Abstract. The long non-coding RNA (lncRNA) PVT1 plays vital roles in the tumorigenesis and development of various types of cancer. However, the potential expression profiling, functions and pathways of PVT1 in HCC remain unknown. PVT1 was knocked down in SMMC-7721 cells, and a miRNA microarray analysis was performed to detect the differentially expressed miRNAs. Twelve target prediction algorithms were used to predict the underlying targets of these differentially expressed miRNAs. Bioinformatics analysis was performed to explore the underlying functions, pathways and networks of the targeted genes. Furthermore, the relationship between PVT1 and the clinical parameters in HCC was confirmed based on the original data in the TCGA database. Among the differentially expressed miRNAs, the top two upregulated and downregulated miRNAs were selected for further analysis based on the false discovery rate (FDR), fold-change (FC) and P-values. Based on the TCGA database, PVT1 was obviously highly expressed in HCC, and a statistically higher PVT1 expression was found for sex (male), ethnicity (Asian) and pathological grade (G3+G4) compared to the control groups (P<0.05). Furthermore, Gene Ontology (GO) analysis revealed that the target genes were involved in complex cellular pathways, such as the macromolecule biosynthetic process, compound metabolic process, and transcription. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that the MAPK and Wnt signaling pathways may be correlated with the regulation of the four candidate miRNAs. The results therefore provide significant information on the differentially expressed miRNAs associated with PVT1 in HCC, and we hypothesized that PVT1 may play vital roles in HCC by regulating different miRNAs or target gene expression (particularly MAPK8) via the MAPK or Wnt signaling pathways. Thus, further investigation of the molecular mechanism of PVT1 in HCC is needed.

Introduction

Hepatocellular carcinoma (HCC) remains one of the main malignancies worldwide with a poor 5-year survival rate (1-4). Generally, patients are diagnosed with HCC at an advanced stage, and a large number of HCC patients show intrahepatic metastasis and postoperative recurrence (5). In the Chinese population, the development of HCC has been associated with the hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in most patients (6). The long-term symptoms of inflammation, chronic hepatitis and cirrhosis contribute to the virus-initiated tumorigenic process (7,8). For the treatment of HCC, liver transplantation or tumor resection is always the most effective treatment. Furthermore, the high rate of metastasis or postsurgical recurrence remains an obstacle to a better prognosis of HCC patients (9,10). Thus, it is imperative to explore the mechanism of HCC, which may lead to novel insights for the diagnosis and treatment of HCC patients.
Long non-coding RNAs (IncRNAs) include the recently identified class of non-protein coding RNA transcripts of 200 nucleotides to 100 kb in length (11-13). Accumulating evidence has demonstrated that IncRNAs may contribute to various biological processes, including proliferation, apoptosis, invasion and metastasis (14-16). However, the particular mechanisms of many IncRNAs remain vague. IncRNA PVT1 is located on chromosomal region 8q24, which is a well-known cancer-related region (17). Previous studies have confirmed that the overexpression of PVT1 accelerates the development and progression of cancer and reduces the chemosensitivity of cancer patients. Although, compared with normal liver tissues, PVT1 showed a high expression in HCC, improved proliferation and predicted recurrence, the precise functions and mechanism of PVT1 in HCC remain to be elucidated (18-20).

miRNAs refer to small non-coding RNAs with nearly 20 nucleotides. Recent studies have confirmed that IncRNAs can affect HCC via combining the expression of miRNAs (21,22). For example, Liu et al (21) found that IncRNA FTX inhibited the proliferation and metastasis of HCC by binding to miR-374a. Zhu et al (22) revealed that IncRNA LINC0052 inhibited the invasion and migration of HCC by binding to miR-452-5p. Therefore, it is of great significance to further explore the miRNA expression profile associated with IncRNA in HCC in order to identify novel therapeutic strategies.

In the present study, we validated the differential PVT1 expression in normal liver and HCC. Furthermore, we combined the miRNA expression profile after silencing PVT1 expression and miRNA target prediction algorithms to explore the underlying target genes related to PVT1 in HCC. Bioinformatics analysis, involving Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), protein-protein interactions (PPIs) and network analyses, was utilized to explore the underlying functions, pathways and networks of the target genes (23-26). Furthermore, the relationship between PVT1 and the clinical parameters in HCC was confirmed based on the original data in the TCGA database. A flow chart of the present study is shown in Fig. 1.

Materials and methods

Cell culture and siRNA transfection. Human HCC cells (SMMC-7721) were obtained from the American Type Culture Collection (ATCC), and the SMMC-7721 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) at 37°C in a humidified incubator with 5% CO2. The Lenti-siRNA vector of PVT1 was produced by GeneChem (Shanghai, China) (sense, 5’-CCCAACAGGAGGACACGUUTT-3’ and antisense, 5’-AAGCGUCCUCCUGUGGGTT-3’). siRNA vectors of PVT1 were transfected into HCC cells according to the manufacturer’s protocol.

miRNA microarray analysis. The sample analysis and miRNA microarray hybridization were completed by Kangchen Bio-tech (Shanghai, China). Briefly, miRNA labeling was performed using the miRCURY™ Array Power Labeling kit (cat. no. 208032-A; Exiqon, Vedbaek, Denmark). Then, the labeled sample was combined with 2X Hybridization buffer (Phalanx Hyb). Assembly and miRCURY™ Array, was used for miRNA array hybridization. miRNA array scanning and analysis were applied via Axon GenePix 4000B microarray scanner and GenePix pro V6.0 software (Molecular Devices, LLC, Sunnyvale, CA, USA). Differentially expressed miRNAs between PVT1 RNAi and the control groups were identified when fold-change (FC) was ≥2 or ≤0.5, and false discovery rate (FDR) <1 and P<0.05.

Validation of the expression of PVT1 in HCC. The TCGA database (http://cancergenome.nih.gov/) is a collection of DNA methylation, RNA-Seq, miRNA-seq, SNP array and exome sequencing (27,28). TCGA can also be used to further explore the expression of complicated cancer genomics and clinical parameters. In the present study, RNA-Seq data of HCC cases, which were calculated on the IlluminaHiSeq RNA-Seq platform, were obtained from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/), containing 374 HCC cases and 50 adjacent normal liver cases up to July 10, 2017. The original expression data of PVT1 were exhibited as reads per million (RPM) and the expression level of PVT1 was normalized by the Deseq package of R language. Prior to applying further analyses, we log transformed the original expression data for PVT1. The difference expression of PVT1 in various clinicopathological parameters in HCC was acknowledged based on the data from the TCGA database. The diagnostic value of PVT1 was evaluated using the receiver operating characteristic (ROC) curve. Additionally, the genetic alteration of PVT1 in HCC was investigated based on TCGA.

Target prediction and functional analysis. Twelve target prediction algorithms were used to predict the probable target genes of miRNAs. The 12 corresponding prediction algorithms were miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/), miRanda (http://www.microrna.org), mirBridge (http://mirsystem.cgm.ntu.edu.tw/), DIANA microF v4 (http://diana.imis.athena-innovation.gr/), miRMap (http://mirmap.ezlab.org/), mirDB (http://www.mirdb.org/), miRNAMap (http://mirnmap.mbc.nctu.edu.tw/), RNA22 (https://cm.jefferson.edu/), Pictar2 (https://www.mdc-berlin.de/), RNAhybrid (http://bibiserv.cebitec.uni-bielefeld.de/), PITA (https://genie.weizmann.ac.il/), and TargetScan (http://www.targetscan.org/), and the overlapping target genes were identified via Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/). In addition, the HPA (http://www.proteinatlas.org) was used to explore the protein expression of target genes in HCC and normal liver tissues.

To further consider the potential functions, pathways and networks of these target genes, bioinformatics analyses (GO, KEGG and network analyses) were performed (31,32). In this process, Database for Annotation, Visualization and Integrated Discovery (DAVID: available online: http://david.abcc.ncifcrf.gov/) was utilized to perform GO and KEGG analyses, and biological process (BP), cellular component (CC) and molecular function (MF) categories were derived from the GO analysis. Additionally, Cytoscape (version 2.8, http://cytoscape.org) was applied to construct the functional network.
Construction of protein-protein interaction (PPI) network.
The interaction pairs of the overlapped target genes were researched by Search Tool for the Retrieval of Interacting Genes (STRING; version 9.0, http://string-db.org) (33). The STRING database provides a worldwide perspective for as many animals and mammals as feasible. The predicted and acknowledged interactions are unified and scored. The interaction pairs in PPI network were selected when the combined score was >0.4.

Statistical analysis. SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. Data were expressed as mean ± standard deviation (SD). Differences in the expression of PVT1 in HCC and normal liver and various clinicopathological parameters were estimated by the Student’s t-test. The comparison between different subgroups was performed by one-way analysis of variance (ANOVA). Kaplan-Meier curves were used to detect the relationship between the PVT1 expression and patient survival in HCC. In addition, the ROC curve was used to predict the clinical diagnostic value of PVT1, which was statistically significant when P<0.05 (two-sided).

Results

miRNA profiling associated with lncRNA PVT1. The transfection efficiency was ~90%, and the knockdown efficiency of PVT1 in SMMC-7721 cells was >75% as detected by RT-qPCR (data not shown). Next, a miRNA microarray assay was applied to detect the differentially expressed miRNAs between PVT1 RNAi and the control groups. We found that 2 miRNAs were upregulated, and 12 miRNAs were downregulated in response to PVT1 knockdown. A summary of the differentially expressed miRNAs is shown in Fig. 2 and Table I. The top 2 upregulated (miR-302b-5p, miR-5191) and Table I. The top 2 upregulated and top 2 downregulated miRNAs.

| Name       | Fold-change | P-value | FDR  |
|------------|-------------|---------|------|
| Upregulated miRNAs |
| miR-302b-5p | 2.832       | 0.020   | 0.441|
| miR-5191   | 2.477       | 0.013   | 0.409|
| Downregulated miRNAs |
| miR-224-5p | 0.372       | 0.009   | 0.398|
| miR-4289   | 0.453       | 0.002   | 0.349|
| miR-UL22A-5p | 0.040     | 0.001   | 0.349|
| miR-548aa/miR-548t-3p | 0.455 | 0.003   | 0.371|
| miR-544b   | 0.076       | 0.006   | 0.379|
| miR-374c-3p | 0.465      | 0.010   | 0.398|
| miR-5009-5p | 0.379      | 0.012   | 0.407|
| miR-138-1-3p | 0.392     | 0.033   | 0.441|
| miR-154-5p | 0.360       | 0.036   | 0.441|
| miR-5003-5p | 0.475      | 0.038   | 0.441|
| miR-195-5p | 0.421       | 0.043   | 0.441|
| miR-3131   | 0.354       | 0.049   | 0.441|
and downregulated miRNAs (miR-224-5p, miR-4289) were finally selected as the most significant differentially expressed miRNAs due to the FC, FDR and P-values. Significance was determined via an FC ≥2 or ≤0.5, FDR <1 and P<0.05 was applied (34). We focused on the top two upregulated and downregulated miRNAs to improve the accuracy and stability of the results. The targets of the top dysregulated miRNAs may play key regulation roles in PVT1-related HCC.
Validation of the expression of PVT1 in HCC. Based on TCGA, 24% cases of PVT1 in HCC were found, which contained amplification, deep deletion and mRNA upregulation (Fig. 3A). To demonstrate the vital role of PVT1 in HCC, a
Figure 4. Validation of PVT1 expression in HCC. (A) Validation of PVT1 expression in the cohort of Chen Liver from Oncomine. Normal liver tissues (n=73) and hepatocellular carcinoma tissues (n=97) were included. (B) Validation of PVT1 expression in the cohort of Wurmbach Liver from Oncomine. Normal liver tissues (n=10) and hepatocellular carcinoma tissues (n=35) were included. (C) Normal liver tissues (n=160) and HCC tissues (n=369) were included based on the GEPIA database. (D) Overall survival of PVT1 expression in HCC based on the GEPIA database. (E) Disease-free survival of PVT1 expression in HCC based on the GEPIA database.

Figure 5. The procedure to achieve 696 genes. a, miR-302b-5p; b, miR-5191; c, miR-224-5p; and d, miR-4289.
A clinical study was performed using the original data in TCGA. The results showed the obvious high expression of PVT1 in HCC compared to that in normal liver tissues (P<0.001, Fig. 3B). Moreover, a statistically significant higher PVT1 expression was observed in sex (male), ethnicity (Asian) and pathological grade (G3+G4) compared with that in the control groups (P<0.05; Fig. 3C-E and Table II). Moreover, the area under curve (AUC) of PVT1 was 0.822 (95% CI, 0.780-0.863), indicating a moderate diagnostic value of the PVT1 expression in HCC (Fig. 3F). Furthermore, we investigated a different PVT1 expression in other clinical parameters of HCC, but no positive results were found based on the TCGA database. In addition, we investigated the relationship between the PVT1 expression and patient survival. A low PVT1 expression was correlated with improved survival (64.31±5.17 months) compared to the high PVT1 expression group (59.59±4.75 months, P=0.241; Fig. 3G) in HCC.

Moreover, the Oncomine and GEPIA databases confirmed the high expression of PVT1 in HCC (Fig. 4A-C). Furthermore, GEPIA demonstrated that patients with a low PVT1 expression have improved overall and disease-free survival, consistent with the results in TCGA (Fig. 4D and E).

### Table III. Top 10 enrichment GO terms (BP, CC and MF) for the target genes of miRNAs.

| GO ID          | Term                                                  | Ontology | Count | Fold enrichment | P-value              |
|----------------|-------------------------------------------------------|----------|-------|----------------|----------------------|
| GO:0010557     | Positive regulation of macromolecule biosynthetic process | BP       | 53    | 2.197          | 1.2412E-07          |
| GO:0051173     | Positive regulation of nitrogen compound metabolic process | BP       | 52    | 2.189          | 1.89479E-07         |
| GO:0045941     | Positive regulation of transcription                   | BP       | 47    | 2.259          | 3.37814E-07         |
| GO:0009891     | Positive regulation of biosynthetic process            | BP       | 54    | 2.106          | 3.51213E-07         |
| GO:0045893     | Positive regulation of transcription, DNA-dependent    | BP       | 42    | 2.387          | 3.84168E-07         |
| GO:0045935     | Positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | BP       | 50    | 2.172          | 4.265E-07           |
| GO:0051254     | Positive regulation of RNA metabolic process           | BP       | 42    | 2.367          | 4.81533E-07         |
| GO:0031328     | Positive regulation of cellular biosynthetic process   | BP       | 53    | 2.098          | 5.24515E-07         |
| GO:0010628     | Positive regulation of gene expression                 | BP       | 47    | 2.193          | 7.8235E-07          |
| GO:0010604     | Positive regulation of macromolecule metabolic process | BP       | 60    | 1.898          | 2.2026E-06          |
| GO:0005635     | Nuclear envelope                                       | CC       | 21    | 2.976          | 2.61292E-05         |
| GO:0030424     | Axon                                                  | CC       | 18    | 3.287          | 3.2788E-05          |
| GO:0030426     | Growth cone                                           | CC       | 10    | 5.380          | 8.24587E-05         |
| GO:0030427     | Site of polarized growth                               | CC       | 10    | 5.282          | 9.56427E-05         |
| GO:0045202     | Synapse                                               | CC       | 28    | 2.291          | 9.57691E-05         |
| GO:0043005     | Neuron projection                                     | CC       | 27    | 2.293          | 0.0001               |
| GO:0031965     | Nuclear membrane                                      | CC       | 11    | 4.377          | 0.0002               |
| GO:0016010     | Dystrophin-associated glycoprotein complex             | CC       | 6     | 10.253         | 0.0002               |
| GO:0031252     | Cell leading edge                                     | CC       | 15    | 3.158          | 0.0003               |
| GO:0044459     | Plasma membrane part                                  | CC       | 105   | 1.385          | 0.0003               |
| GO:0003700     | Transcription factor activity                          | MF       | 71    | 1.857          | 4.45E-07             |
| GO:0030528     | Transcription regulator activity                       | MF       | 95    | 1.603          | 2.72E-06             |
| GO:0043565     | Sequence-specific DNA binding                         | MF       | 45    | 1.891          | 5.87E-05             |
| GO:0008092     | Cytoskeletal protein binding                           | MF       | 36    | 1.822          | 0.0007               |
| GO:0016563     | Transcription activator activity                       | MF       | 31    | 1.929          | 0.0007               |
| GO:0051015     | Actin filament binding                                | MF       | 9     | 4.331          | 0.0010               |
| GO:0003779     | Actin binding                                         | MF       | 26    | 2.034          | 0.0010               |
| GO:0003702     | RNA polymerase II transcription factor activity        | MF       | 20    | 2.091          | 0.0033               |
| GO:0005127     | Ciliary neurotrophic factor receptor binding          | MF       | 3     | 25.507         | 0.0045               |
| GO:0019899     | Enzyme binding                                        | MF       | 34    | 1.658          | 0.0047               |

GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

clinical study was performed using the original data in TCGA. The results showed the obvious high expression of PVT1 in HCC compared to that in normal liver tissues (P<0.001, Fig. 3B). Moreover, a statistically significant higher PVT1 expression was observed in sex (male), ethnicity (Asian) and pathological grade (G3+G4) compared with that in the control groups (P<0.05; Fig. 3C-E and Table II). Moreover, the area under curve (AUC) of PVT1 was 0.822 (95% CI, 0.780-0.863), indicating a moderate diagnostic value of the PVT1 expression in HCC (Fig. 3F). Furthermore, we investigated a different PVT1 expression in other clinical parameters of HCC, but no positive results were found based on the TCGA database. In addition, we investigated the relationship between the PVT1 expression and patient survival. A low PVT1 expression was correlated with improved survival (64.31±5.17 months) compared to the high PVT1 expression group (59.59±4.75 months, P=0.241; Fig. 3G) in HCC.

Moreover, the Oncomine and GEPIA databases confirmed the high expression of PVT1 in HCC (Fig. 4A-C). Furthermore, GEPIA demonstrated that patients with a low PVT1 expression have improved overall and disease-free survival, consistent with the results in TCGA (Fig. 4D and E).

**Target prediction and functional analysis.** In the present study, 12 miRNA target prediction algorithms were utilized to predict the potential target genes of the four miRNAs. The genes predicted by >6 algorithms were selected as the final target genes. Among these target genes, 696 genes
were predicted by >2 miRNAs, and these 696 genes were used for the GO and pathway analyses (Fig. 5). The GO analysis indicated that the target genes were involved in complex cellular pathways, such as macromolecule biosynthetic process, compound metabolic process and transcription (Fig. 6 and Table III). The KEGG pathway analysis revealed that the MAPK and Wnt signaling pathways may be associated with regulation of the four candidate miRNAs (Table IV). To better identify the relationships between PVT1, miRNAs and target genes, a network was constructed via Cytoscape, and the genes were easily observed from the network (Fig. 7).

**PPI network analysis.** The STRING database was applied to construct the PPI network and 1,420 PPI pairs with a combined score of <0.4 were selected. PHLPP2 (degree, 42) and MAPK8 (degree, 26) had the highest degree and interactions in the PPI network. Then, a sub-network of 269 PPI pairs with >20 connectivity degrees was constructed for further analysis (Fig. 8). The number of nodes was 96, accounting for 13.79% of all the target genes. The clustering coefficient of PPI network was 0.634, which indicates that the PPI network had high cluster properties.

In addition, the genes associated with the MAPK and Wnt signaling pathways were selected based on the

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**Figure 6.** A functional network of Gene Ontology (GO) terms for the potential PVT1 genes in HCC. To further elucidate the functions of the overlapping genes, a function network was constructed according to Cytoscape.
KEGG pathway analysis. Five genes (PRKCA, MAPK8, PPP3R2, MAP3K7 and PRKX) were overlapped based on the Venn diagrams. According to the degree of hub genes in PPI network, MAPK8 had a high degree (degree, 26).

Next, we investigated the preliminary expression level of the five genes based on TCGA, and the original expression data of PPP3R2 was censored. Thus, based on TCGA, MAPK8 and PRKX were downregulated, whereas PRKCA...
and MAP3K7 were upregulated in HCC compared to that in normal liver tissues (both \( P < 0.05 \), Fig. 9A-D). Furthermore, negative correlations were found between PVT1 and MAPK8 (\( r = -0.289, P < 0.001 \), Fig. 9E), PRKCA (\( r = -0.140, P = 0.007 \), Fig. 9F) and MAP3K7 (\( r = -0.084, P = 0.106 \), Fig. 9G), whereas a positive correlation was found between PVT1 and PRKX (\( r = 0.154, P = 0.003 \), Fig. 9H). Moreover, based on HPA, weak staining in HCC was observed for MAPK8, whereas moderate staining was observed for PRKCA and PPP3R2 (Fig. 10A-F). Negative staining in both HCC and normal liver tissues was observed for MAP3K7, in contrast with its upregulated expression in TCGA (Fig. 10I and J). Based on these results, PRKCA and MAPK8 were all negatively correlated with PVT1, whereas PRKCA was overexpressed in HCC, in contrast with the correlation of PVT1. Thus, only MAPK8 was selected. We hypothesized that PVT1 may influence MAPK8 expression in the MAPK or Wnt signaling pathways to participate in the different biological processes of HCC. However, the precise molecular mechanism of PVT1 in HCC needs further experimental investigation.

**Discussion**

Previous studies have demonstrated that lncRNAs participates in different biological processes, such as transcription, chromosome remodeling and post-transcriptional processing (35-37). Many studies have verified that lncRNAs are associated with the tumorigenesis and development of various types of cancer through various pathways, including the regulation of cell proliferation, metastasis and invasion (38-40). Thus, lncRNAs have opened an avenue of cancer genomics.

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**Table IV. The top 10 KEGG pathways from the enrichment analysis of the target genes of miRNAs.**

| KEGG ID | KEGG term                        | Count | Fold enrichment | P-value | Gene symbol                  |
|---------|----------------------------------|-------|----------------|---------|-------------------------------|
| hsa04010| MAPK signaling pathway           | 22    | 2.253          | 0.0006  | PRKCA, TAOK1, TGFBR1, PPP3R2, STK4, PRKX, TGFBR2, ATF2, MAP3K7, MAPK1, DUSP4, RP56A3, RASGFR2, ELK4, ARRB1, MAP3K2, MAPT, PDGFR, MAPK8, RAPGFR2, CACNA1B, RASA2 |
| hsa04310| Wnt signaling pathway            | 15    | 2.716          | 0.0011  | PRKCA, TBL1XR1, VANGL1, SMAD4, PPP3R2, SMAD3, FZD3, DAAM1, FZD5, PRKX, MAP3K7, CCND1, PSEN1, PRICKLE2, MAPK8 |
| hsa04360| Axon guidance                    | 13    | 2.755          | 0.0024  | SEMA5A, AB1LIM1, MAPK1, SEMA6A, PLXNA2, NTN4, PPP3R2, ROBO2, EFN, DYSPL2, SLIT1, SRGAP1, EPHA2 |
| hsa05210| Colorectal cancer                | 10    | 3.255          | 0.0032  | MAPK1, CCND1, TGFBR1, PDGFR, SMAD4, SMAD3, FZD3, MAPK8, FZD5, TGFBR2 |
| hsa05212| Pancreatic cancer                | 9     | 3.417          | 0.0043  | MAPK1, CCND1, TGFBR1, SMAD4, RALA, SMAD3, CDK6, MAPK8, TGFBR2 |
| hsa05220| Pathways in cancer               | 21    | 1.750          | 0.01500 | PRKCA, XIP, TGFBR1, MITF, SMAD4, RUNX1, SMAD3, CDK6, EGNL1, FZD3, FZD5, STK4, TGFBR2, MAPK1, CCND1, HADC2, CDKN2B, PDGFR, RALA, MAPK8 |
| hsa05220| Chronic myeloid leukemia         | 8     | 2.916          | 0.01856 | MAPK1, CCND1, HADC2, TGFBR1, SMAD4, SMAD3, CDK6, TGFBR2 |
| hsa04520| Adherens junction                | 8     | 2.840          | 0.0212  | MAP3K7, MAPK1, TGFBR1, SMAD4, SMAD3, CDK6, TGFBR2 |
| hsa04120| Ubiquitin-mediated proteolysis   | 11    | 2.195          | 0.027   | CUL3, UBE2D3, UBE4A, XIAP, NEDD4, UBE2K, UBE2G1, UBA2, UBE2J1, UBE2W, NEDD4L |
| hsa04144| Endocytosis                      | 13    | 1.932          | 0.0350  | DNM3, RAB31, RAB11FIP2, ERBB4, TFRC, NEDD4, ARRB1, TGFBR1, PSD3, PDGFR, EEA1, NEDD4L, PIP4K2B |

*KEGG, Kyoto Encyclopedia of Genes and Genomes.*
To date, several studies have investigated the effect of PVT1 on various cancer types. Xu et al (41) demonstrated that PVT1 overexpression encouraged proliferation and invasion in gastric cancer cells via binding to FOXM1, and a high PVT1 expression was associated with the poor prognosis of gastric cancer patients. Chen et al (42) revealed that the overexpression of PVT1 promoted the invasion of non-small cell lung cancer cells. Additionally, PVT1 functioned as a competitive endogenous RNA to regulate the expression of MMP9 via competitively binding to microRNAs. Liu et al (43) showed that PVT1 was an oncogene in prostate cancer by activating miR-146a methylation to improve tumor growth. Nevertheless, the detailed roles for PVT1 in HCC remain undefinable. In the present study, we combined miRNA microarray analysis and TCGA, as well as Oncomine and GEPIA databases to explore the potential biological functions of PVT1 in HCC. We confirmed that PVT1 was an oncogene and highly expressed in HCC, consistent with Yu et al and Ding et al (18,19). Moreover, Yu et al (18) revealed that the combined upregulation of two IncRNAs (PVT1 and uc002 mbe.2) offered a new method for the diagnosis of HCC, and the expression of these two IncRNAs was positively correlated with tumor size and clinical stage in HCC patients. Furthermore, Ding et al (19) revealed that the overexpression of PVT1 was strongly associated with the AFP level and could predict recurrence. By comparison, the present study showed that PVT1 expression was positively correlated with sex, ethnicity and pathological grade. The AUC of PVT1 indicated a moderate diagnostic value of PVT1 expression in HCC. Furthermore, the genetic alterations of PVT1 were observed in HCC based on TCGA, which may be correlated with the pathogenesis of HCC.
To the best of our knowledge, the present study was the first to identify the differentially expressed miRNAs associated with PVT1 based on miRNA microarray analysis, and 12 miRNA target prediction algorithms were used to predict the underlying target genes of the differentially expressed miRNAs. According to GO analysis, the target genes were involved in complex cellular pathways, such as macromolecule biosynthetic process, compound metabolism, and transcription. The KEGG pathway analysis revealed that the MAPK and Wnt signaling pathways are potentially correlated with the regulation of the four candidate miRNAs. As reported, the MAPK and Wnt signaling pathways were all associated...
with proliferation, migration, invasion and prognosis and HCC (44-48). Consequently, we hypothesized that PVT1 plays a vital role in HCC by regulating the expression of the four miRNAs via the MAPK or Wnt signaling pathways, which requires further investigation on the precise molecular mechanism of PVT1 in HCC. We also investigated the genes from the MAPK and Wnt signaling pathways and the hub genes from PPI. We hypothesized that PVT1 may influence MAPK8 expression to contribute to different biological processes of HCC. Various in vitro and in vivo experiments, including cell proliferation, invasion and metastasis assays, and animal models, are needed to verify this hypothesis. The clinical significance and molecular mechanism of PVT1 in the biological function of HCC are to be further researched at the molecular, cellular, tissue and animal levels. Focusing on the new insight of PVT1 in HCC, the present study aimed to provide a potential biomarker or therapeutic target for HCC.

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Availability of data and materials

Data used in this study are available upon request to the corresponding author.

Authors’ contributions

YZ and WM conceived and designed the study. XW, TZ and YQ performed the experiments. YZ and GC wrote the paper. DW and YD reviewed and edited the manuscript. 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

All experimental protocols were approved by the First Affiliated Hospital of Guangxi Medical University (Nanning, China).

Consent for publication

Not applicable.

Competing interests

The authors DEC that they have no competing interests.

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