ncRNAs-mediated high expression of TICRR promotes tumor cell proliferation and migration and is correlated with poor prognosis and tumor immune infiltration of hepatocellular carcinoma

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TICRR is a regulatory factor of DNA replication with ToPB1 interaction. At present, the underlying function and mechanisms of TICRR remain unclear in LIHC. Our objective was to assess the function and prognosis of TICRR in LIHC. We conducted a differential expression analysis, GO/KEGG, and GSEA enrichment analysis of TICRR in LIHC. We also carried out the gene frequency and SCNA of TICRR. We found that TICRR could serve as an independent prognostic marker in LIHC by univariate and multivariate analysis. In addition, we observed that TICRR was related to immune infiltration, and TICRR had positive correlation with PD1/PD-L1 and CTLA-4 in LIHC. The hsa-miR-126-3p/IPO9-AS1 may be the candidate ncRNAs to regulate the expression of TICRR. The high rate of SCNV of TICRR might have critical effect on the function of CTL cells in LIHC. We further demonstrate through a series of experiments that TICRR facilitated the proliferation and metastasis of liver cancer cells in vitro. Altogether, TICRR might be a potential biomarker and therapeutic target in LIHC.

INTRODUCTION
Hepatocellular carcinoma is one of the most frequent malignancies. Approximately 745,000 people worldwide die from hepatocellular carcinoma,1 making it the second most common tumor-related death.2 According to statistical analyses, the prevention and treatment of liver cancer has become a emergentissue that needs to be addressed. Patients with liver cancer are often detected in advanced tumor stages, resulting in a loss of feasibility for treatment. Owing to a lack of suitable molecular targets for liver hepatocellular carcinoma (LIHC), so systemic chemotherapy is limited.3 The clinical prognosis of patients is poor because the cancer tend to develop resistance to drugs in the advanced cancer. Therefore, searching for new and specific biomarker of liver cancer has important value for guiding clinical treatment and improving the survival rate of patients.

TICRR has strong association with DNA replication and repair. It promotes DNA replication by regulating s-phase.4,5 We performed bioinformatics analyses and observed that overexpression of TICRR in pan-cancer, such as hepatocellular carcinoma and uterine corpus endometrial carcinoma.6 In addition, TICRR is linked to tumorigenesis, chemoresistance, and a worse clinical prognosis. However, the underlying mechanisms of TICRR remain unclear in LIHC.

We comprehensively researched the expression, prognosis, and immunological interaction of TICRR in LIHC. First, we find that TICRR expressed highly in liver cancer. Next, we predicted the association between the TICRR messenger RNA (mRNA) expression and prognosis in LIHC by KM plotter and Cox regression analysis. It is known that the infiltration of immune cells is strongly associated with tumor progression.7–10 This paper analyzed the potential relevance between TICRR and immunocytes infiltration by R package GSVA and single sample GSEA (ssGSEA) algorithm.11 We performed GSEA and gene ontology/Kyoto Encyclopedia of Genes and Genomes (GO/KEGG) analyses to discover important biological pathways based on the database from MSigDB.12 We explored the gene frequency and copy number alteration of TICRR in LIHC. We found that TICRR had a negative correlation with programmed cell death 1/programmed cell death ligand 1 (PD1/PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) in LIHC. We further demonstrate through a series of experiments that TICRR facilitated the proliferation and metastasis of liver cancer cells in vitro.
These findings may provide novel ideas and insights for further search the proliferation mechanism for LIHC. TICRR might be a promising molecular target for clinical treatment of hepatocellular carcinoma.

RESULTS

Differential expression and clinical prognostic of TICRR in LIHC

The mRNA expression of TICRR in multiple tumor types were shown in Figures 1A and 1B. TICRR expression was highly elevated between the tumor and normal tissues of liver by boxplot (N = 50; T = 374) and paired boxplot (N = 50; T = 50) (Figures 1C and 1D). Kaplan-Meier (K-M) survival analyses showed that patients with low TICRR gene expression survived longer than patients with high TICRR gene expression based on overall survival (OS), disease-specific survival (DSS), and progression-free survival (PFI) (Figures 1F, 1G and 1H). The GSE54236 dataset from GEO was selected for verification (Figure S1A). In addition, the receiver operating characteristic (ROC) curves of TICRR associated with and area under the curve (AUC) of 0.970 (Figure 1E), indicating that the predicted efficacy of TICRR gene was accurate.

TICRR expression and clinically relevant association

We used a boxplot to show associations between TICRR and eight clinical characteristics including alpha fetoprotein (AFP), sex, race, pathologic T/N/M stage, and age (Figure 2). The clinical stage, T stage, and AFP were significantly correlated with TICRR mRNA expression. The TICRR mRNA expression among the patients with stages III and IV disease was higher than stages I and II (p < 0.01), T3 and T4 was higher than T1 and T2 (p < 0.01), and an AFP of greater than 400 was higher than an AFP of 400 or less (p < 0.001).

Clinical significance of TICRR in LIHC

We assessed the prognostic impacts between TICRR and clinical characteristics and identified whether these variables were independent prognostic factors by univariate and multivariate analyses based on OS (Figures 3A and 3C). The results showed that TICRR could be used as an independent biomarker for LIHC. Detailed information is provided in Table S1.

We constructed the prognostic nomogram to visually show associations between clinicopathological variables (Figure 3E) and survival rates of patients based on the OS. Calibration curves at 1, 3, and 5 years showed that our results were consistent with the predicted values, indicating that the SCNA of TICRR may play a critical role in LIHC.

We divided 374 LIHC patients into low- and high-expression groups. Next, we screened significantly differentially expressed TICRR-related genes from the two groups, displaying representative genes through heatmaps (Figure 4G). Then we carried out GO/KEGG to predict the pathways of TICRR-related genes (Figures 4H and 4I). There are several TICRR-correlated pathways such as organelle fission (GO:0048285), nuclear division (GO:0000280), spindle (GO:0005819), mitotic nuclear division (GO:0140014), nuclear chromosome segregation (GO:0008813) (Table S2), complement and coagulation cascades (KEGG: hsa04610), and the Fanconi anemia pathway (KEGG: hsa03460) (Table S3).

The SCNA of TICRR had an effect on the immune and clinical prognoses

Furthermore, we analyzed the gene mutation of TICRR in various tumors. The mutational frequency of uterine corpus endometrial carcinoma (UCEC) and skin cutaneous melanoma were higher compared with other tumors types. Mutation frequency in UCEC could reach approximately 8%, although the frequency of liver cancer was only approximately 0.5% (Figure 5A). There was a somatic copy number alteration (SCNA) in a variety of tumors, including deep deletion, arm-level deletion, diploid/normal, arm-level gain, and high amplification. The arm-level deletion, diploid/normal, and arm-level gain were major types of variation in LIHC (Figure 5D). The neutrophils and myeloid dendritic cells were increased when high amplification occurred in LIHC, and the neutrophils were decreased in the group of the arm-level gain compared with the diploid/normal (Figures 5B and 5C). The clinical prognosis of CTL bottom when the SCNA of TICRR was low. The OS was not significantly different when the SCNA of TICRR was high (Figure 5F). This finding indicated that TICRR might have critical effect on the function of cytotoxic T lymphocyte (CTL) cells in LIHC. The high rate of SCNV had a better prognosis in the group of TICRR top (n = 312) compared with the TICRR bottom (n = 27) (Figure 5E). The results indicated that the SCNA of TICRR may play a critical role in LIHC.

Hsa-miR-126-3p/IP09-A51 had an effect on TICRR

We found that microRNA (miRNA) (hsa-miR-126-3p) had a negative correlation with TICRR (r = −0.228, p < 0.05) (Figure 6C). The clinical prognosis was significantly better when hsa-miR-126-3p was highly expressed than hsa-miR-126-3p (p = 0.0078) low expression (Figure 6B), and hsa-miR-126-3p was less expressed in LIHC tissue (Figure 6A). The results indicated that hsa-miR-126-3p might negatively...
Figure 1. The expression levels of TICRR
(A and B) The mRNA expression levels of TICRR in pan-cancers by boxplot and paired boxplot. (C) The TICRR mRNA expression by boxplot in LIHC (N = 50; T = 374). (D) The TICRR mRNA expression by paired boxplot in LIHC (N = 50; T = 50). (E) ROC curves. (F–H) K-M survival analysis of TICRR in LIHC. *p < 0.05; **p < 0.01; ***p < 0.001.
regulate TICRR. The upstream of hsa-miR-126-3p was further explored based on the starBase database. Finally, we found that five long intergenic coding RNA (linRNA) had a better correlation with hsa-miR-126-3p including IPO9-AS1, AC069281.2, LINC01003, AC009403.1, and XIST. The IPO9-AS1, AC069281.2, LINC01003, and AC009403.1 were highly expressed in LIHC (Figure 6D). The clinical prognosis was significantly poorer when IPO9-AS1 and AC009403.1 were highly expressed based on the OS and DSS (Figures 6E–6H). IPO9-AS1 had an obvious association with TICRR ($r=0.510; p<0.001$) and hsa-miR-126-3p ($r=-0.120; p=0.026$).
AC009403.1 was also related to TICRR \( (r = 0.190; p < 0.001) \) and hsa-miR-126-3p \( (r = -0.039; p = 0.452) \) (Figures 6I–6L). It demonstrated that hsa-miR-126-3p/IPO9-AS1 may regulate TICRR expression.

High expression of TICRR was related to immune infiltration in LIHC

We used the R package GSVA and ssGSEA algorithm to infer the immune infiltrating (Figure 7A). We discovered that six immune cell
(legend on next page)
types have significant correlation with TICRR, including T helper type 2 (Th2) cells, neutrophils, dendritic cells (DCs), cytotoxic cells, CD8 T cells, and T helper cells (Figures 7B–7G).

Next, we performed a Spearman test to analyze the correlation with TICRR. The result demonstrated that CD8 T cells, cytotoxic cells, DCs, neutrophils, T gamma delta (Tgd), and plasmacytoid DC (pDC) showed a negative association with TICRR high expression. However, Th2 cells, T follicular helper (Tfh), and T helper cells showed a positive association (Figure 7H). In addition, we observed that chemokine C-C motif ligand (CCL16) and CCL14 had negative correlation with TICRR in LIHC (Figure S2). All p values were less than 0.001.

**TICRR facilitates the proliferation and metastasis of liver cancer cells in vitro**

Given that there was a strong correlation between TICRR expression in LIHC and a worse prognosis of LIHC patients, we further explored whether TICRR facilitates the proliferation and migration of liver cancer cells. We interfered TICRR expressions in the SK-hep-1 and Huh7 cells by using si-TICRR (Figure 8B). CCK8 and colony formation assays showed that TICRR interference significantly disrupted the proliferation and colony formation of SK-hep-1 and Huh7 cells (Figures 8A and 8C). Moreover, transwell assay illustrated that TICRR interference markedly suppressed the invasiveness of SK-hep-1 and Huh7 cells (Figure 8D). In addition, apoptosis was identified as a key factor contributing to liver cancer cell growth, so we conducted flow cytometry to detect the apoptosis level. The results demonstrated that TICRR knockdown by si-TICRR significantly increased the percentage of apoptotic cells (Figure 8E). We also explored the association between TICRR and marker for cell proliferation (MKI67) and metastasis (matrix metalloproteinase 9 [MMP9]). Our results indicated that TICRR was positively correlated with MKI67, MMP9, NOTCH1, and HES1. However, activation of NOTCH1 signaling can contribute to cancer cell stemness, invasion, and metastasis (Figures 8F–8I). Collectively, our data indicated that si-TICRR markedly suppressed the proliferation and metastasis of liver cancer cells, and the proper regulation for its activity might be a promising strategy for LIHC treatment.

**TICRR is positively associated with PD1/PD-L1 and CTLA-4**

PD1/PD-L1 and CTLA-4 are important immune checkpoint. The results of a gene coexpression network demonstrated that the TICRR had a positive association with PD1/PD-L1 and CTLA-4 in LIHC (Figure 9A). We further analyzed the correlation by the Spearman test. PD1/PD-L1 and CTLA-4 showed a positive association with TICRR in liver cancer (Figures 9B–9D). We also analyzed the correlation by GEPIA resource (Figures 9E–9G).

**DISCUSSION**

We explored the expression profile, clinicopathological association, prognostic value, pathway prediction, immune infiltration, and clinical significance of TICRR in LIHC. We observed that TICRR was highly increased in LIHC. Patients with low TICRR gene expression survived longer than those with high TICRR gene expression. TICRR was significantly associated with clinical stage, T, and AFP, and it was significantly related to neuroactive ligand receptor interaction, cell cycle, and DNA replication. In addition, the copy number variation of TICRR had an effect on the immune and clinical prognoses. We predicted that hsa-miR-126-3p and upstream linRNA may regulate the expression TICRR. TICRR shows a significant association with immune infiltration and immune checkpoint (PD1/PD-L1 and CTLA-4). We further demonstrate through a series of experiments that TICRR facilitated the proliferation and metastasis of liver cancer cells in vitro. The results showed that TICRR could serve as a potential biomarker in LIHC.

As an important regulator of DNA replication, the expression of TICRR is increased in other tumors, such as breast invasive carcinoma. TICRR high expression indicated a poor clinical prognosis in LIHC. Uncontrolled cell proliferation is important mechanism of tumorigenesis. In our study, TICRR was significantly associated with the cell cycle by GSEA and GO/KEGG. Studies have reported that lacking TICRR can damage DNA replication and cell cycle checkpoints, which contributes to the induction of consistently increased cell proliferation. In conclusion, TICRR may have important effect on tumorigenesis.

TICRR was significantly related to clinical prognosis in LIHC. TICRR was highly expressed in LIHC and other types of tumors compared with adjacent normal tissues. A wide range of high expression indicates that TICRR may serve as a common predictive prognostic biomarker. A K-M plot demonstrated that patients with TICRR high expression showed a worse clinical prognosis compared with low expression based on OS, DSS, and PFI in LIHC. In addition, the area under ROC curve of TICRR was 0.970, indicating that the predicted efficacy was accurate. TICRR mRNA expression was significantly associated with clinical stage, T, and AFP. We also found that clinical stage and TICRR were strongly correlated based on the OS by univariate and multivariate Cox analyses. Next, we constructed a nomogram to analyze and visualize the prognostic multivariate regression model. Calibration curves at 1, 3, and 5 years indicated the satisfactory performance. We suggest that TICRR could serve as a biomarker of poor prognosis in patients with LIHC.

We also observed the SCNA and mutational frequency in liver cancer. SCNA and gene mutation play critical roles in tumorigenesis and development. The mutation frequency of liver cancer was only
approximately 0.5%, while the arm-level deletion, diploid/normal, and arm-level gain were major types of variation. The high rate of SCNV had a better prognosis. The clinical prognosis was significantly better in the high CTL infiltration when the SCNA of TICRR was low, while the OS was no significant differences when the SCNA of TICRR was high. These results demonstrated that TICRR may have an important influence on the function of CTL cells in LIHC.

We also explored the potential association among TICRR and immune cells. The result indicated that Th2 cells, T helper cells, and Tfh were all positively related to TICRR expression by R package GSVA and ssGSEA algorithms. There are reports in the literature that patients with low anti-tumor lymphocyte (TH1 and CTL) infiltration and high immunosuppressive lymphocyte (TH2) infiltration in the tumor microenvironment had significantly poorer prognosis. It is
Figure 6. hsa-miR-126-3p/IPO9-AS1 might regulate TICRR
(A) hsa-miR-126-3p was low expression in LIHC. (B) The clinical prognosis of hsa-miR-126-3p. (C) The correlation between TICRR and hsa-miR-126-3p. (D) lincRNA expression in upstream of hsa-miR-126-3p in LIHC. (E-H) The clinical prognosis of IPO9-AS1 and AC009403.1 based on OS and DSS in LIHC. (I and L) The correlation of IPO9-AS1 and AC009403.1 with TICRR. (J and K) The correlation of IPO9-AS1 and AC009403.1 with hsa-miR-126-3p.
Figure 7. High expression of TICRR was related to immune infiltration in LIHC

(A) The correlation between TICRR and immune calls. NK, natural killer.

(B–G) TICRR was closely related to Th2 cells, neutrophils, DCs, cytotoxic cells, CD8+ T cells, and T helper cells. (H) The association between TICRR and immune by the Spearman test.
consistent with our results.\textsuperscript{28} However, CD8 T cells, cytotoxic cells, DCs, neutrophils, Tgd, and pDC showed a negative association with TICRR high expression. The CD8$^+$ T cell is one of the most toxic T cells, which have important effect on eliminating cancerous cells.\textsuperscript{29} DCs are one of antigen-presenting cells that mediate antigen cross-presentation to activate CD8$^+$ T cells, playing a critical role in initiating anti-tumor immunity.\textsuperscript{30–32} These results further demonstrate that TICRR high expression could inhibit the antitumor immunity
to some extent. Interestingly, a high TICRR expression phenotype was significantly related to primary immunodeficiency signaling pathways by GSEA. In addition, neutrophils have been demonstrated to have a strong correlation with the clinical prognosis of cancer.33,34 Therefore, overexpressed TICRR may accelerate tumorigenesis by inhibiting tumor immunity. In addition, we observed that immune checkpoint (PD1/PD-L1 and CTLA-4) showed a positive correlation with TICRR, while PD1/PD-L1 could help the tumor to evade immunological surveillance according to some relevant studies.35–37

We further explored the potential pathways of TICRR in LIHC. Studies have reported that Treslin/TICRR have important effects on regulating the DNA replication.38,39 We observed that TICRR was strongly related to cell cycle and DNA replication by GSEA. A GO/KEGG analysis revealed TICRR-related pathways including organelle fission, nuclear division, and spindle and mitotic nuclear division. In another recent study focusing on breast cancer, researchers found that silencing the TICRR expression could significantly suppress DNA replication.21 It is reported that non-coding RNAs have a significant influence in regulating gene expression.40-42 We found that hsa-miR-126-3p had a negative correlation with TICRR, and hsa-miR-126-3p was less expressed in LIHC. Survival was significantly longer when hsa-miR-126-3p was highly expressed. We further predicted five liRNA that were upstream of hsa-miR-126-3p. These data demonstrate that TICRR is a key regulator of DNA replication initiation plays an important role in LIHC. If a molecular drug target of

Figure 9. High TICRR expression could promote tumor immune escape
(A) The results of gene coexpression network. (B–D) The association between TICRR and PD1/PD-L1 and CTLA-4 by the Spearman test. (E and G) The association between TICRR and immune checkpoint by GEPIA database in LIHC.
the TICRR can be developed for the treatment of liver cancer, it may be a great significance.

We further demonstrate through a series of experiments that TICRR facilitated the proliferation and metastasis of liver cancer cells in vitro. We interfered TICRR expressions in the SK-heap-1 and Huh7 cells by using si-TICRR. In addition, CCK8 and colony formation assays showed that TICRR interference significantly disrupted the proliferation and colony formation of SK-heap-1 and Huh7 cells. Moreover, a transwell assay illustrated that TICRR interference markedly suppressed the invasiveness of SK-heap-1 and Huh7 cells. Apoptosis was identified as a key factor contributing to liver cancer cell growth, so we conducted flow cytometry to detect the apoptosis level. The results demonstrated that TICRR knockdown by si-TICRR significantly increased the percentage of apoptotic cells. We also explored the association between TICRR and marker for cell proliferation (MKI67) and metastasis (MMP9). Our results indicated that TICRR was positively correlated with MKI67, MMP9, NOTCH1, and HES1. However, the activation of NOTCH1 signaling can contribute to cancer cell stemness, invasion, and metastasis. These results suggested that TICRR facilitates the proliferation and metastasis of liver cancer cells in vitro.

In conclusion, this study elucidates that TICRR could serve as a potential therapeutic target and independent prognostic indicator for LIHC patients. And TICRR facilitates the proliferation and metastasis of liver cancer cells in vitro. The hsa-miR-126-3p/IPO9-AS1 may be a potential therapeutic target and independent prognostic indicator for LIHC patients. TICRR knockdown by si-TICRR significantly increased the percentage of apoptotic cells. We also explored the association between TICRR and marker for cell proliferation (MKI67) and metastasis (MMP9). Our results indicated that TICRR was positively correlated with MKI67, MMP9, NOTCH1, and HES1. However, the activation of NOTCH1 signaling can contribute to cancer cell stemness, invasion, and metastasis. These results suggested that TICRR facilitates the proliferation and metastasis of liver cancer cells in vitro.

In conclusion, this study elucidates that TICRR could serve as a potential therapeutic target and independent prognostic indicator for LIHC patients. And TICRR facilitates the proliferation and metastasis of liver cancer cells in vitro. The hsa-miR-126-3p/IPO9-AS1 may be the candidate ncRNAs to regulate the expression of TICRR. However, one drawback of the present study was that our results were generated from a bioinformatics analysis, which was not further validated by experiments. Further experiments are planned to resolve this issue. In addition, our results further make us understand the prognostic value of TICRR. It will be of great significance to help health care workers guide clinical decision-making.

MATERIALS AND METHODS
The Cancer Genome Atlas LIHC dataset and difference analysis
Data were mined based on The Cancer Genome Atlas (TCGA), which is an open-access database by bioinformatic approach. RNA sequencing data and relevant clinical information of patients were downloaded from the TCGA database, including 374 tumor samples and 50 adjacent tissues as training sets. Differences in TICRR expression were identified by Wilcoxon rank-sum tests between tumor and normal tissues. We conducted it through version R 3.6.3 and ggplot2.

The analysis of candidate miRNA and pathway prediction
We carried out GO/KEGG analysis via R package clusterProfiler to investigate the major biological effects of TICRR in hepatocellular carcinomas. The results are visualized by ggplot2. A p value of less than 0.05 was considered significant. We also revealed the different pathways between TICRR higher and lower expression groups by GSEA. Gene sets (‘c2.cp.v7.2.symbols.gmt’) from the MSigDB database (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp). We can discover important biological pathways by this way with the threshold of a false discovery rate of less than 0.25. The potential interaction was predicted among TICRR and other relevant genes by online STRING and GeneMANIA databases. We further explored the miRNA and upstream lncRNA of TICRR in the starBase resource.

Clinical implications of TICRR in LIHC
We also explored the prognostic efficacy of TICRR in hepatocellular carcinoma by ROC curve. The results were analyzed and visualized by R package pROC and ggplot2. Diagnostic accuracy was calculated by the AUC. We evaluated the prognostic value of TICRR based on disease-free survival, PFI, and OS by K-M survival curves. The results are analyzed by the R package survminer and survival. Cox regression was used for univariate and multivariate analyses, which evaluate the prognostic factors in LIHC. A prognostic nomogram is used to analyze and visualize the prognostic multivariate regression model. The R package rms is used to analyze the OS of LIHC patients at 1, 3, 5 years and generate a prognostic nomogram based on the clinical information. At the same time, the prediction effect is evaluated by constructing calibration model. The above statistical analysis is carried out by R version 3.6.3.

TICRR is related to immune infiltrating, gene mutations, and copy number variations
Gene mutations of TICRR were analyzed in pan-cancer by cBioPortal database. We further explored the copy number variations of TICRR in the TIMER database. We performed the clinical prognosis and T cell dysfunction based on the copy number variations of TICRR by TIDE database. R package GSVA and performed ssGSEA algorithm to calculate and infer the immune infiltrating by spearman test. We further performed the relation among TICRR and immune cells in the TISIDB database. We also explored the association between TICRR and PD1/PD-L1 and CTLA-4 by R package GSVA. The above statistical analysis is carried out by R version 3.6.3.

Cell culture and transfection
SK-heap-1 and Huh7 cell lines without mycoplasma were provided by Procell (Wuhan, China). SK-heap-1 cell line was grown in MEM (Gibco, Grand Isle, NY) medium and Huh7 cell line was incubated in DMEM (Gibco) containing 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen, Waltham, MA) at 37°C with 5% CO₂. Lipofectamine 2000 (Invitrogen) was used to transfect si-TICRR and negative control si-NC. After 24 or 48 h of transfections, the cells were harvested. All of the above small interfering RNA sequences are listed in Table S4.

Cell proliferation and migration assays
The CCK8 method was used to detect cell viability thereby assessing cell proliferation. In brief, the transfected SK-heap-1 and Huh7 cells (4,000 cells/well) with si-TICRR were seeded in 96-well plates. Next, cell viability was detected every 24 h based on CCK8 kit protocols. For colony formation assay, 400 cells were inoculated into six-well plates and cultured at 37°C for 14 days. Then, the colonies were fixed with methanol and stained with hematoxylin. All trials
were conducted in triplicate. Transwell migration assay was carried out to measure cell migration. Transwell chambers (Corning, Corning, NY) have a membrane pore size of 8 μm. A total of 3 × 10^4 were inoculated in the upper chambers. Then, the lower chamber was filled using the medium with 10% fetal bovine serum. The cells were fixed, stained, and counted in an inverted microscope after 24 h incubation. All trials were conducted in triplicate. Total RNA was extracted from liver cancer cells with TRIzol (Invitrogen). All of the primer sequences are listed in Table S4.

**Flow cytometry analysis for apoptosis**

After 48 h transfection with si-TICRR or si-NC, SK-hep-1 and Huh7 cells were harvested. Next, these cells were stained based on the protocols of a FITC Annexin V Apoptosis Detection kit (Meilunbio, Dalian, China), and were detected by flow cytometry (Beckman Coulter, Brea, CA). Finally, the results were analyzed with FCP Array software.

**Statistical analyses**

All trials were independently conducted at least three times, with the samples in triplicate. All statistical analyses were carried out with GraphPad Prism 8.0 software. The Student t-test or one-way ANOVA, followed by the Tukey test for multiple comparisons, was used to compare biological themes among gene clusters. OMICS A J. Integr. Biol. 6, 284–287.

**DATA AVAILABILITY**

The raw data supporting the conclusions of this article will be made available by the authors, and further inquiries can be directed to the corresponding authors. The related data, figures and tables has not been previously published and that the manuscript is not under consideration elsewhere.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2022.09.007.

**ACKNOWLEDGMENTS**

This study was supported by the National Natural Science Foundation of China (no. 81970460, 81770548) in China, and the Key Laboratory of intestinal microbiome and human health in Xiamen, China.

**AUTHOR CONTRIBUTIONS**

H.K.J. designed this work. H.K.J., O.X.M., G.G.Y., and Z.Y.F. performed bioinformatic analyses. H.K.J., C.X.S., L.Y. and L.Y. wrote the manuscript. G.B. revised the manuscript and supervised the whole experiment. All authors have read and approved the final submitted manuscript.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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