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Transcription factor 7 like 2 promotes metastasis in hepatocellular carcinoma via NEDD9-mediated activation of AKT/mTOR signaling pathway

Linsong Tang^{1,6†}, Shengjun Xu^{2,4,6†}, Rongli Wei⁴, Guanghan Fan⁴, Junbin Zhou⁵, Xuyong Wei^{4,6} and Xiao Xu^{2,3,6*} 

Abstract

Background Hepatocellular carcinoma (HCC) is one of the most common malignant tumors of the digestive system, and the exact mechanism of HCC is still unclear. Transcription factor 7 like 2 (TCF7L2) plays a pivotal role in cell proliferation and stemness maintenance. However, the exact mechanism of TCF7L2 in HCC remains unclear.

Methods Clinical samples and public databases were used to analyze the expression and prognosis of TCF7L2 in HCC. The function of TCF7L2 in HCC was studied in vitro and in vivo. ChIP and luciferase assays were used to explore the molecular mechanism of TCF7L2. The relationship between TCF7L2 and NEDD9 was verified in HCC clinical samples by tissue microarrays.

Results The expression of TCF7L2 was upregulated in HCC, and high expression of TCF7L2 was associated with poor prognosis of HCC patients. Overexpression of TCF7L2 promoted the metastasis of HCC in vitro and in vivo, while Knockdown of TCF7L2 showed the opposite effect. Mechanically, TCF7L2 activated neural precursor cell expressed developmentally downregulated protein 9 (NEDD9) transcription by binding to the -1522/-1509 site of the NEDD9 promoter region, thereby increasing the phosphorylation levels of AKT and mTOR. The combination of TCF7L2 and NEDD9 could distinguish the survival of HCC patients.

Conclusions This study demonstrated that TCF7L2 promotes HCC metastasis by activating AKT/mTOR pathway in a NEDD9-dependent manner, suggesting that potential of TCF7L2 and NEDD9 as prognostic markers and therapeutic targets for HCC.

Keywords Hepatocellular carcinoma, TCF7L2, NEDD9, AKT/mTOR pathway, Tumor metastasis

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Background

HCC accounts for ~80% of liver cancer and represents the third cancer-related mortality globally (Sung et al. 2021). Surgery is still the most important treatment for early-stage HCC patients, with a five-year survival rate exceeding 70% (Tang et al. 2022). Multidisciplinary comprehensive treatment including radiotherapy, targeted therapy and immunotherapy is currently the main therapeutic strategy of advanced-stage HCC patients (Tang et al. 2020). Although great progress has been made in the treatment of HCC, some advanced-stage HCC patients do not benefit significantly from the current treatment options (Zhang et al. 2022a, b). Therefore, further elucidation of the mechanisms of HCC is required.

The Wnt/ β -catenin pathway is one of the most frequently mutated pathways in HCC, with about half of HCC showing activation of the Wnt/ β -catenin pathway (Vilchez et al. 2016). After Wnt activation, β -catenin enters the nucleus and binds to TCF/LEF family proteins to activate the transcription of downstream target genes, which plays an important role in the progression and metastasis of HCC (Anastas and Moon 2013). TCF7L2, also known as transcription factor 4 (TCF4), plays a crucial role in embryonic development, cell stemness regulation and tissue renewal (Hrckulak et al. 2016). TCF7L2 contains a β -catenin binding domain that functions as a downstream effector of the Wnt pathway (Jin 2016). Previous studies have linked TCF7L2 to diseases such as diabetes, obesity, and metabolic syndrome (Zhang et al. 2021). Indeed, TCF7L2 is considered to be the strongest risk factor for type 2 diabetes (Lee et al. 2023). Recent studies have also shown that TCF7L2 can affect the progression of cancer. For example, functional deficiency of TCF7L2 promotes metastasis of colorectal cancer (Wenzel et al. 2020). In gastric cancer, TCF7L2 promotes the proliferation and metastasis of cancer cell by activating downstream target genes (Zhang et al. 2022a, b). TCF7L2 has also been reported to be involved in the tumorigenesis of prostate cancer and breast cancer (Wang et al. 2021; Drake et al. 2014). It has been found that the tumorigenic ability of HCC cell lines is decreased after knocking down the expression of TCF7L2 (Malakar et al. 2019). However, the specific mechanism of TCF7L2 in HCC remains unclear.

In present study, we found that the expression of TCF7L2 was upregulated in HCC. Knockdown of TCF7L2 could significantly inhibit the invasion and metastasis of HCC. Furthermore, we clarified that TCF7L2 promotes the progression of HCC via NEDD9-dependent regulation of AKT/mTOR pathway. The combination of TCF7L2 and NEDD9 could distinguish the survival of HCC patients.

Materials and methods

Data collection pre-processing

The mRNA expression data of TCF7L2 in GSE14520, GSE46465 were afforded from GEO (Gene expression omnibus) database and processed by GEO2R. The expression and clinical information of HCC patients were obtained from TCGA (The Cancer Genome Atlas) cohort (<https://portal.gdc.cancer.gov/>). The data of the interaction between transcription factors and target genes were obtained from three databases: hTFtarget (<http://bioinfo.life.hust.edu.cn/hTFtarget/>), CHEA (<https://maayanlab.cloud/Harmonizome/>) and ChIPBase (<http://rna.sysu.edu.cn/chipbase/>), respectively (Zhang et al. 2020; Lachmann et al. 2010; Rouillard et al. 2016; Zhou et al. 2017a, b).

Clinical samples

The cancer tissues and normal liver tissues in this study were obtained from HCC patients who underwent surgical resection in the First Affiliated Hospital, Zhejiang University School of Medicine. Patients were included with criteria: pathological diagnosis of HCC; did not receive preoperative radiotherapy, chemotherapy and other treatment; adjacent normal liver tissues were isolated from at least 2 cm away from the cutting edge; had complete postoperative follow-up data. Samples from a total of 87 HCC patients were fabricated into tissue microarray for subsequent expression and survival analysis. The clinical characteristics of the 87 patients were shown in Table S1. The patients involved in this study had signed an informed consent form. This study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine.

Cell culture

Human cell lines of HCC, including Hep3B, Li-7, HepG2, Huh7, PLC/PRF/5 and SK-Hep-1 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. HCCLM3, MHCC97-H, MHCC97/L and SNU-449 were purchased from American Type Culture Collection, USA. HCC cell lines were cultured in appropriate medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in humidified air containing 5% CO₂.

Western blotting

Total cell protein was extracted from HCC cells and lysed in lysis buffer. The protein samples were separated by SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis), transferred to PVDF (Polyvinylidene Fluoride) membranes, and blocked in TBST containing 5% skim milk. Then the membranes were probed with primary antibodies and secondary antibodies separately. The membranes were detected by Image Lab software (Bio-Rad, Hercules, CA). In total,

primary antibodies included: β -actin (66009-1-Ig, Proteintech), TCF7L2(#2565, Cell Signaling Technology), NEDD9(#4044, Cell Signaling Technology), phospho-AKT (66444-1-Ig, Proteintech), total-AKT (60203-2-Ig, Proteintech), phospho-mTOR (67778-1-Ig, Proteintech), total-mTOR (66888-1-Ig, Proteintech).

Quantitative real-time PCR

Total RNA of HCC cells was extracted by Cell/Tissue Total RNA Kit (19221ES50, Yeasen), and quantitative real-time PCR was performed. All operations followed the manufacturer's protocol. Primer sequences (Tsingke Biological Technology) used in this study were listed in Table S2.

Immunohistochemical (IHC) staining

The immunohistochemical staining was performed as the previously described (Zhuo et al. 2021). The primary antibodies against TCF7L2(#2565, Cell Signaling Technology), NEDD9(AP14592c, Abcepta) were used at concentrations of 1:500 and 1:100, respectively. IHC score was calculated by cell staining intensity multiplied the proportion of positive cells. Cell staining intensity was defined as follows: 0 (none), 1 (pale yellow), 2 (brownish yellow) and 3 (brown). Proportion of positive cells was defined as: 1 (0–25%), 2 (26–50%), 3 (51–75%) and 4 (>75%). IHC score less than 7 (≤ 7) was defined as low expression, whereas the others represented high expression. All stained slides were determined independently by two pathologists.

Gene overexpression or knockdown

TCF7L2 overexpression lentivirus was produced by OBiO Technology. Small hairpin RNA (shRNA) targeting TCF7L2 knockdown lentivirus was produced by GeneChem. Small interfering RNA (siRNA) targeting NEDD9 was designed by GenePharma. For stable cell clone, after the growth of lentivirus-transfected cells reached 80–90%, puromycin was used for screening. siRNA was transfected by jetPRIME[®] (Polyplus) following the manufacturer's protocol. The sequences were listed in Table S2.

Cell growth and metastasis assays

Cell proliferation was assessed by Cell counting kit-8 (CCK8) assay (Yeasen). Cell metastasis was assessed by wound healing assay, transwell migration and invasion assays using 24-well Transwell chamber system (Corning). The experimental method was described in the previous study (Chen et al. 2021).

ChIP-qPCR

SimpleChIP[®] Enzymatic Chromatin IP Kit (Cell Signaling Technology) was used for Chromatin

Immunoprecipitation (ChIP). The chromatin complexes were co-immunoprecipitated by anti-TCF7L2 or IgG antibodies, and the bound DNA fragments were analyzed by qPCR. Primer sequences of human NEDD9 promoter were provided in Table S2.

Luciferase reporter assays

A 2000 bp of NEDD9 promoter fragment was cloned into the pGL4.21 Vector (NEDD9-WT), and a NEDD9 promoter fragment with random mutation of binding site was constructed into pGL4.21 Vector (NEDD9-MUT), both of them were synthesized and sequenced by Synbio Biotechnology Co., LTD. The two plasmids and pRL-TK Vector (with renilla luciferase) were transfected into cells, respectively. After 24 h, cells were lysed and then assayed for luciferase activities with the Dual-Glo[®] Luciferase Assay System (Promega). The relative luciferase intensity of the reporter gene was the ratio of firefly luciferase to renilla luciferase.

Animal experiments

Orthotopic HCC implantation models and lung metastasis models were performed as previous study reported (Zuo et al. 2021). All animal experiments followed the guidelines of the First Affiliated Hospital, Zhejiang University School of Medicine.

Statistical analysis

Analysis of data were performed using SPSS 22 and GraphPad Prism 8. All data were presented as the mean \pm SD. Student's t-test was used for comparing the differences of two groups. The survival of HCC patients was evaluated by log-rank test and Kaplan-Meier analysis. Comparison of clinical information was performed by χ^2 test. In present study, $P < 0.05$ was considered statistically significant.

Results

High expression of TCF7L2 correlates with poor prognosis of HCC patients

The expression level of TCF7L2 in different tumors tissues and normal tissues from TCGA database was obtained by the TIMER 2.0 (Li et al. 2020). The expression of TCF7L2 was downregulated in breast cancer, colorectal cancer and prostate cancer, while it was upregulated in cholangiocarcinoma, HCC, and gastric cancer (Fig. 1A). Paired analysis of patients from the GSE14520 cohort ($n=19$) and TCGA ($n=50$) also yielded the same results (Fig. 1B and Figure S1A). Then, we performed immunohistochemical staining on the tissue microarrays of 87 HCC patients, which exhibited a significant upregulation of TCF7L2 in HCC tissues compared with adjacent normal liver tissues (Fig. 1C).

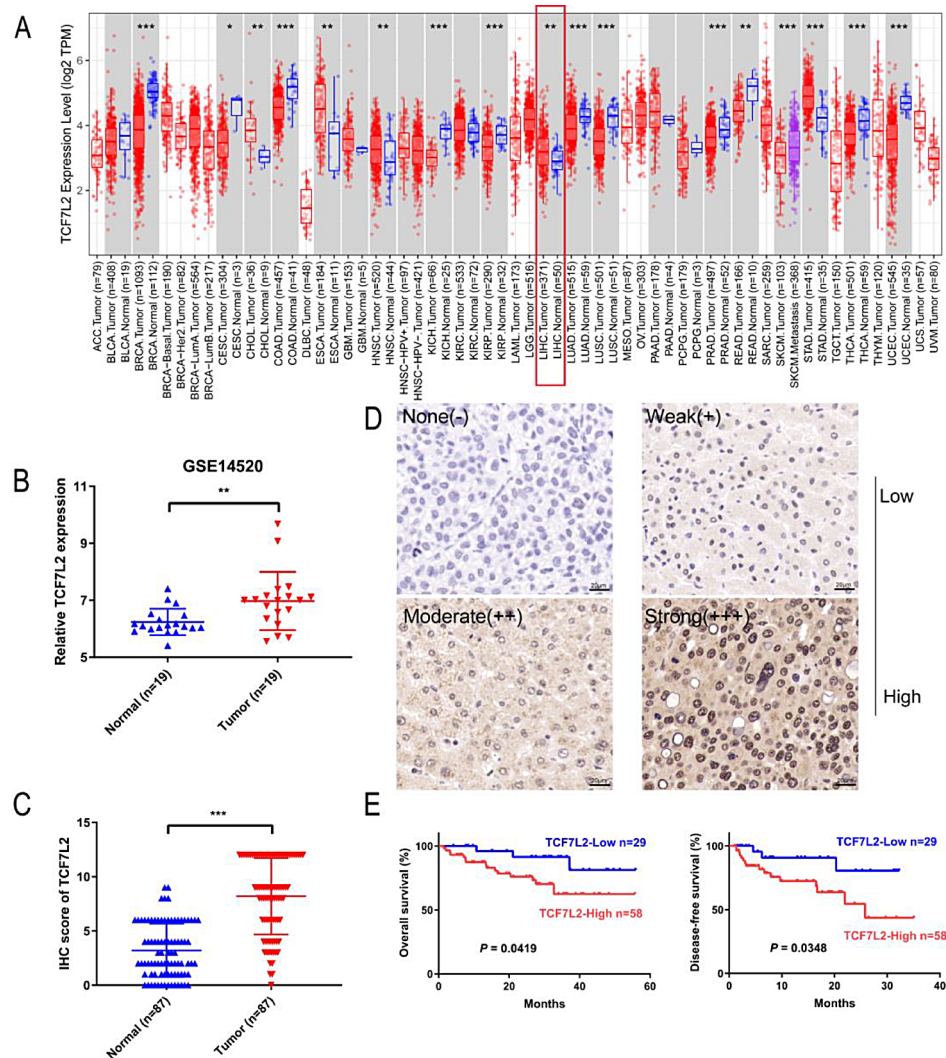


Fig. 1 High expression of TCF7L2 correlates with poor prognosis in HCC. **A** and **B**. The mRNA expression level of TCF7L2 in different tumors tissues and normal tissues from TCGA database (**A**) and GSE14520 cohort (**B**). **C**. Immunohistochemical staining and IHC scores of TCF7L2 on the tissue microarrays of 87 HCC patients ($n=87$). **D** and **E**. 87 HCC patients were divided into TCF7L2-low expression group and TCF7L2-high expression group according to IHC scores (**D**), and Kaplan-Meier survival analysis was performed on overall survival and disease-free survival of the two groups (**E**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

87 HCC patients were categorized into TCF7L2-low expression group ($n=29$) and TCF7L2-high expression group ($n=58$) based on immunohistochemical scores (Fig. 1D). The overall survival and disease-free survival of HCC patients with TCF7L2-high expression were significantly lower than those with TCF7L2-low expression (Fig. 1E). We also found that high TCF7L2 expression was significantly associated with vascular invasion ($P=0.0003$) (Table 1). Taken together, these data link high TCF7L2 expression to a worse prognosis in HCC patients.

Knockdown of TCF7L2 inhibits metastasis of HCC cells in vitro and in vivo

Owing to the expression of TCF7L2 varying from different HCC cell lines (Figure S1B), we selected HepG2 and HCCLM3 cell lines for TCF7L2 knockdown experiments, SK-Hep-1 (abbreviated as SK1) and Huh7 cell lines for TCF7L2 overexpression experiments. Stable cell models of TCF7L2 knockdown or overexpressed were validated at mRNA (Figure S2A) and protein levels (Fig. 2A). CCK8 assays indicated that TCF7L2 did not significantly affect the proliferation of HCC cells in vitro (Figure S2B, C). However, the wound healing assay, transwell migration and invasion assays uncovered that in HepG2 and HCCLM3 cell lines, the migration and invasion ability of cells was significantly inhibited after TCF7L2

Table 1 nCorrelation between the expression level of TCF7L2 and clinicopathologic characteristics of 87 HCC patients

Characteristics	TCF7L2 expression		P value	
	High(n=58)	Low(n=29)		
Gender	Male	54	26	0.577
	Female	4	3	
Age(years)	> 50	41	17	0.260
	≤ 50	17	12	
Tumor size(cm)	> 5	29	11	0.287
	≤ 5	29	18	
Tumor number	Single	45	22	0.857
	Multiple	13	7	
Cirrhosis	Yes	30	15	0.999
	No	28	14	
HBsAg	Positive	54	24	0.135
	Negative	4	5	
AFP(ng/mL)	> 400	27	12	0.647
	≤ 400	31	17	
Vascular invasion	Yes	40	8	0.0003***
	No	18	21	
TNM stage	I&II	31	21	0.089
	III&IV	27	8	

Note χ^2 test were used for comparing the clinical characteristics in TCF7L2-low expression group and TCF7L2-high expression group, *** $P < 0.001$. Abbreviations HBsAg, hepatitis B virus surface antigen; AFP, α -Fetoprotein

knockdown (Fig. 2B-E). Next, HCCLM3 cells with stable TCF7L2 knockdown were injected into the liver of mice to construct orthotopic HCC implantation models (Fig. 2F), and the tumor metastasis in the liver of mice was observed 8 weeks later. We found that the number of intrahepatic metastases was significantly reduced after TCF7L2 knockdown (Fig. 2G).

Overexpression of TCF7L2 promotes metastasis of HCC cells in vitro and in vivo

To further validate the role of TCF7L2 in HCC, cell lines with stable TCF7L2 overexpression were used to perform wound healing assay, transwell migration and invasion assays. We found that in Huh7 and SK1 cell lines, the metastasis ability of cells was significantly enhanced after TCF7L2 overexpression (Fig. 3A-D). In addition, SK1 cells with stable TCF7L2 overexpression were injected into the tail veins of mice to construct lung metastasis models (Fig. 3E), and the tumor metastasis in the lung of mice was observed 8 weeks later. The number of lung metastases was significantly increased after TCF7L2 overexpression compared to the control group (Fig. 3F). Collectively, these data suggest that TCF7L2 promotes HCC metastasis in vivo and in vitro.

TCF7L2 regulates HCC migration and invasion by activating AKT/mTOR pathway

To elucidate the mechanism of TCF7L2 regulation in HCC, we analyzed the differentially expressed genes

(DEGs) between the TCF7L2 knockdown group and the control group in the GSE46465 dataset (Fig. 4A). Further Gene set enrichment analysis (GSEA) of DEGs revealed that TCF7L2 could upregulate the mTORC1 signaling pathway (Fig. 4B). AKT is directly upstream of the mTORC1 signaling pathway, and the AKT/mTOR pathway is activated in a variety of tumors (Popova and Jücker 2021). Therefore, we further investigated whether TCF7L2 can activate the AKT/mTOR pathway in HCC. Pearson correlation was used to analyze the correlation between TCF7L2 and key genes of AKT/mTOR pathway in TCGA, and we found that TCF7L2 was associated with AKT1 and MTOR (Fig. 4C). We further detected AKT/mTOR pathway activation in TCF7L2 knockdown and overexpression cell lines. The results showed that TCF7L2 knockdown decreased the phosphorylation levels of AKT and mTOR, while the phosphorylation levels of AKT and mTOR increased after TCF7L2 overexpression (Fig. 4D). These results suggest that TCF7L2 activates the AKT/mTOR pathway in HCC. To determine whether TCF7L2 regulates migration and invasion via AKT/mTOR pathway in HCC, SK1 and Huh7 cells overexpressing TCF7L2 were treated with the AKT inhibitor MK-2206 and the mTOR inhibitor Rapamycin. We found that both MK-2206 and Rapamycin could significantly inhibit the migration and invasion ability in TCF7L2 overexpressed cell lines (Fig. 4E, F). These results suggest that TCF7L2 promotes HCC migration and invasion by activating AKT/mTOR pathway.

TCF7L2 regulates transcription of NEDD9

Given that TCF7L2 acts as a transcription factor, it mainly plays a role in regulating the transcription of downstream genes. We attempted to find out the directly downstream target genes of TCF7L2. The target genes of TCF7L2 were predicted by three transcription factor databases (hTFtarget, CHEA and ChIPBase), and intersected with the DEGs obtained from GSE46465, we finally identified the TCF7L2 target gene NEDD9 (Fig. 5A). The expression data in the TCGA database also showed that there is a significant correlation between the expression of TCF7L2 and NEDD9 in HCC (Fig. 5B). In addition, the protein level of NEDD9 was altered after TCF7L2 knockdown or overexpression (Figure S2D). Next, we predicted the binding site of TCF7L2 to NEDD9 gene promoter in the JASPAR database (Castro-Mondragon et al. 2022). Four TCF7L2 binding sites with the highest score on the NEDD9 promoter was selected for subsequent analysis: "GAAGATGAAAAAAC" (site A), "AAGCATAAAAGGAC" (site B), "CTACTTGAAAGCAC" (site C) and "AAAC TACAAAGTGT" (site D) (Fig. 5C). We designed primers at both ends of each site and analyzed the abundance of each site by agarose gel electrophoresis and ChIP-qPCR. The results showed that compared with IgG group, the

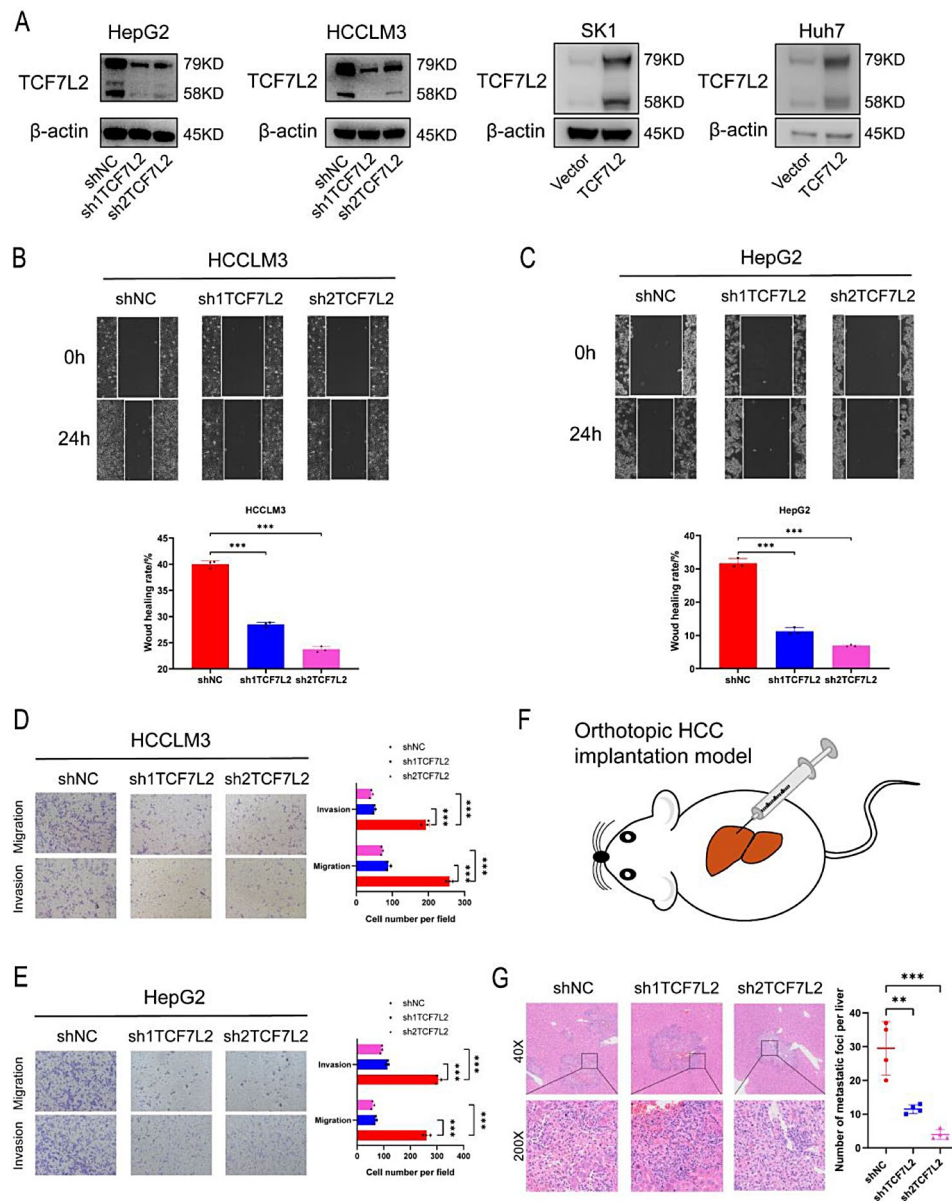


Fig. 2 Knockdown of TCF7L2 inhibits metastasis of HCC cells in vitro and in vivo. **A**. The knockdown and overexpression effects of TCF7L2 in HCC cell lines were verified at the protein level. **B** and **C**. Knockdown of TCF7L2 inhibits migration of HCCLM3 (**A**) and HepG2 (**B**) cells detected by wound healing assay ($n = 3$ biological replicates). **D** and **E**. Knockdown of TCF7L2 attenuates the ability of HCCLM3 (**C**) and HepG2 (**D**) cells to migrate and invade detected by transwell assays ($n = 3$ biological replicates). **F**. The schematic illustration for establishing orthotopic HCC implantation models. **G**. The number of intrahepatic metastases was significantly reduced after TCF7L2 knockdown ($n = 3$ biological replicates). ** $P < 0.01$, *** $P < 0.001$

abundance of site B was significantly increased (Fig. 5D, E). Meanwhile, luciferase report assays showed that TCF7L2 knockdown significantly inhibited the activity of luciferase carrying site B, while the activity of luciferase carrying site B mutation was not affected by TCF7L2 expression (Fig. 5F). These results indicate that TCF7L2 can directly bind to the promoter region of NEDD9 and positively regulate the transcription of NEDD9.

TCF7L2 promotes HCC migration and invasion via activating AKT/mTOR pathway through NEDD9

Previous studies have found that NEDD9 can activate the AKT/mTOR pathway and affect tumor progression, but no relevant studies have been reported in HCC (Izumchenko et al. 2009; Xue et al. 2020). To further clarify the function of NEDD9 and its relationship with AKT/mTOR pathway, we constructed NEDD9 small interfering RNA and selected the best interfering effect for subsequent experiments. Rescue experiments were performed in SK1 and Huh7 cell lines with TCF7L2 overexpression, and

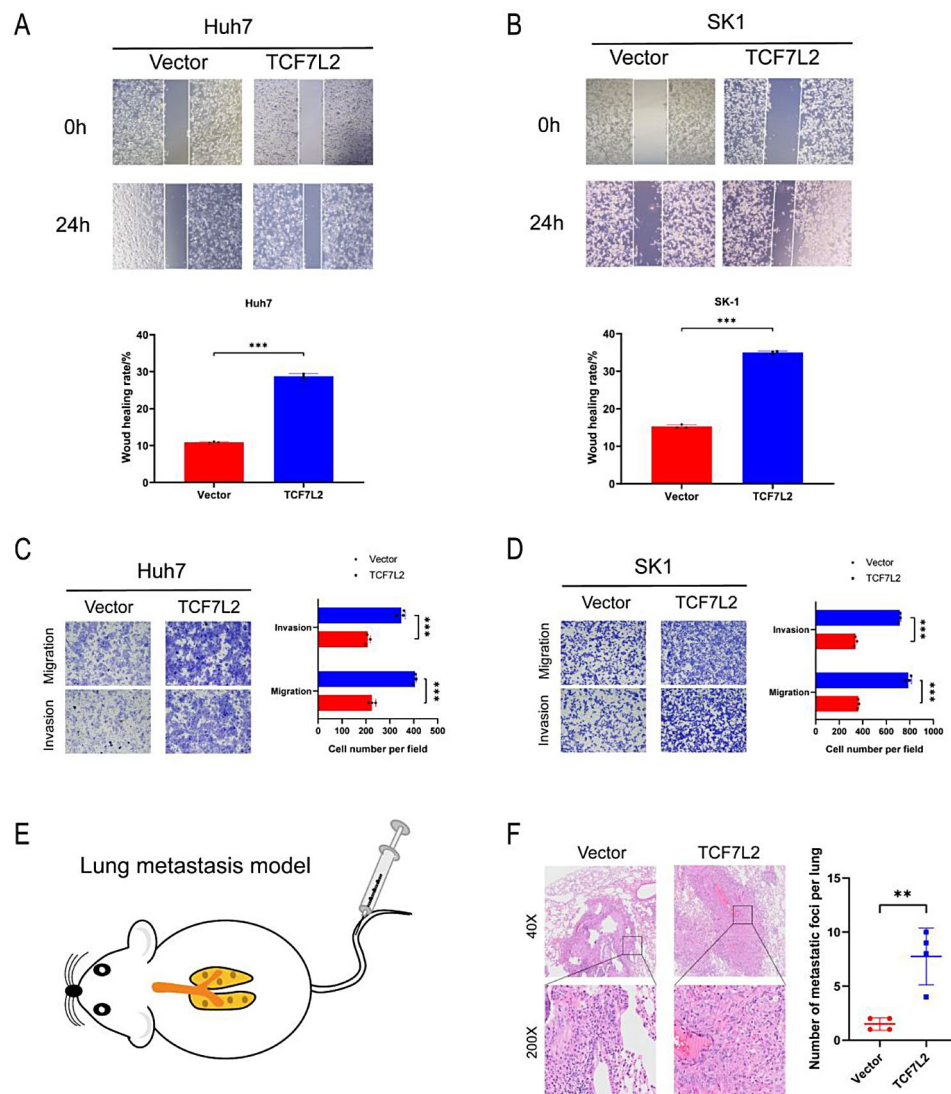


Fig. 3 Overexpression of TCF7L2 promotes metastasis of HCC cells in vitro and in vivo. **A** and **B**. Overexpression of TCF7L2 promotes migration of Huh7 (**A**) and SK1 (**B**) cells detected by wound healing assay ($n=3$ biological replicates). **C** and **D**. Overexpression of TCF7L2 attenuates the ability of Huh7 (**C**) and SK1 (**D**) cells to migrate and invade detected by transwell assays ($n=3$ biological replicates). **E**. The schematic illustration for establishing lung metastasis models. **F**. The number of lung metastases was significantly increased after TCF7L2 overexpression ($n=4$ biological replicates). ** $P < 0.01$, *** $P < 0.001$

we found that NEDD9 knockdown significantly inhibited the activation of AKT/mTOR pathway by TCF7L2 overexpression (Fig. 6A). Meanwhile, analysis from the TCGA database also showed the positive correlation of NEDD9 with AKT1 and MTOR (Fig. 6B). To further clarify whether NEDD9 is involved in the pro-metastasis effect of TCF7L2, transwell migration and invasion assays were performed on TCF7L2 overexpressed cells with or without interference of NEDD9. The results showed that NEDD9 interference significantly reduced the migration and invasion ability of SK1 and Huh7 cells after overexpression of TCF7L2 (Fig. 6C-F). These evidences above indicate that TCF7L2 promotes HCC migration and invasion in a NEDD9-dependent manner.

NEDD9 is associated with TCF7L2 in HCC

We further performed expression and survival analysis in 87 patients with tissue microarray. The results suggested that HCC patients with high NEDD9 expression had significantly higher overall and disease-free survival (Fig. 7A, B). We further analyzed the correlation between NEDD9 and TCF7L2 in 87 HCC patients, and found that NEDD9 was positively correlated with TCF7L2 (Fig. 7C). Furthermore, we investigated the predictive ability of the combination of the two genes on the prognosis of HCC patients. 87 HCC patients were divided into two groups: those with high expression of both TCF7L2 and NEDD9 ($n=25$) and those with low expression of at least one of them ($n=62$). The overall and disease-free survival were significantly lower in the group with high expression of

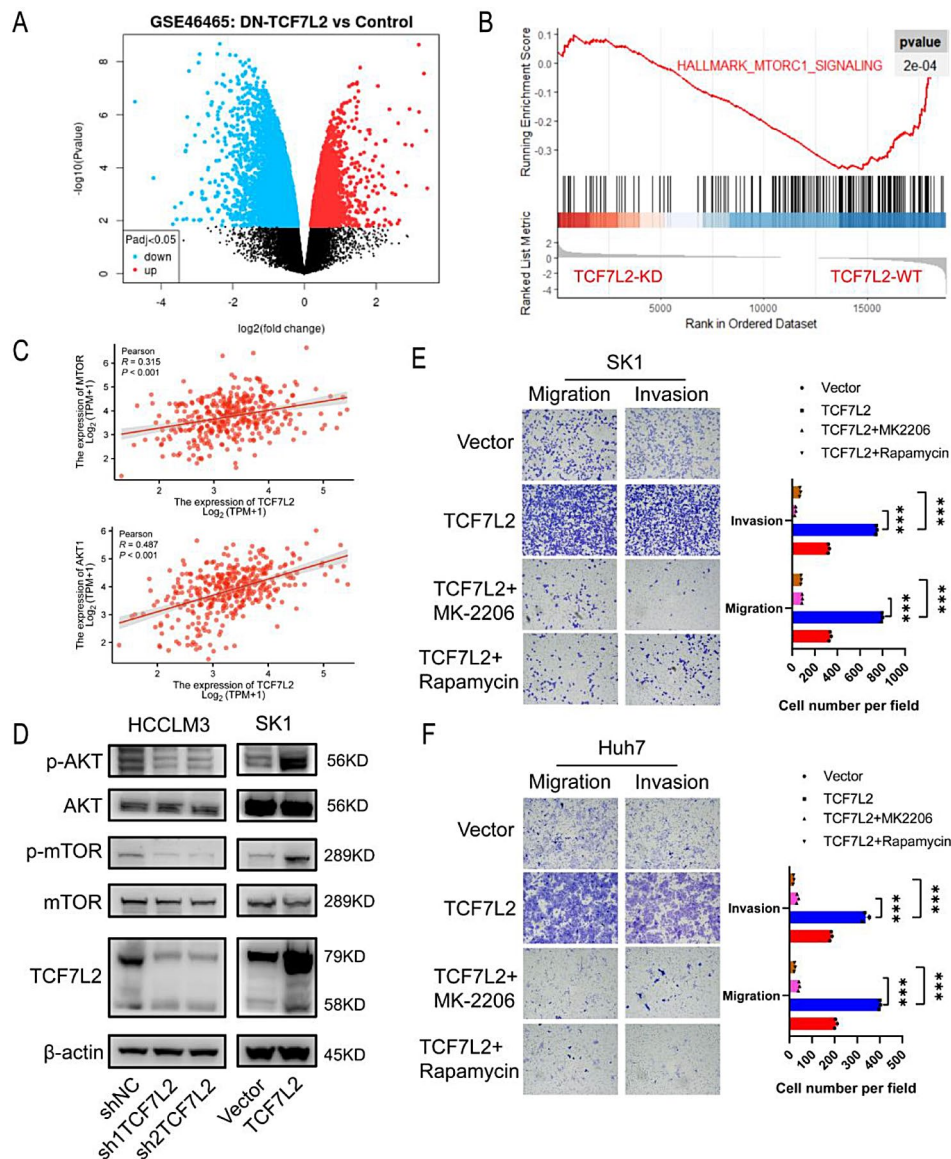


Fig. 4 TCF7L2 promotes HCC migration and invasion by activating AKT/mTOR pathway. **(A)** Differentially expressed genes (DEGs) between the TCF7L2 knockdown group and the control group in the GSE46465 dataset. **(B)** Gene set enrichment analysis (GSEA) of DEGs showed the relationship between TCF7L2 and mTORC1 signaling pathway. **(C)** The correlation between TCF7L2 and key genes of AKT/mTOR pathway in TCGA. **(D)** The dysregulation of AKT/mTOR pathway at the protein level in TCF knockdown and overexpression cell lines. **E** and **F**. AKT inhibitor MK-2206 and mTOR inhibitor Rapamycin were used for rescue experiments in SK1 **(E)** and huh7 **(F)** cells ($n = 3$ biological replicates). *** $P < 0.001$

both genes than in the group with low expression of at least one gene (Fig. 7D, E), indicating that the combined detection of TCF7L2 and NEDD9 can well predict the prognosis of HCC patients.

Discussion

As one of the most common malignant tumors, HCC has a very high mortality rate. At present, the treatment of HCC gradually expanded, but the prognosis of patients has not been significantly improved (Couri and Pillai 2019). Therefore, elucidating the mechanism of the progression of HCC and seeking new therapeutic targets

for HCC are still the focus of current research. TCF7L2 has been associated with glucose metabolism in the past, especially in type 2 diabetes (Bosque-Plata et al. 2022). However, its role in the development of HCC remains unclear. In present study, TCF7L2 is found to be upregulated in HCC, portending a worse prognosis for these patients. Mechanically, TCF7L2 activates NEDD9 transcription by directly binding to NEDD9 promoter region, and NEDD9 can activate the AKT/mTOR pathway, thereby promoting the migration and invasion of HCC.

TCF7L2 plays a vital role in cell proliferation and stemness maintenance (Hrckulak et al. 2018; Es et al. 2012).

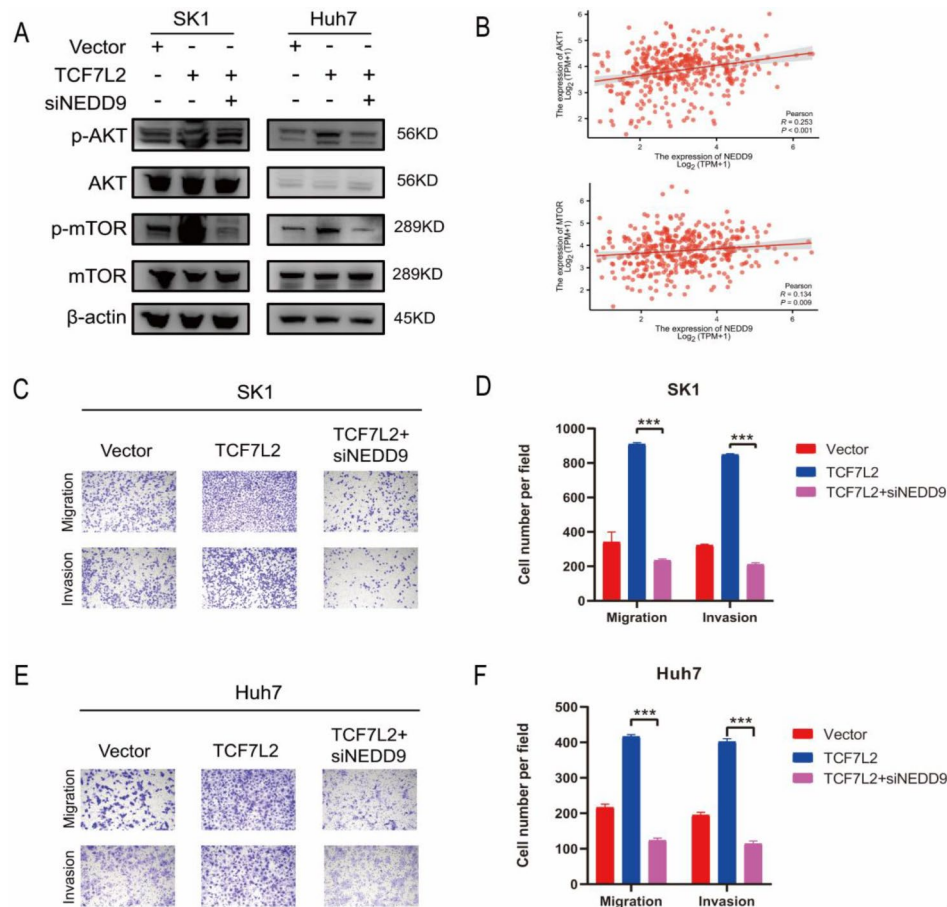


Fig. 6 TCF7L2 promotes HCC migration and invasion via activating AKT/mTOR pathway through NEDD9. **A**. Western blotting was performed in SK1 and Huh7 cells to detect the effect of the addition of siNEDD9 on the AKT/mTOR pathway; **B**. The correlation between the expression of NEDD9 and AKT1 (above) and mTOR (below) in HCC was analyzed in TCGA database. **C** and **D**. Transwell migration and invasion assays were performed on TCF7L2 overexpressed SK1 cells with or without siNEDD9 ($n = 3$ biological replicates). **E** and **F**. Transwell migration and invasion assays were performed on TCF7L2 overexpressed Huh7 cells with or without siNEDD9 ($n = 3$ biological replicates). *** $P < 0.001$

enriched in the mTORC1 pathway by GSEA analysis. We demonstrated that upregulation of TCF7L2 activated the AKT/mTOR pathway at the protein level, while AKT/mTOR pathway was inhibited after TCF7L2 knock-down. Functionally, we found that the pro-metastatic effect of TCF7L2 on HCC could be reversed by AKT or mTOR inhibitors, indicating that TCF7L2 plays a role in HCC by activating the AKT/mTOR pathway. Our findings are consistent with those of Wu et al., who found that TCF7L2 could activate the AKT/mTOR pathway in breast cancer (Wu et al. 2021). Our study suggests the crosstalk between the Wnt pathway and the AKT/mTOR pathway in HCC, and the combined targeting of these two pathways for the treatment of HCC deserves further investigation.

NEDD9 is a scaffold protein involved in cell signal transduction, which can regulate a variety of cellular processes, including cell growth, proliferation, injury and metastasis (Shagisultanova et al. 2015; Tikhomirova et al. 2022). A series of previous studies have found that

NEDD9 can affect epithelial mesenchymal transformation and promote tumor metastasis (Li et al. 2016; Gabbasov et al. 2018; Deneka et al. 2022; Wang et al. 2019). Zhou et al. found that NEDD9 is upregulated in HCC, and the high expression of NEDD9 could promote the metastasis of HCC (Zhou et al. 2017a, b). In our clinical sample, patients with high NEDD9 expression had significantly worse overall survival and disease-free survival than patients with low NEDD9 expression. The expression of NEDD9 is regulated by upstream genes. In melanoma, TCF7L2 regulates the expression of NEDD9 through miRNA to affect tumor progression (Rambow et al. 2016). Our study found that NEDD9 is a direct target gene of TCF7L2, and TCF7L2 can directly bind to the -1522/-1509 site of the NEDD9 promoter and positively regulate the transcription of NEDD9. Downstream of NEDD9 is often associated with the activation of several kinases, including FAK, Src, and AURKA (Izumchenko et al. 2009; Sima et al. 2013; Pugacheva et al. 2007). Deneka et al. found that NEDD9 was involved in the activation

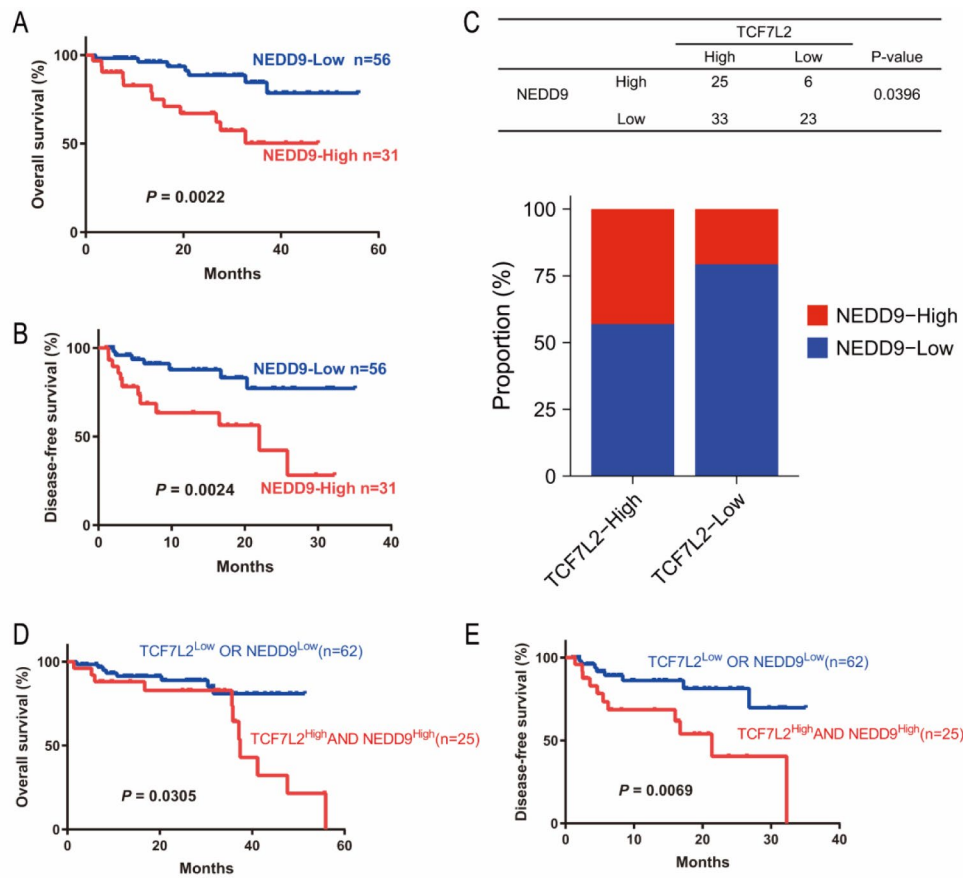


Fig. 7 Correlation of NEDD9 and TCF7L2 in HCC. **A** and **B**. Kaplan-Meier survival analysis was performed on overall survival (**A**) and disease-free survival (**B**) of HCC patients with high NEDD9 expression and low NEDD9 expression. **C**. The correlation between the expression of NEDD9 and TCF7L2 in 87 HCC patients. χ^2 test were used for assessment of correlation. **D** and **E**. Kaplan-Meier survival analysis was performed on overall survival (**D**) and disease-free survival (**E**) of HCC patients with high expression of both TCF7L2 and NEDD9 ($n = 25$) and those with low expression of at least one of them

of AKT in lung cancer (Deneka et al. 2021). Our results suggest that TCF7L2 activates AKT/mTOR pathway by regulating NEDD9, thereby promoting HCC metastasis. However, how NEDD9 activates the AKT/mTOR pathway in HCC remains unclear which deserves further exploration.

Conclusions

In summary, this study elucidates the role and mechanism of TCF7L2 in HCC metastasis. TCF7L2 targets NEDD9 transcription to activate AKT/mTOR pathway, thereby promoting HCC migration and invasion. Our findings elucidate the mechanism of action of TCF7L2 in HCC, suggesting the potential of TCF7L2 as a prognostic marker and therapeutic target for HCC.

Abbreviations

TCF7L2	Transcription factor 7 like 2
HCC	Hepatocellular Carcinoma
NEDD9	Neural precursor cell Expressed Developmentally Downregulated protein 9
TCF/LEF	T cell factor/Lymphoid Enhancer Factor
GEO	Gene Expression Omnibus
TCGA	The Cancer Genome Atlas

FBS	Fetal Bovine Serum
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
shRNA	Small hairpin RNA
siRNA	Small interfering RNA
CCK8	Cell Counting kit-8
ChIP	Chromatin Immunoprecipitation
GSEA	Gene Set Enrichment Analysis

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s10020-024-00878-9>.

- Supplementary Material 1
- Supplementary Material 2
- Supplementary Material 3
- Supplementary Material 4

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Not applicable.

Author contributions

X Xu and LS Tang designed the study. LS Tang was responsible for completing the experiment and writing the paper. SJ Xu, RL Wei, JB Zhou and GH Fan were responsible for collecting experimental data. XY Wei helped analyze the data.

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Data availability

Data from the current study are all available.

Declarations

Ethics approval and consent to participate

The patients involved in this study had signed an informed consent form. This study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine and was in accordance with the Helsinki Declaration. All animal experiments followed the guidelines of the First Affiliated Hospital, Zhejiang University School of Medicine.

Consent for publication

The final manuscript was approved by all authors for publication.

Competing interests

The authors declare that they have no competing interests.

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