association between *TEAD2* expression and prognosis in HCC. The purpose of the present study was to investigate the mRNA expression status of *TEAD2* in HCC tumors, as well as the prognostic clinical significance of *TEAD2* in HCC. Therefore, the role of *TEAD2* as a possible prognostic marker and therapeutic target for treating HCC was evaluated.

Materials and methods

Gene expression profiling. Level 3 mRNA expression aggregated data and clinical data from 50 normal and 377 HCC

samples were acquired from The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) data portal (https://portal.gdc.cancer.gov/) and RNAseqV2-RSEM_genes were obtained from Firebrowse v.1.1.39. (http://firebrowse.org/) for gene expression analysis. All 377 dataset samples were included for profiling of mRNA expression.

Analysis of mRNA expression. R software (v.3.6.1; http://www.r-project.org) was used to analyze the raw data. mRNA expression levels were analyzed with Firebrowse (http://firebrowse.org) and Gene Expression Profiling Interactive Analysis software (GEPIA; http://gepia.cancer-pku.cn/). GeneNeighbors and ClassNeighbors, which are modules in GenePattern (http://broadinstitute.org/cancer/software/genepattern), were used to calculate the nearest gene neighbors and identify genes most significantly correlated with a class template for a specific Hippo pathway gene, respectively. The dataset, which is Illuminahiseq_maseqv2-RSEM_genes_normalized RNA-Seq data obtained from Firebrowse (http://firebrowse.org/?cohort=LIHC&download_dialog=true), was processed using R software (v.3.6.1; http://www.r-project.org) in GeneNeighbors and ClassNeighbors analyses. The 100 genes most strongly associated with *TEAD2* and *VGLL4* were selected for classification according to Gene Ontology Enrichment Analysis (GO terms) using Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov). Differentially expressed genes (DEGs) were classified by GO terms on the basis of their molecular function, biological process, or cellular component. DAVID provided not only an overview of extensive pathways (www.biocarta.com) with various gene interactions but also the number of DEGs per pathway with a gene enrichment P-value. Gene enrichment score with $P<0.05$ considered to indicate a strong association rather than random probability (17).

Functional enrichment analysis. Gene set enrichment analysis (GSEA) was performed to identify enriched genes predicted to be associated with pathways in the hallmark and curated gene sets derived from Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg) information. A P-value <0.05 and false discovery rate <0.1 were considered to indicate a statistically significant gene.

Survival analysis. Cutoff Finder (http://molpath.charite.de/cutoff) was used to determine the cut-off values for LIHC mRNA expression. Illuminahiseq_maseqv2-RSEM_genes_normalised RNA-seq data of Hippo pathway genes were acquired from a tab-separated files, and the columns represented variables and the rows represented patients (http://molpath.charite.de/cutoff/load.jsp). The determination of optimal cut-off point in Cutoff Finder was based on overall survival rate for significance on the basis of the log-rank test for outcome of patient (http://molpath.charite.de/cutoff/assign.jsp). The Kaplan-Meier method was performed to estimate the cumulative event (death) rate from the date of operation until death used as the outcome variable. Survival curves stratified by high and low expression groups on each Hippo pathway genes were compared using the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Human liver samples. In total, 79 patients with HCC were retrospectively screened who were treated by surgical resection between 1999 and 2016 at Chungnam National University Hospital (Daejeon, South Korea). The median age of patients was 59 years (range, 18-78 years). All tissue samples were obtained from specimens removed during lobectomy or segmentectomy. Clinical data was obtained by reviewing the medical records of all patients. Clinicopathological review of all cases performed was by JSJ and HSE. The histologic grade of HCC was determined according to the Edmondson and Steiner grading system and the Tumor-Node-Metastasis (TNM) staging system for HCC was determined according to the 8th edition of the American Joint Committee on Cancer TNM staging system at the time of surgery (18,19).

Of the 79 HCC cases, fresh-frozen 79 primary HCC and paired 79 non-tumor liver tissues were acquired for quantitative (q)PCR from the National Biobank of Korea, Chungnam National University Hospital, a member of the Korea Biobank Network. In total, one vial (100 mg) of tumor sample and one vial (100 mg) of non-tumor sample was obtained from the Biobank of Chungnam National University Hospital. The present research was approved by the Institutional Review Board of Chungnam National University Hospital (approval no. CNUH 2017-05-013).

Reverse transcription (RT)-qPCR. Total RNA was extracted from liver tissues with the RNeasy Mini kit (Qiagen, Inc.; cat. no. 74106) or TRIzol reagent (Thermo Fisher Scientific, Inc.) in accordance with the manufacturers' instructions. We extracted RNA from all liver tissues used in this experiment using RNeasy Mini kit. Reverse transcription was performed using the same quantity of total RNA to generate cDNA using amfiRivert cDNA synthesis master mix (GenDEPOT). The following temperature protocol were used for RT: Annealing, 25˚C for 5 min and extension at 50˚C for 60 min. After that, Reverse Transcriptase inactivation was performed at 70˚C for 15 min. Finally, the samples were held at 4˚C. qPCR was performed using SYBR Green Real-time PCR Master mix (Toyobo Life Science). The qPCR thermocycling condition were as follows: First, pre-denaturation step is performed at 95˚C for 10 min. This is followed by 40 cycles of denaturation at 95˚C for 45 sec, annealing at 60˚C for 45 sec and extension for 60 sec at 72˚C. After that, the final denaturation reaction is performed for 15 sec at 95˚C followed by a final extension for 5 sec at 65˚C. To quantify transcription, the mRNA expression levels of the target genes were normalized to those of β-actin. Table SI shows the primers used in this study. All samples were evaluated in duplicate, and the relative fold-changes in gene expression levels were calculated as $2^{-\Delta\Delta Cq}$ (20).

Experimental and recurrence, survival analyses. SPSS 13.0 (SPSS, Inc.) and Prism v.5.0 (GraphPad Software, Inc.) were used for data analysis. In our experimental analysis, complement DNA (cDNA) was synthesized from paired liver tissues (tumor and paired non-tumor tissue) and gene expression was analyzed by amplifying *TEAD2* and β-actin on the cDNA. The mRNA expression data of *TEAD2* was analyzed in tumors and normalized to β-actin. Arbitrary values were then obtained by defining (Tumor *TEAD2*/Tumor β-actin)/(Non-tumor *TEAD2*/Non-tumor β-actin). Groups stratified into high and

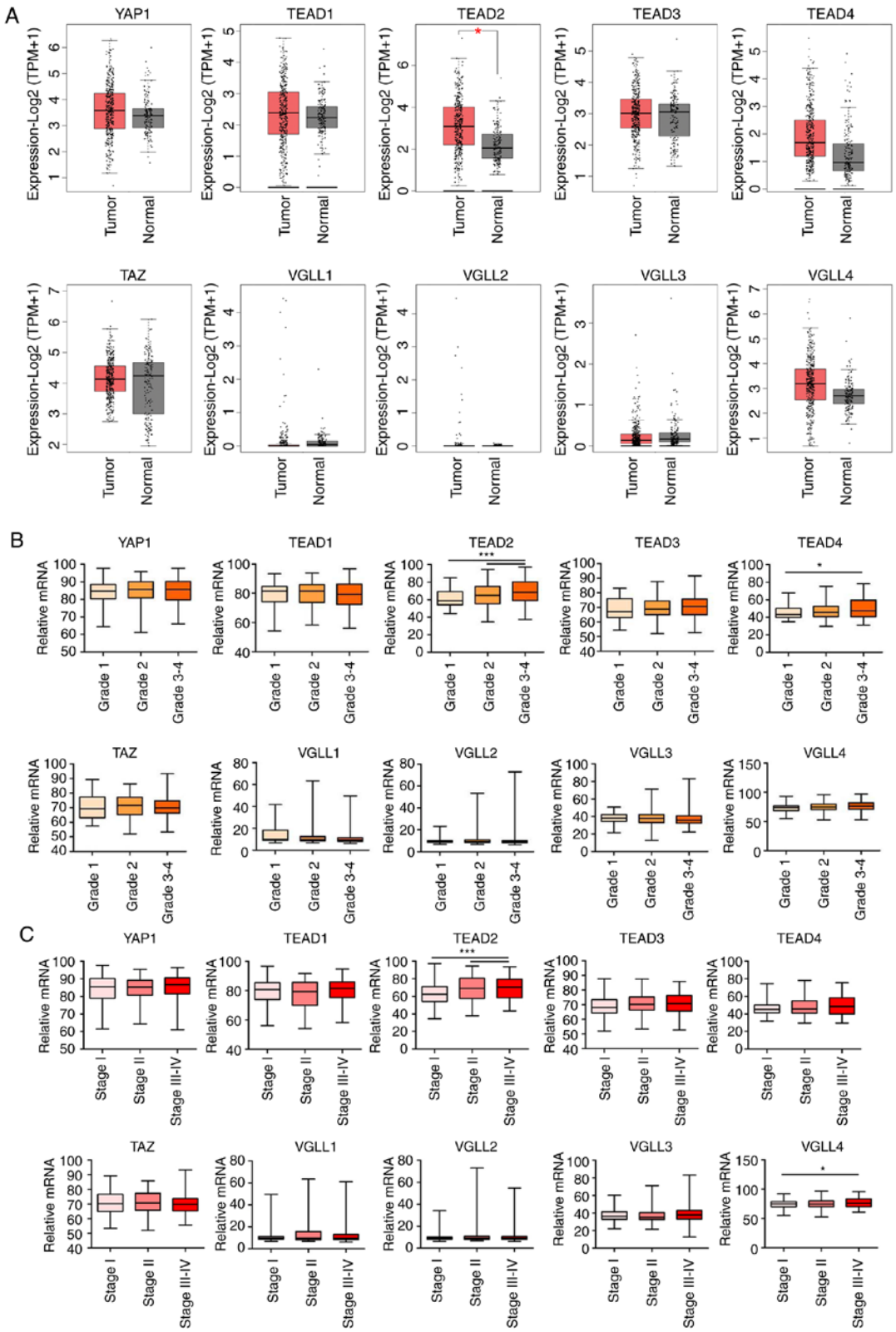

Figure 1. Gene expression of Hippo pathway in HCC. (A) Gene expression of the Hippo pathway in HCC, based on the Gene Expression Profiling Interactive Analysis database was compared between normal and tumor samples and is given as Log2 (Transcripts per kilobase million + 1). *P<0.01. (B) Relative mRNA expression differences of Hippo pathway genes in HCC according to histological grade (Edmondson-Steiner grade). (C) Relative mRNA expression differences of Hippo pathway genes in HCC according to Tumor-Node-Metastasis stage. One-way ANOVA was conducted in more than two groups. *P<0.05; **P<0.01; ***P<0.001. HCC, hepatocellular carcinoma; *TEAD*, transcriptional enhancer associated domain; *VGLL*, vestigial like family; *TAZ*, tafazzin; *YAP*, yes-associate protein; TNM, Tumor-Node-Metastasis.

low expression according to the median values and these groups were converted to categorical variables based on this. Survival analysis was performed for recurrence and overall survival related with these two groups. Overall survival (OS) was defined as the duration between the date of diagnosis and the date of death/final follow up, from any cause. Recurrence-free

Table I. Clinicopathologic information of patients with hepatocellular carcinoma derived from TCGA data portal.

Characteristics	Total, n (%)
Patients	377 (100.0)
Sex	
Female	122 (32.3)
Male	255 (67.7)
Age, years	
≤60	180 (47.7)
>60	196 (52.0)
NA	1 (0.3)
TNM stage (8th AJCC staging system)	
Stage I	175 (46.4)
Stage II	87 (23.1)
Stage III	86 (22.8)
Stage IV	5 (1.3)
NA	24 (6.4)
Histological grade (Edmondson-Steiner grade)	
Grade 1	55 (14.6)
Grade 2	180 (47.7)
Grade 3	124 (33.0)
Grade 4	13 (3.4)
NA	5 (1.3)
Vital status	
Alive	245 (65.0)
Dead	132 (35.0)
Child-Pugh classification	
A	223 (59.1)
B	21 (5.6)
C	1 (0.3)
NA	132 (35.0)
Histological type	
Hepatocholangiocarcinoma	7 (1.9)
Hepatocellular carcinoma	367 (97.3)
Fibrolamellar carcinoma	3 (0.9)
Adjacent hepatic tissue inflammation extent type	
Mild	101 (26.8)
Severe	19 (5.0)
None	119 (31.6)
NA	138 (36.6)
Ishak fibrosis score	
0-no fibrosis	76 (20.1)
1,2-portal fibrosis	31 (8.2)
3,4-fibrous septa	30 (8.0)
5-nodular formation and incomplete cirrhosis	9 (2.4)
6-established cirrhosis	72 (19.1)
NA	159 (42.2)
Thrombocytopenia (<150x10^9/l)	
Yes	76 (20.1)
No	234 (62.1)
NA	67 (17.8)
Albumin level, g/dl	
>3.5	217 (57.6)
≤3.5	86 (22.8)
NA	74 (19.6)
AFP, ng/ml	
≤20	152 (40.3)
>20	132 (35.0)
NA	93 (24.7)
History of hepatocellular carcinoma risk	
Hepatitis B	105 (27.9)
Hepatitis C	51 (13.5)
Hepatitis B + C	7 (1.9)
Alcohol consumption	118 (31.3)
Non-alcoholic fatty liver disease	18 (4.8)
NA	78 (20.6)

TNM, Tumor-Node-Metastasis; AJCC, American Joint Committee on Cancer; AFP, alpha-fetoprotein; NA, not applicable.

survival (RFS) was defined as the interval between the date of surgery and the date of first recurrence or the date of the last follow-up. In the experiment, the gene expression value obtained from duplicated results for each gene and set the expression value of the patient using the mean value of the duplicated results. The experiment was performed once using the paired tissue of the patients. Comparison of the distributions between two groups were analyzed using the χ^2 test (or Fisher's exact test when the expected frequency in any group was <5) for categorical variables and by paired Student's t-test (or by Kolmogorov-Smirnov test when the expected frequency in any group was <5) for continuous variables. One-way ANOVA followed by Student Newman-Keuls post-hoc comparisons were used to compare ≥3 groups. P<0.05 was considered to indicate a statistically significant difference.

Results

mRNA expression of Hippo pathway genes in HCC. Table I exhibits the clinicopathological characteristics of patients included in the present study. The expression of Hippo pathway genes was examined in patients with HCC using the GEPIA database (Fig. 1A and Table II). Comparison of mRNA expression levels between HCC and normal control samples revealed that *TEAD2* expression was significantly higher in HCC tumor tissue compared with normal control samples. The mRNA expression levels of *YAP*, *TEAD4* and *VGLL4* were higher in HCC tissue compared with normal control tissue, although the differences were not significant (Fig. 1A and Table II). Specifically, the mRNA expression of *TEAD2* was significantly increased in histologic grades 3-4 samples compared with histologic grades 1-2 samples and the mRNA expression of *TEAD4* was significantly increased in histologic grades 3-4 samples compared with histologic grades 1 samples (Fig. 1B). The mRNA expression of *TEAD2* was significantly increased in patients with TNM stage III and IV compared with TNM stage I and II, and the mRNA expression of *VGLL4* was also significantly increased in TNM stage III and IV compared with

Table II. Genes regulating the Hippo pathway hepatocellular carcinoma. The gene alteration contains gene amplification, deep deletion, missense mutation (unknown significance), mRNA upregulation and truncating mutation (unknown significance) of each analysed gene on HCC tissues.

Symbol	Gene name	Chromosome location	Gene alteration (%)
YAP1	Yes-Associated Protein 1	11q22.1	7
TAZ	Tafazzin	Xq28	9
TEAD1	TEA Domain Transcription Factor 1	11p15.3	4
TEAD2	TEA Domain Transcription Factor 2	19q13.33	6
TEAD3	TEA Domain Transcription Factor 3	6p21.31	13
TEAD4	TEA Domain Transcription Factor 4	12p13.33	8
VGLL1	Vestigial Like Family Member 1	Xq26.3	3
VGLL2	Vestigial Like Family Member 2	6q22.1	3
VGLL3	Vestigial Like Family Member 3	3p12.1	4
VGLL4	Vestigial Like Family Member 4	3p25.2	6

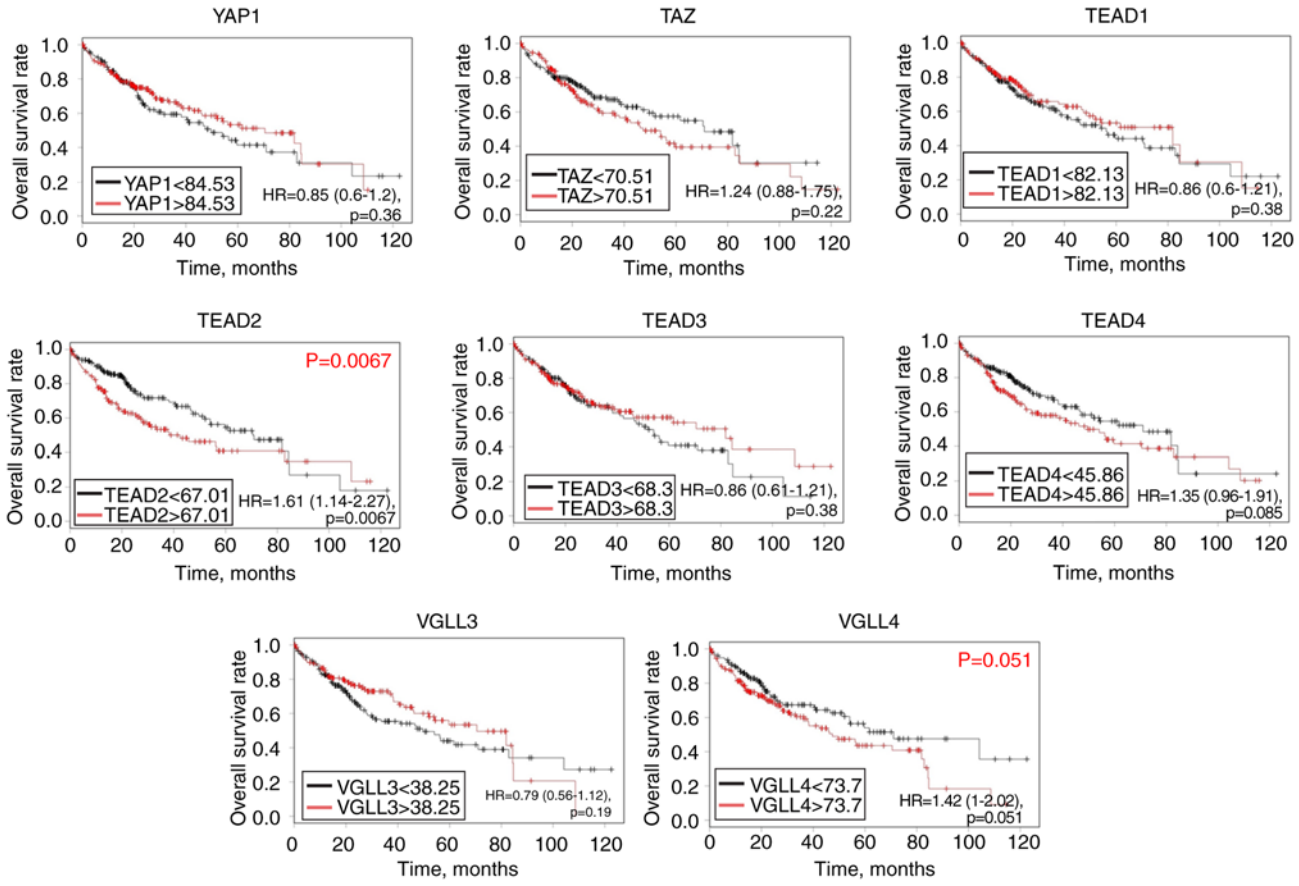

Figure 2. Survival analysis of Hippo pathway genes in hepatocellular carcinoma. Kaplan-Meier analysis of the association between mRNA expression of Hippo pathway genes and overall survival rate of patients. *TEAD*, transcriptional enhancer associated domain; *VGLL*, vestigial like family; *TAZ*, tafazzin; *YAP*, yes-associated protein.

in TNM stage I (Fig. 1C). Among the ten genes evaluated, *TEAD2* exhibited higher expression in HCC tumor tissue compared with normal control tissue, and this was significantly associated with histological grade or stage of HCC (Fig. 1B and C).

Poor prognosis of patients with HCC with high expression of TEAD2 and VGLL4. Based on the log-rank test and TCGA database, abundant mRNA expression of *TEAD2* [hazard ratio (HR), 1.61; 95% confidence interval (CI), 1.14-2.27; P=0.0067] was significantly associated with a poor OS rate in patients with HCC, and there was a tendency towards significance between *TEAD4* (HR, 1.35; 95% CI, 0.95-1.91; P=0.085) and *VGLL4* mRNA expression (HR, 1.42; 95% CI, 1-2.02; P=0.051), and a poor prognosis (Fig. 2). However, *YAP1*, *TAZ*, *TEAD1*, *TEAD3*

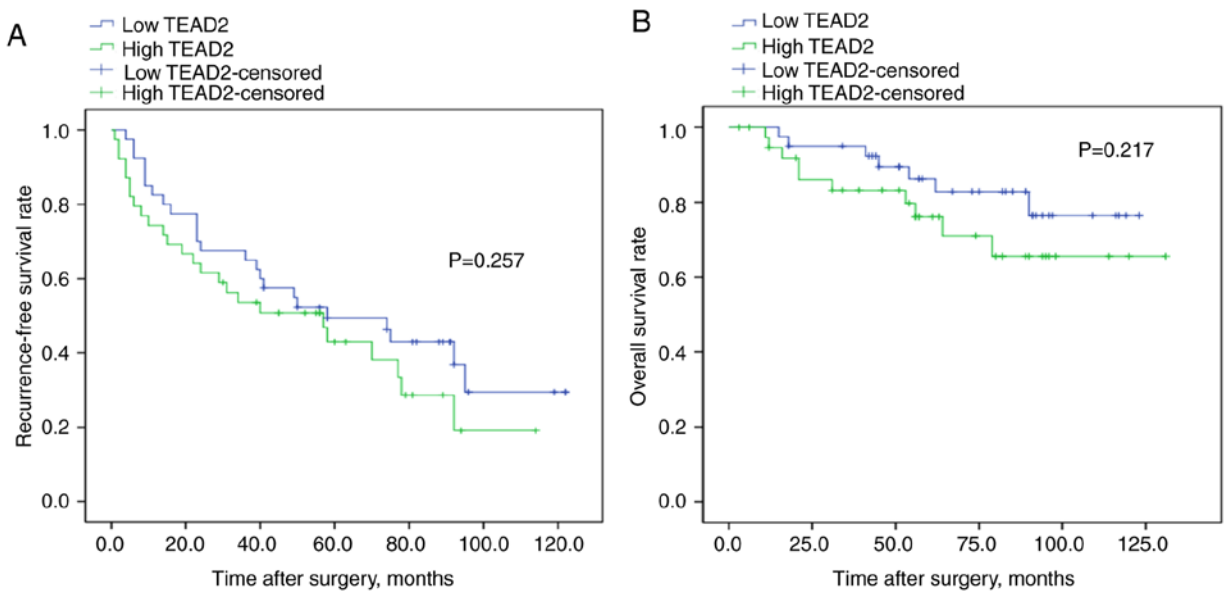

Figure 3. OS rate and RFS rate analysis of patients with HCC based on *TEAD2* mRNA expression. Groups were stratified according to median expression level and were converted to categorical variables accordingly. Survival curves were compared by the log-rank test. (A) Kaplan-Meier analysis of RFS rate. Patients who did not exhibit recurrence at either the date of death or the date of last follow-up were censored. (B) Kaplan-Meier analysis of OS rate. Patients who were still alive at the end of the follow-up period were censored. HCC, hepatocellular carcinoma; OS, overall survival; RFS, recurrence-free survival; *TEAD*, transcriptional enhancer associated domain.

and *VGLL3* mRNA levels were not significantly associated with the prognosis of patients with HCC. To validate the association between the mRNA expression of *TEAD2* and prognosis, RT-qPCR was performed and a log-rank test was conducted on 79 clinical HCC tissues samples. The clinicopathological characteristics of the patients with HCC are summarized in Table SII. Although the results were not significant, there was a tendency for a less favorable prognosis in patients with increased *TEAD2* mRNA expression compared with those with decreased *TEAD2* mRNA expression, affecting both in recurrence-free and overall survival rate (P=0.257, P=0.217, respectively; Fig. 3).

GeneNeighbors analysis of TEAD2 and VGLL4. The present data revealed that *TEAD2* and *VGLL4* are closely associated with a poor prognosis in overall survival rate analysis using TCGA database, and that *TEAD2* is associated with a poor recurrence-free survival rate according to the current clinical sample-based analyses (Figs. 2 and 3). Therefore, GeneNeighbors analysis was performed to determine the effect of *TEAD2* and *VGLL4* on the survival of patients with HCC. The 100 genes most strongly associated with *TEAD2* and *VGLL4* were identified via GeneNeighbors analysis (Fig. 4A and B) and classified using the DAVID (https://david.ncifcrf.gov/). The selected genes were divided into three categories of biological processes, molecular functions and cellular components according to gene ontology (GO) terms. In GeneNeighbors analysis of *TEAD2*, highly expressed genes in HCC were primarily associated with 'positive regulation of small GTPase', 'vasculogenesis', 'positive regulation of transcript', 'regulation of cell migration', 'regulation of VEGF signaling', 'actin cytoskeleton organization', 'peptidyl-arginine methylation', 'B cell apoptotic process' and 'positive regulation of vascular permeability' in biological processes (Fig. 4A). For cellular components, highly expressed genes in HCC were strongly associated with 'focal adhesion', 'cytoplasm', 'cytosol' and 'RNA polymerase II transcription factor'. For molecular functions, highly expressed genes in HCC were mainly associated with 'transcription factor binding', 'protein heterodimerization activity', 'protein-arginine methyltransferase activity', 'profilin binding' and 'protein binding' (Fig. 4A).

In GeneNeighbors of *VGLL4*, highly expressed genes in HCC were primarily associated with 'integrin-mediated signaling', 'cell adhesion mediated by integrin', 'extracellular matrix (ECM) organization', 'cell-matrix adhesion', 'cell-cell adhesion', 'mesodermal cell differentiation', 'cell motility', 'focal adhesion assembly', 'cell adhesion' and 'regulation of cell migration in biological processes' (Fig. 4B).

For cellular components, highly expressed genes in HCC were associated with 'focal adhesion', 'cell-cell adherent junction', 'integrin complex', 'extracellular exosome' and 'ruffle'. For molecular functions, highly expressed genes in HCC were mainly associated with 'cadherin binding involved in cell-cell adhesion', 'integrin binding', 'laminin binding', 'protein binding' and 'protein kinase C binding' (Fig. 4B).

ClassNeighbors analysis of TEAD2 and VGLL4. Analysis by ClassNeighbors yielded two classes of HCC samples: Class A included the highest 10% expressed *TEAD2* and *VGLL4*-upregulated HCC samples and class B included the lowest 10% expressed *TEAD2* and *VGLL4*-downregulated HCC samples (Fig. 4C and D). The 150 most highly expressed genes in classes A and B were identified from 20,502 probe sets. These genes were divided into three categories; biological processes, molecular functions and cellular components based on gene ontology (GO) terms (Fig. 4C and D). In ClassNeighbors of *TEAD2*, highly expressed genes in class A were primarily associated with 'drug transmembrane

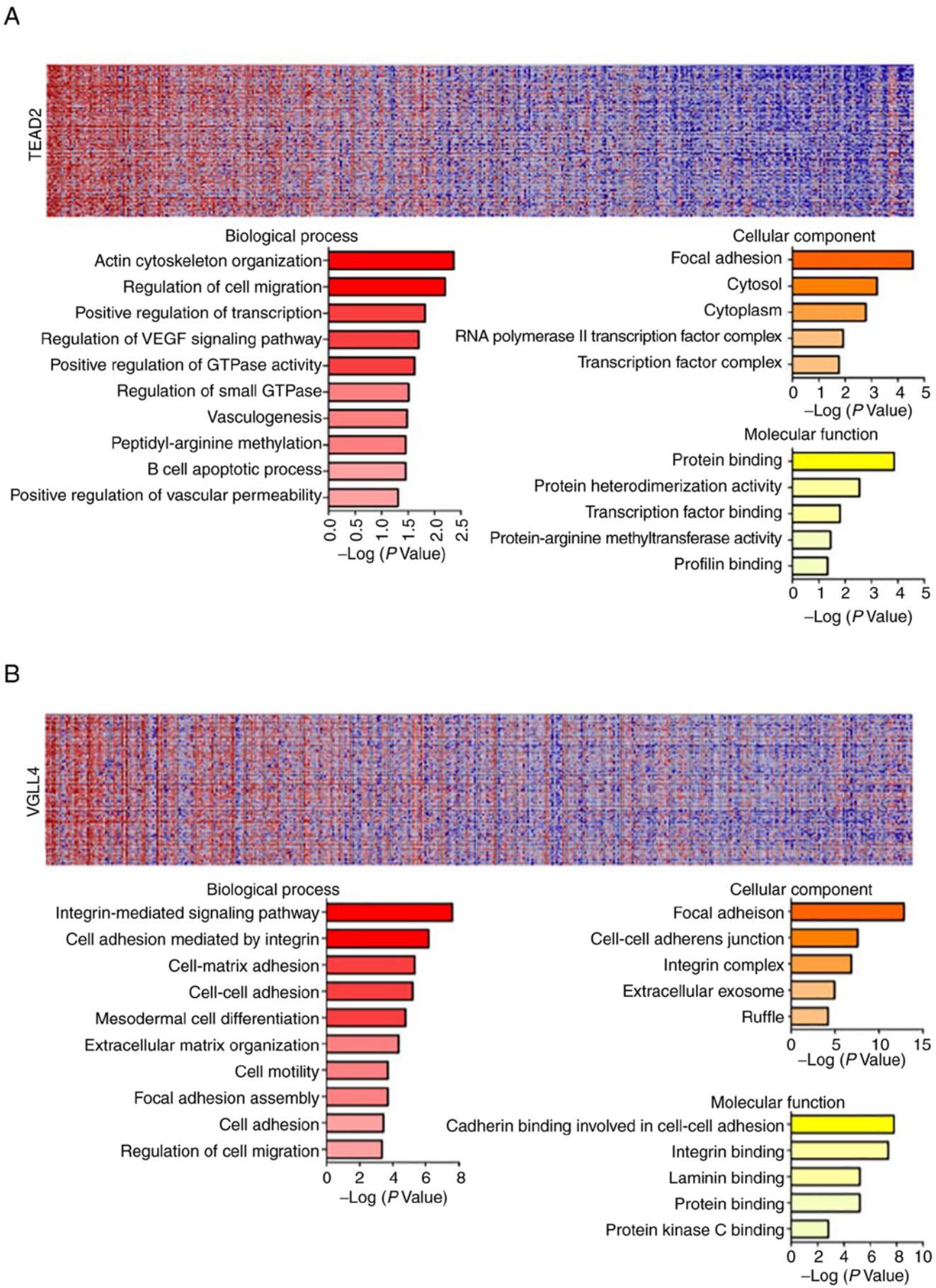

Figure 4. GeneNeighbors analysis and ClassNeighbors analysis of *TEAD2* and *VGLL4* in 377 HCC samples. Class A comprised the highest 10% *TEAD2* and *VGLL4*-upregulated HCC samples and class B included the 10% of HCC samples with the lowest expression levels of *TEAD2* and *VGLL4*. (A) GeneNeighbors analysis of *TEAD2*. (B) GeneNeighbors analysis of VGLL4.

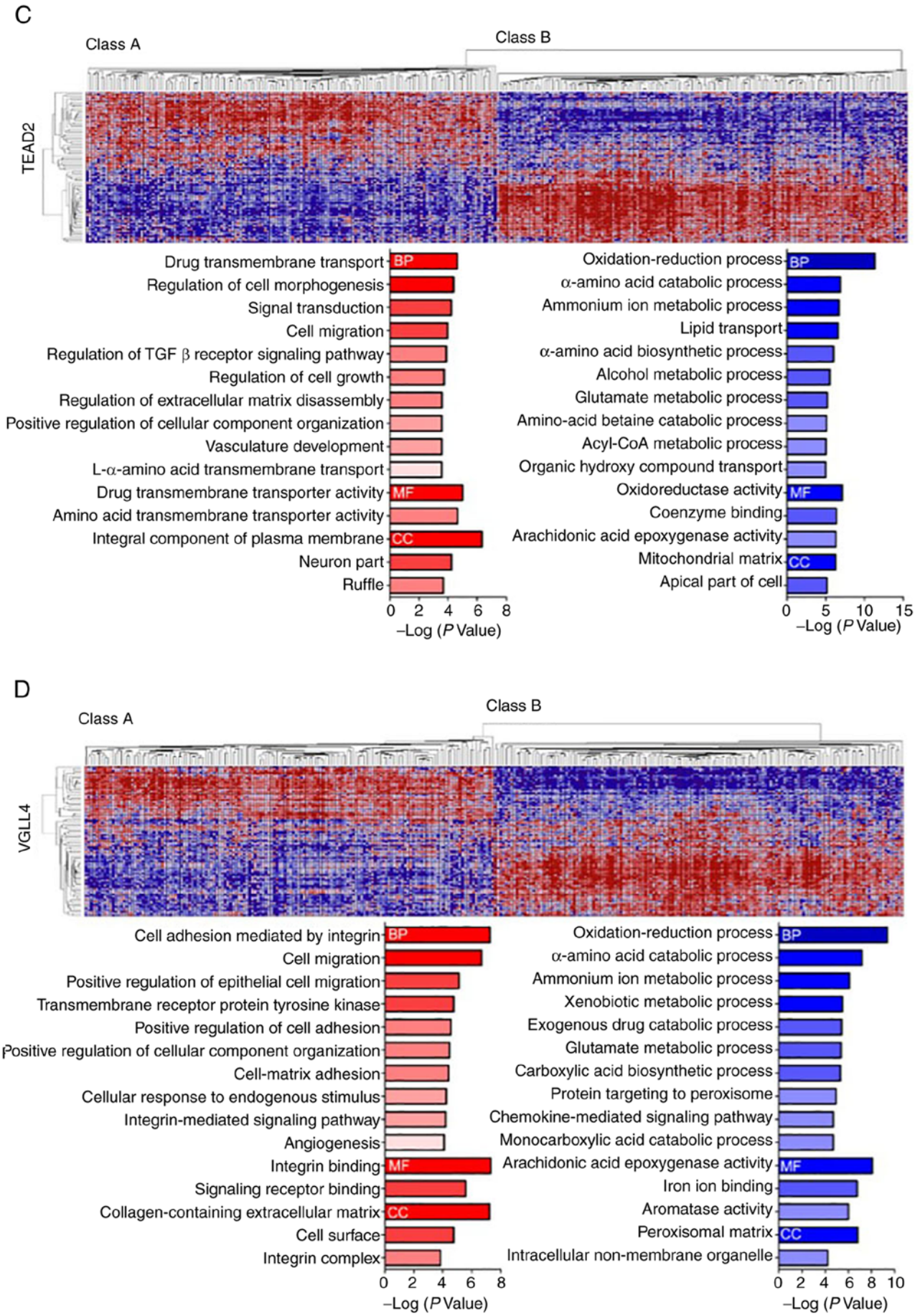

Figure 4. Continued. (C) ClassNeighbors analysis of *TEAD2*. (D) ClassNeighbors analysis of *VGLL4*. HCC, hepatocellular carcinoma; *TEAD2*, transcriptional enhancer associated domain 2; *VGLL4*, vestigial like family member 4.

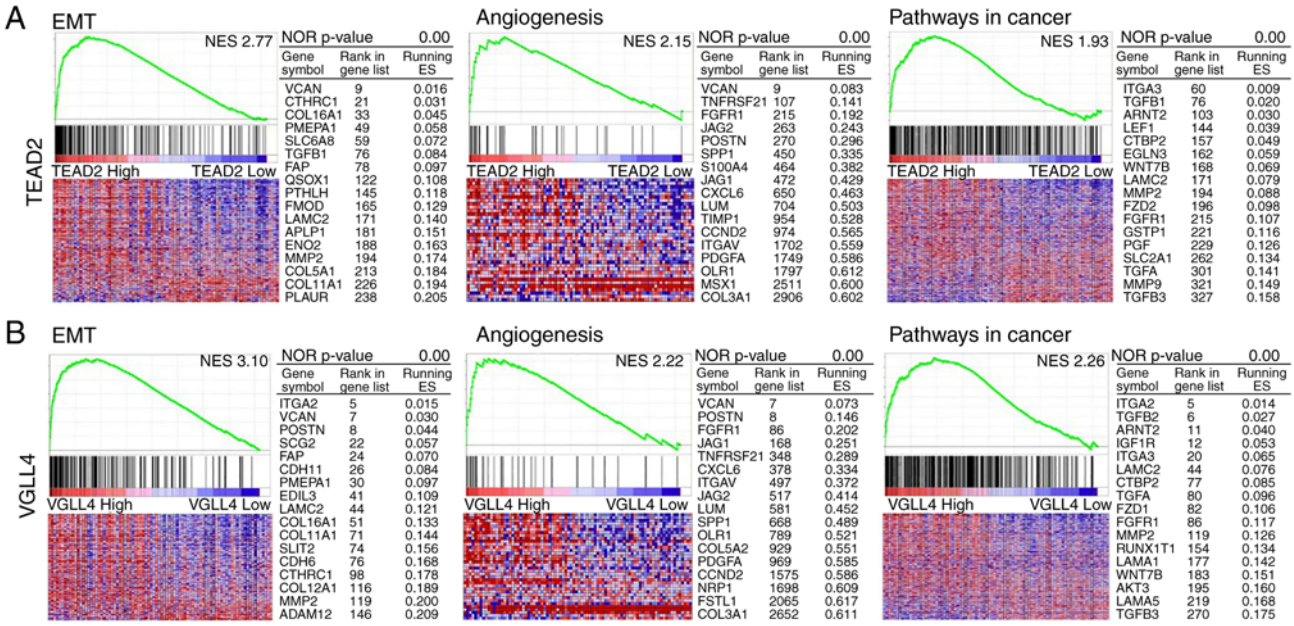

Figure 5. Gene Set Enrichment Analysis (GSEA) results for HCC with high *TEAD2* and *VGLL4* expression. Representative GSEA data with P-values are exhibited for (A) *TEAD2* and (B) *VGLL4*. HCC, hepatocellular carcinoma; *TEAD2*, transcriptional enhancer associated domain 2; *VGLL4*, vestigial like family member 4; EMT, epithelial-mesenchymal transition; NOM, nominal; ES, enrichment score; NES, normalized enrichment score.

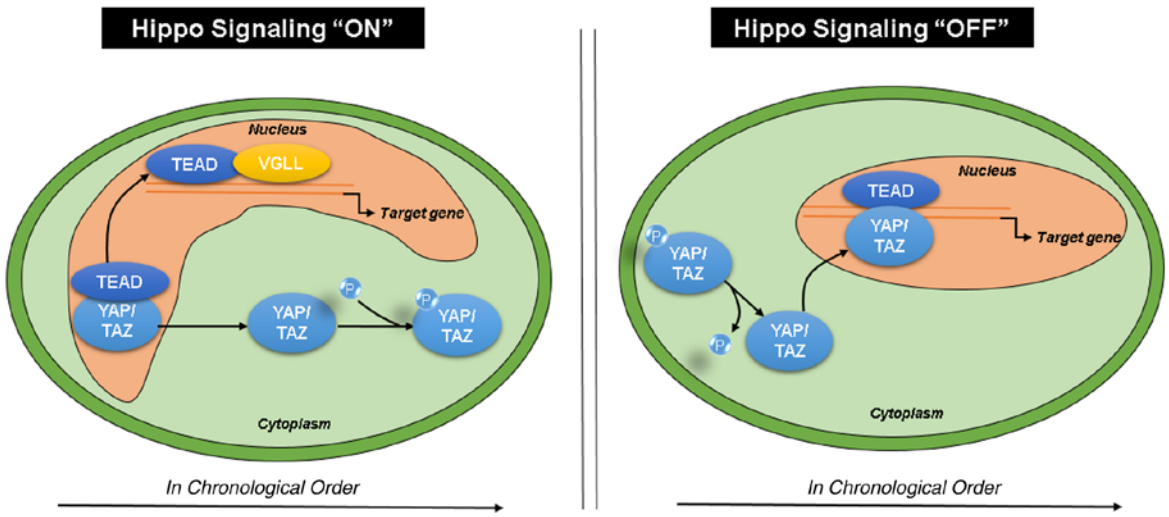

Figure 6. Hippo signaling pathway. Upon activation of Hippo signaling, *YAP/TAZ* are phosphorylated and sequestered in the cytoplasm, whereas *TEAD* and *VGLL* bind in the nucleus. When Hippo signaling is deactivated, *YAP/TAZ* are dephosphorylated and translocate to the nucleus to bind TEAD, activating the downstream transcription of target genes. *TEAD*, transcriptional enhancer associated domain; *VGLL*, vestigial like family; *HCC*, hepatocellular carcinoma; *TEAD2*, transcriptional enhancer associated domain 2; *VGLL4*, vestigial like family member 4; *YAP*, yes-associated protein.

transport', 'regulation of cell morphogenesis', 'signal transduction', 'cell migration', 'regulation of transforming growth factor beta receptor', 'cell growth', 'ECM disassembly', 'cellular component organization', 'vasculature development' and 'L-alpha-amino acid transmembrane transport' in biological processes; 'integral component of plasma membrane', 'neuron part' and 'ruffle' in cellular components; and 'drug transmembrane transporter activity' and 'amino acid transmembrane transporter activity' in molecular functions. Highly expressed genes in class B were closely related to 'oxidation-reduction', 'alpha-amino acid catabolic process', 'ammonium ion metabolic process', 'alcohol metabolic process', 'glutamate metabolic process', 'amino-acid betaine catabolic process', 'acyl-CoA metabolic process', 'alpha-amino acid biosynthetic process', 'lipid transport' and 'organic hydroxyl compound transport' in biological process; oxidoreductase activity and coenzyme binding in molecular function; mitochondrial matrix and apical part of cell in cellular components (Fig. 4C).

In ClassNeighbors of *VGLL4*, highly expressed genes in class A were closely associated with 'cell adhesion mediated by integrin', 'cell migration', 'epithelial cell migration', 'tyrosine kinase', 'cell adhesion', 'cellular compartment organization', 'cell-matrix adhesion', 'cellular response to endogenous stimulus', 'integrin-mediated signaling pathway' and 'angiogenesis' in biological processes; 'collagen-containing ECM', 'cell

surface' and 'integrin complex' in cellular components; and 'integrin binding' and 'signaling receptor binding' in molecular functions. Highly expressed genes in class B were closely associated with 'oxidation-reduction', 'xenobiotic metabolic process', 'ammonium ion metabolic process', 'glutamate metabolic process', 'carboxylic acid biosynthetic process', 'protein targeting to peroxisome', 'chemokine-mediated signaling', 'monocarboxylic acid catabolic process', 'exogenous drug catabolic process' and 'alpha-amino acid catabolic process' in biological process; 'arachidonic acid epoxygenase activity', 'iron ion binding' and 'aromatase activity' in molecular function; and 'peroxisomal matrix' and 'intracellular non-membrane organelle' in cellular components (Fig. 4D).

GSEA for TEAD2 and VGLL4. Using GSEA of mRNAs associated with hallmark pathways and KEGG pathways (Fig. 5), the top 10% of HCC samples with upregulated *TEAD2* and *VGLL4* and bottom 10% of samples with downregulated *TEAD2* and *VGLL4* were investigated. In the hallmarks pathways, high mRNA expression of *TEAD2* and *VGLL4* was strongly associated with genes related to 'epithelial-mesenchymal transition (EMT)' and 'angiogenesis'. In KEGG pathway analysis, increased mRNA expression of *TEAD2* and *VGLL4* was closely associated with genes involved in cancer pathways (Fig. 5A and B).

Discussion

TEAD protein serves an essential role in the early stage of the developmental process and cellular ageing (21-23). It has been demonstrated that specific *TEAD* proteins influence cancer as activators of transcription of pro-growth genes (24). In previous studies, *TEAD* was revealed to be upregulated in gastric cancer (16), breast cancer (11,25) and renal cell carcinoma (15). In prostate cancer, an increased *TEAD1* level was correlated with unfavorable clinical outcomes (12), and in colorectal cancer and gastric cancer an increased *TEAD4* expression level was associated with poor outcomes (13,14,26). Regarding HCC, expression of *YAP-TEAD* was reported to be upregulated, with most studies examining the expression of *TEAD1* and *TEAD4 (*26-28). According to previous cellular-based assays, inhibition of *LATS2*, an upstream regulator of *YAP/TAZ* in the Hippo signaling pathway, induces nuclear localization of *YAP1* and *YAP1/TEAD2* interactions, which in turn promotes HCC progression (26). However, this study only explained the molecular mechanism underlying the action of *YAP1/TEAD2* via *LATS2* but did not reveal the clinical significance of *TEAD2* in HCC. In the present study, based on analyses of TCGA database and clinical samples, as the HCC stage and histologic grade of tumor increased, *TEAD2* expression adversely affected overall survival rate and recurrence-free survival rate.

In TCGA data analysis, increased mRNA expression of *TEAD2* was significantly associated with a less favorable prognosis in patients with HCC in overall survival rate, and the potential of *TEAD2* as a prognostic marker was predicted. Moreover, clinical data analysis revealed a similar tendency as the results of TCGA data analysis. However, additional studies are needed to confirm the association between mRNA expression of *TEAD2* and prognosis.

To investigate the function and mechanism of *TEAD2* in HCC, bioinformatics analysis was performed. GeneNeighbors analyses demonstrated that 'angiogenesis-associated genes' (VEGF, vasculogenesis and vascular permeability), 'regulation of cell migration' and 'actin cytoskeleton organization' were highly correlated with *TEAD2* in HCC. ClassNeighbors analysis classified *TEAD2*-expressing HCC into Class A, which expresses genes associated with 'signal transduction', 'cell migration', 'ECM disassembly', 'cell morphogenesis', 'transforming growth factor β signaling' and 'vasculature development'. It was also classified into Class B, which expresses genes associated with metabolic pathways. Class A genes enhance EMT and angiogenesis. GSEA was performed on *TEAD2*, which was significantly associated with the prognosis of patients with HCC. EMT, angiogenesis and cancer pathway-related genes were strongly associated with high mRNA expression of *TEAD2*. During EMT, cancer cells lose their apical basal polarity and cell-cell adhesions and develop a more invasive phenotype (29). Diepenbruck *et al* (30) reported that increased *TEAD2* transcriptional activity promotes the induction of EMT in breast cancer cells and mammary gland epithelial cells. Moreover, angiogenesis also serves an important role in tumor growth and metastasis (31,32). For a tumor to grow above a certain size the development of new blood vessels is required, and tumors induce angiogenesis by secreting various growth factors including VEGF (33,34).

In the current study, the mRNA expression of *VGLL4* was higher in HCC compared with normal control samples although the difference was not significant, and there was a tendency towards significance between *VGLL4* expression and a poor prognosis (P=0.051). GeneNeighbors analyses demonstrated that 'ECM organization', 'integrin-mediated signaling pathway' and 'cell adhesion' associated genes were highly correlated with *VGLL4* expression in HCC. ClassNeighbors analysis classified *VGLL4*-expressing HCC into Class A, which includes genes associated with 'integrin', 'cell adhesion' and 'angiogenesis', and Class B, which includes genes associated with 'metabolic pathways'. Class A genes enhance EMT and angiogenesis. Remodeling of the ECM and changes in cell-ECM interactions are necessary for the induction and progression of EMT (35). Integrin complexes allow cells to receive signals from ECM proteins via interactions with signal transduction mediators. Some integrins play essential roles in EMT progression (35). GSEA revealed that EMT-, angiogenesis- and cancer pathway-associated genes were strongly associated with high mRNA expression of *VGLL4*. Previous studies have reported that *VGLL4* suppresses multiple cancer types, such as gastric, lung, colon and breast carcinoma by suppressing the WNT and Hippo signaling pathways (36-40). Xie *et al* (41) demonstrated that *VGLL4* causes G_2/M phase arrest in HCC cells, and Shu *et al* (42) reported that the *YAP/VGLL4* ratio is higher in patients with HCC, which is associate with tumor progression and a poor prognosis (41,42). By contrast to previous studies, the present study reported tendency towards significance between increased *VGLL4* mRNA expression and the poor prognosis of patients with HCC. Unlike other cancer types, unknown mechanisms specific to HCC may have contributed to this discrepancy, and further studies are needed.

According to previous studies, when Hippo signaling is activated, *YAP/TAZ* are phosphorylated and sequestered in the cytoplasm, whereas *TEAD* and *VGLL* bind in the nucleus. When Hippo signaling is deactivated, *YAP/TAZ* are dephosphorylated and translocate to the nucleus to bind *TEAD*, activating the downstream transcription of target genes (Fig. 6) (43,44). In the present study, mRNA expression and poor prognosis on overall survival rate for both *TEAD2* and *VGLL4* exhibited similar tendencies in patients with HCC. Generally, when Hippo signaling pathway is 'On', *TEAD* and *VGLL* maintain their binding status; therefore, the similar tendency between *TEAD2* and *VGLL4* is likely related to our findings. However, further mechanistic studies are needed to validate this prediction. The present analysis did not reveal that *TEAD2* and *VGLL4* significantly influence each other. However, *TEAD2* and *VGLL4* expression were significantly correlated in TCGA HCC cohorts ($P<0.001$) (data not shown). Therefore, further experimental studies are required to determine which factors are the most significant.

The present study demonstrated that an increase in expression of *TEAD4* was associated with an increase in histological grade; however, survival tended to decrease with increasing *TEAD4* expression. Thus, in addition to histological grades, other factors may influence patient survival.

In conclusion, the present study was the first to evaluate the association between *TEAD2* and the survival of patients with HCC. The current data revealed that a high expression level of *TEAD2* in resected tumor tissue from patients with HCC was positively associated with a poor prognosis. Therefore, *TEAD2* may represent a useful prognostic marker and therapeutic target for HCC.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Datasets were both generated and presented in the present study (patient tissue analysis) and retrieved from online databases (TCGA). TCGA-LIHC dataset is available in GDC data portal (https://portal.gdc.cancer.gov/) and Firebrowse (http://firebrowse.org/?cohort=LIHC&download_dialog=true).

Authors' contributions

BSL and HSE designed the study and conducted critical revision of the manuscript. JSJ, SYC, WSR, HSE, JSK, SHKang, ESL, HSM, SHKim and JKS conducted acquisition, analysis and interpretation of the data. BSL carried out supervision of the analysis. JSJ and SYC wrote the manuscript. ISK verified and contributed statistical analysis. All authors read and approved the final 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

This research was approved by the Institutional Review Board of Chungnam National University Hospital (approval no. CNUH 2017-05-013).

Patient consent of publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Yang LY, Fang F, Ou DP, Wu W, Zeng ZJ and Wu F: Solitary large hepatocellular carcinoma: A specific subtype of hepatocellular carcinoma with good outcome after hepatic resection. Ann Surg 249: 118-123, 2009.
3. Aravalli RN, Cressman EN and Steer CJ: Cellular and molecular mechanisms of hepatocellular carcinoma: An update. Arch Toxicol 87: 227-247, 2013.
4. Farazi PA and DePinho RA: Hepatocellular carcinoma pathogenesis: From genes to environment. Nat Rev Cancer 6: 674-687, 2006.
5. Hong W and Guan KL: The YAP and TAZ transcription co-activators: Key downstream effectors of the mammalian Hippo pathway. Semin Cell Dev Biol 23: 785-793, 2012.
6. Zhao B, Li L, Lu Q, Wang LH, Liu CY, Lei Q and Guan KL: Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. Genes Dev 25: 51-63, 2011.
7. Chen HH, Maeda T, Mullett SJ and Stewart AF: Transcription cofactor Vgl-2 is required for skeletal muscle differentiation. Genesis 39: 273-279, 2004.
8. Chen HH, Mullett SJ and Stewart AF: Vgl-4, a novel member of the vestigial-like family of transcription cofactors, regulates alpha1-adrenergic activation of gene expression in cardiac myocytes. J Biol Chem 279: 30800-30806, 2004.
9. Günther S, Mielcarek M, Kruger M and Braun T: VITO-1 is an essential cofactor of TEF1-dependent muscle-specific gene regulation. Nucleic Acids Res 32: 791-802, 2004.
10. Koontz LM, Liu-Chittenden Y, Yin F, Zheng Y, Yu J, Huang B, Chen Q, Wu S and Pan D: The Hippo effector Yorkie controls normal tissue growth by antagonizing scalloped-mediated default repression. Dev Cell 25: 388-401, 2013.
11. Lamar JM, Stern P, Liu H, Schindler JW, Jiang ZG and Hynes RO: The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. Proc Natl Acad Sci USA 109: E2441-E2450, 2012.
12. Knight JF, Shepherd CJ, Rizzo S, Brewer D, Jhavar S, Dodson AR, Cooper CS, Eeles R, Falconer A, Kovacs G, *et al*: TEAD1 and c-Cbl are novel prostate basal cell markers that correlate with poor clinical outcome in prostate cancer. Br J Cancer 99: 1849-1858, 2008.
13. Lim B, Park JL, Kim HJ, Park YK, Kim JH, Sohn HA, Noh SM, Song KS, Kim WH, Kim YS, *et al*: Integrative genomics analysis reveals the multilevel dysregulation and oncogenic characteristics of TEAD4 in gastric cancer. Carcinogenesis 35: 1020-1027, 2014.
14. Liu Y, Wang G, Yang Y, Mei Z, Liang Z, Cui A, Wu T, Liu CY and Cui L: Increased TEAD4 expression and nuclear localization in colorectal cancer promote epithelial-mesenchymal transition and metastasis in a YAP-independent manner. Oncogene 35: 2789-2800, 2016.
15. Schütte U, Bisht S, Heukamp LC, Kebschull M, Florin A, Haarmann J, Hoffmann P, Bendas G, Buettner R, Brossart P and Feldmann G: Hippo signaling mediates proliferation, invasiveness, and metastatic potential of clear cell renal cell carcinoma. Transl Oncol 7: 309-321, 2014.
16. Zhou GX, Li XY, Zhang Q, Zhao K, Zhang CP, Xue CH, Yang K and Tian ZB: Effects of the hippo signaling pathway in human gastric cancer. Asian Pac J Cancer Prev 14: 5199-5205, 2013.

17. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC and Lempicki RA: The DAVID Gene Functional Classification Tool: A novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 8: R183, 2007.
18. Edmondson HA and Steiner PE: Primary carcinoma of the liver: A study of 100 cases among 48,900 necropsies. Cancer 7: 462-503, 1954.
19. Ghassan K A-A and Timothy M: Pawlik, Junichi Shindoh and Jean-Nicolas Vauthey editors: AJCC Cancer Staging Manual. (Eighth ed). Chicago, IL, Springer, 287-293, 2017.
20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
21. Chen Z, Friedrich GA and Soriano P: Transcriptional enhancer factor 1 disruption by a retroviral gene trap leads to heart defects and embryonic lethality in mice. Genes Dev 8: 2293-2301, 1994.
22. Sawada A, Kiyonari H, Ukita K, Nishioka N, Imuta Y and Sasaki H: Redundant roles of Tead1 and Tead2 in notochord development and the regulation of cell proliferation and survival. Mol Cell Biol 28: 3177-3189, 2008.
23. Kaneko KJ, Kohn MJ, Liu C and DePamphilis ML: Transcription factor TEAD2 is involved in neural tube closure. Genesis 45: 577-587, 2007.
24. Holden JK and Cunningham CN: Targeting the Hippo pathway and cancer through the TEAD family of transcription factors. Cancers (Basel) 10: pii: E81, 2018.
25. Wang C, Nie Z, Zhou Z, Zhang H, Liu R, Wu J, Qin J, Ma Y, Chen L, Li S, *et al*: The interplay between TEAD4 and KLF5 promotes breast cancer partially through inhibiting the transcription of p27Kip1. Oncotarget 6: 17685-17697, 2015.
26. Guo C, Wang X and Liang L: LATS2-mediated YAP1 phosphorylation is involved in HCC tumorigenesis. Int J Clin Exp Pathol 8: 1690-1697, 2015.
27. Mao B, Hu F, Cheng J, Wang P, Xu M, Yuan F, Meng S, Wang Y, Yuan Z and Bi W: SIRT1 regulates YAP2-mediated cell proliferation and chemoresistance in hepatocellular carcinoma. Oncogene 33: 1468-1474, 2014.
28. Bai N, Zhang C, Liang N, Zhang Z, Chang A, Yin J, Li Z, Luo N, Tan X, Luo N, *et al*: Yes-Associated Protein (YAP) increases chemosensitivity of hepatocellular carcinoma cells by modulation of p53. Cancer Biol Ther 14: 511-520, 2013.
29. Sánchez-Tilló E, Liu Y, de Barrios O, Siles L, Fanlo L, Cuatrecasas M, Darling DS, Dean DC, Castells A and Postigo A: EMT-activating transcription factors in cancer: Beyond EMT and tumor invasiveness. Cell Mol Life Sci 69: 3429-3456, 2012.
30. Diepenbruck M, Waldmeier L, Ivanek R, Berninger P, Arnold P, van Nimwegen E and Christofori G: Tead2 expression levels control the subcellular distribution of Yap and Taz, zyxin expression and epithelial-mesenchymal transition. J Cell Sci 127: 1523-1536, 2014.
31. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW and Terstappen LW: Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 10: 6897-6904, 2004.
32. Folkman J: Tumor angiogenesis: Therapeutic implications. N Engl J Med 285: 1182-1186, 1971.
33. McDougall SR, Anderson AR and Chaplain MA: Mathematical modelling of dynamic adaptive tumour-induced angiogenesis: Clinical implications and therapeutic targeting strategies. J Theor Biol 241: 564-859, 2006.
34. Hicklin DJ and Ellis LM: Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 23: 1011-1027, 2005.
35. Lamouille S, Xu J and Derynck R: Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15: 178-196, 2014.
36. Zhang W, Gao Y, Li P, Shi Z, Guo T, Li F, Han X, Feng Y, Zheng C, Wang Z, *et al*: VGLL4 functions as a new tumor suppressor in lung cancer by negatively regulating the YAP-TEAD transcriptional complex. Cell Res 24: 331-343, 2014.
37. Jiao S, Li C, Hao Q, Miao H, Zhang L, Li L and Zhou Z: VGLL4 targets a TCF4-TEAD4 complex to coregulate Wnt and Hippo signalling in colorectal cancer. Nat Commun 8: 14058, 2017.
38. Jin HS, Park HS, Shin JH, Kim DH, Jun SH, Lee CJ and Lee TH: A novel inhibitor of apoptosis protein (IAP)-interacting protein, Vestigial-like (Vgl)-4, counteracts apoptosis-inhibitory function of IAPs by nuclear sequestration. Biochem Biophys Res Commun 412: 454-459, 2011.
39. Jiao S, Wang H, Shi Z, Dong A, Zhang W, Song X, He F, Wang Y, Zhang Z, Wang W, *et al*: A peptide mimicking VGLL4 function acts as a YAP antagonist therapy against gastric cancer. Cancer Cell 25: 166-180, 2014.
40. Zhang Y, Shen H, Withers HG, Yang N, Denson KE, Mussell AL, Truskinovsky A, Fan Q, Gelman IH, Frangou C and Zhang J: VGLL4 Selectively represses YAP-dependent gene induction and tumorigenic phenotypes in breast cancer. Sci Rep 7: 6190, 2017.
41. Shu B, Zhai M, Miao X, He C, Deng C, Fang Y, Luo M, Liu L and Liu S: Serotonin and YAP/VGLL4 balance correlated with progression and poor prognosis of hepatocellular carcinoma. Sci Rep 8: 9739, 2018.
42. Xie W, Hao J, Zhang K, Fang X and Liu X: Adenovirus armed with VGLL4 selectively kills hepatocellular carcinoma with G2/M phase arrest and apoptosis promotion. Biochem Biophys Res Commun 503: 2758-2763, 2018.
43. Zhou Y, Huang T, Cheng AS, Yu J, Kang W and To KF: The TEAD family and its oncogenic role in promoting tumorigenesis. Int J Mol Sci 17: pii: E138, 2016.
44. Lin KC, Park HW and Guan KL: Regulation of the Hippo pathway transcription factor TEAD. Trends Biochem Sci 42: 862-872, 2017.