Long non-coding RNA HOTTIP promotes hepatocellular carcinoma tumorigenesis and development: A comprehensive investigation based on bioinformatics, qRT-PCR and meta-analysis of 393 cases

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Abstract. HOTTIP functions as an independent biomarker in multiple cancers. However, the role of HOTTIP in hepatocellular carcinoma (HCC) remains unclear. In this study, we sought to investigate the HOTTIP expression in HCC and normal liver. We combined quantitative reverse transcription-polymerase chain reactions (qRT-PCR), Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA), Multi Experiment Matrix (MEM) and Oncomine database to assess the clinical role and the potential molecular mechanism of HOTTIP in HCC. Furthermore, a meta-analysis was performed to evaluate the relationship between HOTTIP and HCC tumorigenesis and development. Additionally, bioinformatics analysis, which contained Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and network analysis, were applied to investigate the underlying functions, pathways and networks of the potential genes. HOTTIP was obviously upregulated in HCC. A statistically significant higher expression of HOTTIP was found in TNM (III +IV), age (≥60), sex (male), race (white) and cirrhosis (no) compared to the control groups (P<0.05). Furthermore, the meta-analysis of 393 cases from multiple centers indicated that HOTTIP had high diagnostic value in HCC. Additionally, according to GO and KEGG analyses, we found that the most strongly enriched functional terms were gland development, transcription factor activity and extrinsic to membrane. Also, the HOTTIP co-expressed genes were significantly related to PPAR signaling pathway. We speculate that HOTTIP might play a vital part in HCC via regulating various pathways, especially PPAR signaling pathway. However, the detailed mechanism should be confirmed by functional experiments.

Introduction

Hepatocellular carcinoma (HCC) is known as the most common liver malignancy worldwide, with extremely high incidence and mortality rate (1-5). In China, HCC frequently occurs owing to chronic infection of hepatitis B virus (HBV) (6-9). Other conditions, such as alcoholic hepatitis, non-alcoholic fatty liver disease, hemochromatosis and diabetes, also contribute to the development of HCC (10-12). Up to now, liver transplantation and tumor resection still have been the most effective treatments for HCC, whereas the high metastasis and postoperative recurrence rates barri- cade the prognosis of HCC patients, especially HCC patients in advanced stage (13,14). As the current treatment options are limited, it is of great significance to investigate the underlying mechanism of HCC, which might provide novel insights into the diagnosis and treatment of HCC patients.

Long non-coding RNAs (lncRNAs) represent the non-protein coding RNAs with the length from 200 nucleotides to 100 kb (15-17). Recent evidence has clarified that various
In the present study, we investigated the expression of HOTTIP in HCC and normal liver. Furthermore, we combined Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA), Multi Experiment Matrix (MEM) and Oncomine database, quantitative reverse transcription-polymerase chain reactions (qRT-PCR), and meta-analysis to assess the clinical role and the potential molecular mechanism of HOTTIP in HCC. Additionally, bioinformatics analysis, which contain Genes and Genomes (KEGG) and network analysis, were applied to explore the underlying functions, pathways and networks of the potential genes (46-48). A flow chart of this study is shown in Fig. 1.

Materials and methods

Quantitative real-time PCR. A total of 41 HCC cases, between January 2012 and March 2013, were collected from the Department of Pathology, First Affiliated Hospital of the Guangxi Medical University (Nanning, Guangxi, China). The samples were collected randomly from patients undergoing surgical resection without treatment. All methods were performed based on the relevant regulations and guidelines. Also, all experimental protocols have been approved by the Ethical Committee of the First Affiliated Hospital of Guangxi Medical University, and the clinicians and patients have signed the consent forms for the use of their tissues in the study. In this study, the total RNA was extracted via a Takara PrimeScript RT reagent kit based on the manufacturer’s instructions. Then, the total RNA was used for cDNA synthesis through the Takara PrimeScript RT reagent kit according to the instructions. Then, qRT-PCR was operated using a LightCycler 480 Real-time PCR system (Roche, Shanghai, China). The specific primers were employed as follows: HOTTIP forward, 5'-CACACTCACATTCGCACACT-3'; reverse, 5'-TCCAGAACTAAGCCAGCATA-3'. GAPDH (internal control) forward, 5'-AGTGGAAGGAAGATGAGGATT-3'; reverse, 5'-GTGGAGTACATCTGGAACA-3' (49). Results were normalized to the GAPDH expression and calculated based on the ∆∆Ct method (50,51).

HOTTIP and HCC: a meta-analysis. HCC-related HOTTIP microarray and RNA-seq datasets were downloaded from TCGA, the National Center of Biotechnology Information (NCBI) GEO (http://www.ncbi.nlm.nih.gov/geo/), ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) and Oncomine (https://www.oncomine.org/resource/main.html). In addition, publications associated with the diagnostic value of HOTTIP in HCC were also selected from 12 online databases: PubMed, Web of Science, Science Direct, Google Scholar, Ovid, LILACS, Wiley Online Library, EMBASE, Cochrane Central Register of Controlled Trials, Chong Qing VIP, Chinese CNKI, Wan Fang and China Biology Medicine disc. The retrieval date was up to April 20, 2017 with the following keywords: (HOTTIP or HOXA-AS6 or HOXA13-AS1 or NCRNA00213) and (malignan* OR cancer OR tumor OR tumor OR neoplas* OR carcinoma) and (hepatocellul* OR liver OR hepatic OR HCC). The literature retrieval was assessed and cross-checked by two independent investigators (Jia-Cheng Huang and Wen-Ya Pan). Group discussion was carried out if there was disagreement. The number of true-positives (tp), true-negatives (tn), false-positives (fp) and false-negatives (fn) was extracted. When no direct data was found from a study, a basic formula (such as ‘sensitivity’ = tp/ (tp+fn), ‘specificity’ = tn/ (tn+fp)) would be used to calculate the incidence.

Validation of the expression of HOTTIP in HCC. TCGA (http://cancergenome.nih.gov/) is a collection of SNP arrays, DNA methylation, miRNA-seq, exome sequencing, RNA-seq, and more (52,53). TCGA can be also applied to investigate the complicated cancer genomics expression and clinical parameters. In this study, RNA-Seq data from individuals with HCC, which were calculated on Illumina HiSeq RNASeq platform, were achieved from TCGA data portal (https://tcga-data.nci.nih.gov/tcga/), containing 171 HCC tissues and 28 adjacent normal liver tissue samples up to April 1, 2017. The expression data of HOTTIP were displayed as reads per million (RPM) and the HOTTIP expression level was normalized by Deseq package of R language for further analysis. Student’s t-test (SPSS Inc., Chicago, IL, USA) was performed for the statistical analysis of the differential expression of HOTTIP between HCC and non-cancerous liver tissues. Also, the relationship between HOTTIP and the clinicopathological parameters in HCC was identified based on the original data from TCGA database. Then, the clinical diagnostic value of HOTTIP was analyzed by a receiver operating characteristic (ROC) curve. Furthermore, the original data in Oncomine database was also applied to verify the HOTTIP expression in HCC (54).

The potential functions and pathways associated with HOTTIP. To further investigate the co-expressed genes associated with HOTTIP, MEM (http://biit.cs.ut.ee/mem/index.cgi), an open-access resource, was utilized to explore the co-expressed genes of HOTTIP based on Affymetrix Gene Chip Human Genome
U133 Plus 2.0 Array platform (55). Differentially expressed genes in TCGA were extracted via R language package DESeq based on the following criteria: adjusted P<0.05 and the absolute log2 fold change >1 (56,57). Also, the genes differentially expressed in GEO (http://www.ncbi.nlm.nih.gov/geo/) database were selected using the GEO2R online tool (58-61). The overlapping genes were identified and compared using Venn diagrams (available online: http://bioinformatics.psb.ugent.be/webtools/Venn/). Then, bioinformatics analyses including GO, KEGG and network analysis were applied to explore the potential functions, pathways and networks of the overlapping genes as described (62,63). In this process, Database for Annotation, Visualization and Integrated Discovery (DAVID: available online: http://david.abcc.ncifcrf.gov/) was applied to perform GO and KEGG analyses. In addition, three independent categories [biological process (BP), cellular component (CC) and molecular function (MF)] were derived from GO analysis. Besides, a functional network was constructed via Cytoscape (version 2.8, http://cytoscape.org).

**Construction of protein-protein interaction (PPI) network.**

The interaction pairs of these co-expressed genes were explored with Search Tool for the Retrieval of Interacting Genes (STRING; version 9.0, http://string-db.org) (64). The STRING database supplies a global perspective for as many organisms as feasible. The known and predicted interactions are integrated and scored. The interaction pairs in PPI network were selected with the combined score >0.4.

**Statistical analysis.**

The high-throughput expression data were log2-transformed. The mean ± standard deviation (mean ± SD) was calculated through SPSS 22.0 (IBM, NY, USA) to estimate the HOTTIP expression level in each dataset. The HOTTIP expression between normal liver tissue and HCC was evaluated by Student’s t-test. Student’s t-test was also used to evaluate the relationships between HOTTIP expression and the clinicopathological parameters. The comparison between subgroups was performed via one-way analysis of variance (ANOVA). A Mann-Whitney U test or Kruskal-Wallis H test was conducted for non-normally distributed variables. Mining for co-expression genes across hundreds of datasets was carried out via novel rank aggregation and visualization methods. A P-value of <0.05 was identified to be statistically significant (two sides) using SPSS 22.0.

In the diagnostic meta-analysis, all the statistical analysis was performed by STATA 14.0 (STATA Corp., College, TX, USA). Heterogeneity between the included studies was assessed by Cochrane's Q test and I^2 statistic, and I^2 > 50% represented significant heterogeneity. Publication bias was evaluated by Deek’s funnel plot asymmetry test. A P-value of <0.05 was regarded significant publication bias. To investigate the underlying diagnostic performance of HOTTIP in HCC, we performed summary receiver operating characteristic (SROC) curves and calculated area under the curve (AUC) with 95% CIs and the corresponding sensitivity and specificity by using Meta-DISc software (65). An AUC value of 0.5-0.7 was regarded as low diagnostic capability; an AUC of 0.7-0.9 represented a moderate diagnostic ability; an AUC of >0.9 indicated a high diagnostic accuracy. We also applied STATA 14.0 (STATA Corp.) to conduct continuous variable meta-analysis. Fixed model (Mantel-Haenszel method) was applied at first and random model (Der Simonian and Laird method) was used when there existed significant heterogeneity. Funnel plot was described to test publication bias.

**Results**

The clinical value of HOTTIP expression in HCC. In the present study, an upregulated trend in HOTTIP level in HCC tissues (4.67-fold) was found when compared to normal liver tissues (P=0.002, Fig. 2A) based on qRT-PCR. We also investigated the relationship between different expression of HOTTIP and clinicopathological parameters. As a result, HOTTIP expression was highly expressed in III+IV and
no cirrhosis groups (P<0.05, Table I and Fig. 2B and C) in HCC, but no statistical significance was found in other clinico-pathological parameters. In addition, the diagnostic value of the HOTTIP level in HCC was assessed by a ROC curve and the AUC of HOTTIP was 0.762 (95% CI, 0.660-0.864, P-value of <0.001, Fig. 2D), indicating a moderate diagnostic value of the HOTTIP level in HCC.

| Clinicopathological features | N  | Fold change | T     | P-value |
|-----------------------------|----|-------------|-------|---------|
| Tissues Tissues             |    |             |       |         |
| Normal liver                | 41 | 1.00        | 3.226 | 0.002   |
| HCC                         | 41 | 4.67        |       |         |
| Age                         |    |             |       |         |
| <50                         | 19 | 3.33        | 1.233 | 0.226   |
| ≥50                         | 22 | 6.00        |       |         |
| Sex                         |    |             |       |         |
| Male                        | 31 | 5.17        | 0.775 | 0.443   |
| Female                      | 10 | 3.17        |       |         |
| Cirrhosis Cirrhosis         |    |             |       |         |
| No                          | 27 | 6.00        | -2.212| 0.035   |
| Yes                         | 14 | 2.17        |       |         |
| TNM                         |    |             |       |         |
| I+II                        | 25 | 2.50        | -2.255| 0.037   |
| III +IV                     | 16 | 8.17        |       |         |
| Metastasis Metastasis       |    |             |       |         |
| No                          | 38 | 4.17        | -0.754| 0.528   |
| Yes                         | 3  | 10.83       |       |         |
| Tumor diameter (cm) Tumor diameter (cm) |    |             |       |         |
| <7                          | 11 | 2.67        | -1.126| 0.267   |
| ≥7                          | 30 | 5.50        |       |         |
| Vascular invasion Vascular invasion |    |             |       |         |
| No                          | 35 | 5.00        | 0.772 | 0.445   |
| Yes                         | 6  | 2.67        |       |         |
| Complete capsule Complete capsule |    |             |       |         |
| No                          | 16 | 4.67        | 0.050 | 0.961   |
| Yes                         | 25 | 4.83        |       |         |
| Differentiation Differentiation |    |             |       |         |
| Low                         | 10 | 3.17        | F=2.194| 0.125  |
| Moderate                    | 27 | 4.67        |       |         |
| High                        | 4  | 8.33        |       |         |
| AFP                         |    |             |       |         |
| Negative                    | 22 | 5.50        | -1.409| 0.169   |
| Positive                    | 13 | 2.67        |       |         |
| Embolus Embolus             |    |             |       |         |
| No                          | 39 | 4.83        | 0.791 | 0.433   |
| Yes                         | 2  | 0.83        |       |         |
Figure 2. Clinical significance of HOTTIP in HCC based on qRT-PCR. (A) Differential expression of HOTTIP between HCC and non-cancerous liver tissue. (B) Differential expression of HOTTIP in I+ II vs. III+IV. (C) With cirrhosis vs. without cirrhosis. (D) ROC curve of HOTTIP in HCC.

Figure 3. Forest plot showing the pooled sensitivity and specificity of the included studies. The pooled sensitivity and specificity of HOTTIP was 0.88 (0.70-0.96) and 0.59 (0.20-0.89), respectively.
DLR-positive and DLR-negative were 2.13 (0.79-5.75) and 0.20 (0.07-0.59, Fig. 4), respectively. The diagnostic score and odds ratio were 2.35 (0.55-4.15) and 10.45 (1.73-63.16, Fig. 5), respectively. The AUC of SROC was 0.87 (0.83-0.89, Fig. 6), which indicated a moderate diagnostic value of HOTTIP in HCC. The pre-test probability was 20% when the positive and
negative pre-test probability was 35 and 5% (Fig. 7), respectively. As for the publication bias, no significant publication bias was found (P=0.15, Fig. 8).

As for the expression of HOTTIP in HCC compared to non-cancerous group, a fix-effect model was selected to calculate the pooled standard mean deviation (SMD) and 95% CI and the combined SMD reached 0.83 (0.57, 1.09), indicating a statistically significant higher expression of HOTTIP could be found in HCC than in normal control groups (P<0.001, Fig. 9A). In addition, no report bias was found in our study (P>0.05, Fig. 9B). Above all, a flow chart of this meta-analysis is shown in Fig. 10.

Validation of the expression of HOTTIP in HCC. To further explore the differential expression of HOTTIP between HCC and non-cancerous liver tissues, we performed a clinical research based on the original data in TCGA. One HCC cohort, which was comprised of 171 HCC cases and 28 non-cancerous liver cases, was extracted. Increased expression of HOTTIP was observed in HCC tissues (5.001±2.453) compared with the non-cancerous tissues (0.182±0.459, P<0.001, Fig. 11A). With regard to the clinicopathological parameters, we found that HOTTIP expression was highly expressed in age (≥60), sex (male), race (white) compared to that in the control group (P-value of <0.05, Table II and Fig. 11B-D). For the other clinicopathological characteristics, no statistical significance was found based on TCGA database. Moreover, the AUC of HOTTIP reached 0.982 (95% CI, 0.966-0.998, P<0.0001, Fig. 11E), which indicated a high diagnostic value of the HOTTIP level in HCC.

Additionally, two probe sets (1564069_at and 1564070_s_at) of Wurmbach Liver in Oncomine were used to validate the HOTTIP expression. However, an opposite trend was found in the two probe sets (Fig. 12). HOTTIP was downregulated in 1564070_s_at probe set compared to the normal liver, which was inconsistent with the results of qRT-PCR and TCGA.
Also, the opposite trend of HOTTIP expression might be a source of heterogeneity in meta-analysis.

The potential pathways associated with HOTTIP. Based on GEO, TCGA and MEM database, 287 overlapped genes were selected (Fig. 13). In addition, GO and pathway analyses were performed using these 287 genes. The most strongly enriched GO terms were identified as follows: embryonic morphogenesis, gland development, transcription factor activity and extrinsic to membrane (Table III). To better affect the functions of these genes, a function network was constructed according to GO analysis (Fig. 14). Besides, the KEGG pathway analysis confirmed that these genes were significantly involved in PPAR signaling pathway, FcγR-mediated phagocytosis and endocytosis (Table IV). Altogether, the GO and KEGG pathway analysis revealed that HOTTIP might participate in the biological mechanism of HCC. In addition, a gene network of the 287 genes was
Figure 11. Clinical significance of HOTTIP in HCC based on TCGA database. (A) Differential expression of HOTTIP between HCC and non-cancerous liver tissue. (B) Differential expression of HOTTIP in <60 vs. ≥60. (C) Male vs female. (D) Asian vs. Black vs. White. (E) ROC curve of HOTTIP in HCC.

Figure 12. Validation of HOTTIP expression in the cohort of Wurmbach Liver from Oncomine. (A) Normal liver tissues (n=10) and hepatocellular carcinoma tissues (n=35) were included in the cohort of Wurmbach Liver (1564069_at). (B) Normal liver tissues (n=10) and hepatocellular carcinoma tissues (n=35) were included in the cohort of Wurmbach Liver (1564070_s_at).
Table II. Differential expression of HOTTIP of clinicopathological parameters in HCC tissue based on TCGA database.

| Clinicopathological features | N     | Mean ± SD  | T    | P-value |
|-----------------------------|-------|------------|------|---------|
| Tissues                     |       |            |      |         |
| Normal liver                | 28    | 0.182±0.459| 8.16 | <0.0001 |
| HCC                         | 171   | 5.001±2.453|      |         |
| Age                         |       |            |      |         |
| <60                         | 66    | 4.405±2.513| -2.563| 0.011   |
| ≥60                         | 106   | 5.376±2.349|      |         |
| Sex                         |       |            |      |         |
| Male                        | 114   | 5.457±2.393| 3.556| 0.0005  |
| Female                      | 57    | 4.089±2.332|      |         |
| Race                        |       |            |      |         |
| White                       | 84    | 5.077±2.379|      | 0.049   |
| Black                       | 11    | 6.522±1.740|      |         |
| Yellow                      | 70    | 4.622±2.540|      |         |
| T (tumor)                   |       |            |      |         |
| T1+ T2                      | 133   | 5.099±2.372| 1.236| 0.218   |
| T3+ T4                      | 37    | 4.539±2.657|      |         |
| Vascular invasion           |       |            |      |         |
| No                          | 95    | 4.898±2.405| 0.521| 0.603   |
| Yes                         | 51    | 5.118±2.509|      |         |
| Patological grade           |       |            |      |         |
| g1+g2                       | 107   | 5.068±2.434| 0.569| 0.570   |
| g3+g4                       | 61    | 4.847±2.382|      |         |
| Stage                       |       |            |      |         |
| I+ II                       | 126   | 5.065±2.333| 1.544| 0.125   |
| III +IV                     | 32    | 4.341±2.520|      |         |
| Recurrence                  |       |            |      |         |
| No                          | 151   | 4.942±2.468| 1.334| 0.184   |
| Yes                         | 10    | 6.008±2.029|      |         |

Table III. Top 5 enrichment GO terms (BP, CC and MF) of the potential genes of HOTTIP.

| GO ID               | Term                                | Ontology | Count | P-value   |
|---------------------|-------------------------------------|----------|-------|-----------|
| GO:0048732          | Gland development                    | BP       | 12    | 5.32E-06  |
| GO:0007389          | Pattern specification process        | BP       | 16    | 1.08E-05  |
| GO:0003002          | Regionalization                      | BP       | 13    | 3.84E-05  |
| GO:0048598          | Embryonic morphogenesis              | BP       | 16    | 5.50E-05  |
| GO:0051216          | Cartilage development                | BP       | 8     | 1.12E-04  |
| GO:0043565          | Sequence-specific DNA binding        | MF       | 23    | 3.61E-04  |
| GO:0003700          | Transcription factor activity        | MF       | 31    | 5.20E-04  |
| GO:0008289          | Lipid binding                        | MF       | 17    | 2.81E-03  |
| GO:0004385          | Guanylate kinase activity            | MF       | 3     | 1.31E-02  |
| GO:0042803          | Protein homodimerization activity    | MF       | 12    | 2.08E-02  |
| GO:0019898          | Extrinsic to membrane                | CC       | 23    | 3.54E-06  |
| GO:0045177          | Apical part of cell                  | CC       | 13    | 1.32E-05  |
| GO:0016324          | Apical plasma membrane               | CC       | 11    | 2.63E-05  |
| GO:0044459          | Plasma membrane part                 | CC       | 52    | 4.28E-04  |
| GO:0005624          | Membrane fraction                    | CC       | 22    | 7.97E-03  |

GO, Gene Ontology. BP, biological process. CC, cellular component. MF, molecular function.
constructed in the present study (Fig. 15), from which we could easily observe relationships between HOTTIP and these potential genes.

The PPI network was constructed through STRING online and a total of 72 PPI pairs with combined score >0.4 were noted (Fig. 16). Also, PPARA had the highest degree (degree 6) according to the PPI network.

In addition, a total of four genes (PPARA, PPARG, FABP2, SCP2) related to PPAR signaling pathway were detected based on KEGG pathway analysis. Moreover, we investigated the expression of these four genes and their correlations with HOTTIP based on the original data in TCGA. We found that both PPARG and FABP2 were highly expressed in HCC compared to normal liver (P<0.05, Fig. 17A and B), whereas SCP2 was highly expressed in normal liver (14.99±0.082 vs. 13.74±0.064, P<0.001, Fig. 17C). However, only a minor difference was found in PPARA expression between normal liver and HCC (11.88±0.098 vs. 11.73±0.054, P=0.174, Fig. 17D). As for the correlation between HOTTIP

Figure 13. A flow chart to screen the co-expressed genes based on Venn diagrams.

Figure 14. A function network of Gene Ontology (GO) terms for the potential genes of HOTTIP in HCC. Based on GEO, TCGA and MEM database, 287 overlapped genes were selected. In addition, GO analysis was performed using these 287 genes. To further reflect the functions of these genes, a function network was constructed based on GO analysis.
and these genes, we discovered that HOTTIP had a positive correlation with PPARA or SCP2 (P<0.001, Fig. 17E and F), whereas a negative correlation was found between HOTTIP and PPARG and FABP2 (P>0.05, Fig. 17G and H). Based on the aforementioned results, we hypothesized that HOTTIP may influence the SCP2 expression of PPAR signaling.
pathway to participate in different biological processes of HCC.

Discussion

Up to now, countless studies have confirmed that lncRNAs could participate in various chemical and biological processes, such as chromosome remodeling, transcription, cancer metastasis and posttranscriptional processing (66,67). Many studies have demonstrated that lncRNAs were related to tumorigenesis and development of HCC through various pathways, including regulation of apoptosis, cell cycle and chemotherapy resistance in HCC tissue or cell lines (68-70). lncRNAs have opened an avenue of cancer genomics.

To date, several studies have investigated the effect and potential mechanism of HOTTIP on HCC. Quagliata et al (71) found that the HOTTIP expression was associated with HCC progression and HOTTIP could be a predictive biomarker in HCC based on the snap-frozen needle HCC biopsies and their matched non-neoplastic counterparts. They also clarified that HOTTIP could directly control the expression of HOXA locus gene by interacting with the WDR5/MLL complex, but

Figure 16. The PPI network of the co-expressed genes. The PPI network was constructed via STRING online and 72 PPI pairs was chosen for further analysis.
Figure 17. Clinical significance of four related genes (PPARA, PPARG, FABP2, SCP2) in HCC based on TCGA database. (A) Differential expression of PPARG between HCC and non-cancerous liver tissue. (B) Differential expression of FABP2 between HCC and non-cancerous liver tissue. (C) Differential expression of SCP2 between HCC and non-cancerous liver tissue. (D) Differential expression of PPARA between HCC and non-cancerous liver tissue. (E) Positive correlation between HOTTIP and PPARA. (F) Positive correlation between HOTTIP and SCP2. (G) Negative correlation between HOTTIP and PPARG. (H) Negative correlation between HOTTIP and FABP2.
the specific relationship between HOTTIP and HOX genes is still vague. Tsang et al (45) found that HOTTIP was an oncogenic IncRNA and highly expressed in HCC tissues based on qRT-PCR, and HOTTIP could contribute to hepatocarcinogenesis via targeting tumor suppressor miR-125b, which was verified by luciferase reporter assay and functional analysis. Also, the migratory ability of HCC cells could be inhibited after silencing HOTTIP expression. Ge et al (72), using dual luciferase reporter assays, confirmed that HOTTIP was a significant oncogene in HCC and miR-192/-204-HOTTIP axis was a significant molecular pathway during tumorigenesis of HCC. They also demonstrated the prognostic and potential therapeutic roles of HOTTIP. In comparison, we designed this study using RT-qPCR, meta-analysis and bioinformatics to further investigate the effect of HOTTIP in HCC. Interestingly, we discovered that HOTTIP was a tumorigenic gene and HOTTIP expression was highly expressed in TNM (III +IV), age (≥60), sex (male), race (white) and cirrhosis (no) compared to that in the control groups. In the present study, ROC curve was applied to evaluate the association between HOTTIP expression and the diagnostic value, and the AUC of HOTTIP indicated the potential diagnostic value of HOTTIP level in HCC. Moreover, this is the first meta-analysis to investigate the expression and diagnostic value of HOTTIP in HCC. As a result, the SMD of the meta-analysis validated the higher expression of HOTTIP in HCC. Furthermore, in the diagnostic meta-analysis, 393 cases from GEO, TCGA, Oncomine and publications were included. The meta-analysis was performed to evaluate the validity of HOTTIP for the detection of HCC. The sensitivity of the HOTTIP assay in the included parts ranged from 70 to 96%, and the specificity of HOTTIP range from 20 to 89%. The combined values of sensitivity (0.88) and specificity (0.59) demonstrated the accuracy of HOTTIP for the detection of HCC. Also, our results clarified that the SROC curve was located near the upper left corner. The AUC was 0.87, which indicated a moderate diagnostic accuracy (73). The PLR and NLR were presented to measure the diagnostic potential of HOTTIP than HOTTIP in tissues. We also intend to perform in-depth exploration on the circulating HOTTIP in HCC patients in the future. Focusing on the new insight of HOTTIP, this study aimed to provide a new biomarker or therapeutic target for HCC.

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References

1. Li C, Miao R, Liu S, Wan Y, Zhang S, Deng Y, Bi J, Qu K, Zhang J and Liu C: Down-regulation of miR-146b-5p by long noncoding RNA MALAT1 in hepatocellular carcinoma promotes cancer growth and metastasis. Oncotarget 8: 28683-28695, 2017.
2. Lu PH, Chen MB, Liu YY, Wu MH, Li WT, Wei MX, Liu CY and Qiu SK: Identification of sphenogine kinase 1 (SphK1) as a primary target of iscaritin in hepatocellular carcinoma cells. Oncotarget 8: 22800-22810, 2017.
3. He R, Gao L, Ma J, Peng Z, Zhou S, Yang L, Feng Z, Deng Y and Chen G: The essential role of MTDH in the progression of HCC: A study with immunohistochemistry, TCGA, meta-analysis and in vitro investigation. Am J Transl Res 9: 1561-1579, 2017.
4. Mo Z, Zheng S, Lv Z, Huang Y, Lan X, Wang F, Liu X, Zhao Y and Zhou S: Senescence marker protein 30 (SMP30) serves as a potential prognostic indicator in hepatocellular carcinoma. Sci Rep 6: 39576, 2016.
5. Huang WT, Wang HL, Yang H, Ren FH, Luo YH, Huang CQ, Liang YY, Liang HW, Chen G and Yang YW: Lower expressed miR-198 and its potential targets in hepatocellular carcinoma: A clinicopathological and in silico study. Onco Targets Ther 9: 5163-5180, 2016.
6. Torres LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
7. Xu Y, Qi Y, Luo J, Yang J, Xie Q, Deng C, Su N, Wei W, Shi D, Xu F, et al: Hepatitis B Virus X protein stimulates proliferation, wound closure and inhibits apoptosis of HuH-7 cells via CDC42. Int J Mol Sci 18: 18, 2017.
8. Yu C, Cao Q, Chen P, Yang S, Gong X, Deng M, Ruan B and Li L: Tissue transglutaminase 2 exerts a tumor-promoting role in hepatitis B virus-related hepatocellular carcinoma. Tumour Biol 37: 16269-16274, 2016.
9. Gong X, Wei W, Chen L, Xia Z and Yu C: Comprehensive analysis of long non-coding RNA expression profiles in hepatitis B virus-related hepatocellular carcinoma. Oncotarget 7: 42422-42430, 2016.
52. Bornstein S, Schmidt M, Choonoog G, Levin T, Gray J, Thomas CR Jr, Wong M and McWeeny S: IL-10 and integrin signaling pathways are associated with head and neck cancer progression. BMC Genomics 17: 38, 2016.

53. Zeng JH, Xiong DD, Pang YY, Zhang Y, Tang RX, Luo DZ and Chen G: Identification of molecular targets for esophageal carcinoma diagnosis using miRNA-seq and RNA-seq data from The Cancer Genome Atlas: A study of 187 cases. Oncotarget 8: 35681-35699, 2017.

54. Rhodes DR, Kalyavana-Sundaram S, Mahavivino V, Varambally R, Yu J, Briggs BB, Barrette TR, Anstet MJ, Kinead-Beal C, Kulkarni P, et al: Oncomine 3.0: Genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia 9: 166-180, 2007.

55. Adler P, Kolde R, Kull M, Tkachenko A, Peterson H, Reimand J et al: STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 41D: D808-D815, 2013.

56. Rhodes DR, Kalyana-Sundaram S, Mahavisno v, varambally R, et al: Comprehensive analysis of The Cancer Genome Atlas reveals a unique gene and non-coding RNA signature of fibrolamellar carcinoma. Sci Rep 10: R139, 2009.

57. Li CQ, Huang GW, Wu ZY, Xu YJ, Li XC, Xue YJ, Zhu Y, Zhao JM, Li M, Zhang J, et al: Integrative analyses of transcriptome sequencing identify novel functional IncRNAs in esophageal squamous cell carcinoma. Oncogenesics 6: e297, 2017.

58. Dinh TA, Vitucci EC, Wauthier E, Graham RP, Pitman WA, et al: The clinical value of IncRNA NEAT1 in digestive system malignancies: A comprehensive investigation based on 57 microarray and RNA-seq datasets. Oncotarget 8: 17665-17683, 2017.

59. Li J, Huang Q, Long X, Zeng Z, Yu Z, Zeng W and Wang K: Assessing the clinical value of microRNA sequencing in the clinical diagnosis of hepatocellular carcinoma. Mol Med Rep 16: 3371-3378, 2017.

60. Gao H, Wang H and Yang W: Identification of key genes and pathways using novel rank aggregation and visualization methods. Genome Biol 10: R139, 2009.