Life Sciences 224 (2019) 1–11

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

MicroRNA-424-5p acts as a potential biomarker and inhibits proliferation and invasion in hepatocellular carcinoma by targeting TRIM29

Huimin Du[a], Qing Xu[b], Sheng Xiao[c], Zhenru Wu[b], Jianping Gong[d], Changan Liu[d], Guosheng Ren[e], Hao Wu[d,*]

[a] Department of Oncology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China
[b] Laboratory of Pathology, Key Laboratory of Transplant Engineering and Immunology, NHFPC, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China
[c] Department of Breast Surgery, Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646000, China
[d] Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China
[e] Chongqing Key Laboratory of Molecular Oncology and Epigenetics, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China

ARTICLE INFO

Keywords:
miR-424-5p
Tripartite motif-containing 29
Hepatocellular carcinoma
Tumor progression
Molecular marker

ABSTRACT

Background: miRNA-424-5p (miR-424-5p) has been implicated in the development and progression of various tumors. However, the functional mechanisms of miR-424-5p in hepatocellular carcinoma (HCC) are unclear. In this study, we investigated the specific biological functions of miRNA in HCC.

Methods: The expression of miR-424-5p was measured by qRT-PCR in HCC tissues and cell lines. Western blot and immunohistochemistry were used to detect the protein expression level of TRIM29. The relationship between miR-424-5p and the clinicopathological features of HCC patients was analyzed. Cell function experiments were performed to examine proliferation and invasion in HCC cells. The miRNA database was used to predict downstream target genes of miR-424-5p, which were verified by a luciferase reporter assay. Furthermore, cell and animal experiments confirmed that miR-424-5p exerts its biological function through the target gene TRIM29.

Results: miR-424-5p expression was decreased in HCC tissues and cell lines, and correlated with AFP, TNM stage, intrahepatic metastasis and poor overall survival in HCC. The upregulation of miR-424-5p inhibited cell proliferation and invasion in vitro and suppressed HCC tumor growth in vivo. TRIM29 was confirmed to be the downstream target gene of miR-424-5p. Finally, rescue experiments suggested that the upregulation of TRIM29 could rescue inhibitory effect of miR-424-5p overexpression on cell proliferation and migration.

Conclusion: miR-424-5p is a tumor suppressor miRNA that inhibits cell proliferation and invasion via directly modulating TRIM29, which is related to cell proliferation and invasion in HCC. Thus, miR-424-5p may be a potential therapeutic and new prognostic marker for HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor that causes a large number of deaths every year worldwide [1]. With the increased intake of alcohol and the spread of hepatitis viruses, China is already one of the countries with the highest incidence of HCC [2]. Despite continuous improvements in diagnostic and therapeutic techniques, the recurrence rate of HCC patients after treatment is still very high, resulting in a low 5-year survival rate [3]. In addition, the progression of cancer is a complex multifactor process involving complex signaling pathways in the tumor itself and in the microenvironment. Therefore, it is urgent to find important molecular mechanisms involved in the malignant progression of HCC to improve the diagnosis and treatment efficiency of HCC and to improve the quality of life of HCC patients.

Tripartite motif-containing 29 (TRIM29) is a member of the TRIM protein family. It has multiple zinc finger patterns and a leucine zipper pattern that can participate in the formation of nucleic acid-bound homodimers or heterodimers. Therefore, it can act as a transcriptional regulator in carcinogenesis and differentiation [4,5]. TRIM29 has been found to be highly expressed in a variety of malignant tumors including lung [6], bladder [7], and pancreatic [8] cancers, and the higher its expression level, the worse the prognosis. Recent studies have reported that TRIM29 regulates the levels of cyclin and Bcl family proteins through the NF-κB signaling pathway, promotes the growth of bladder

* Corresponding author.
E-mail address: whwh1hero@163.com (H. Wu).

https://doi.org/10.1016/j.lfs.2019.03.028
Received 24 January 2019; Received in revised form 8 March 2019; Accepted 11 March 2019
Available online 12 March 2019
0024-3205/

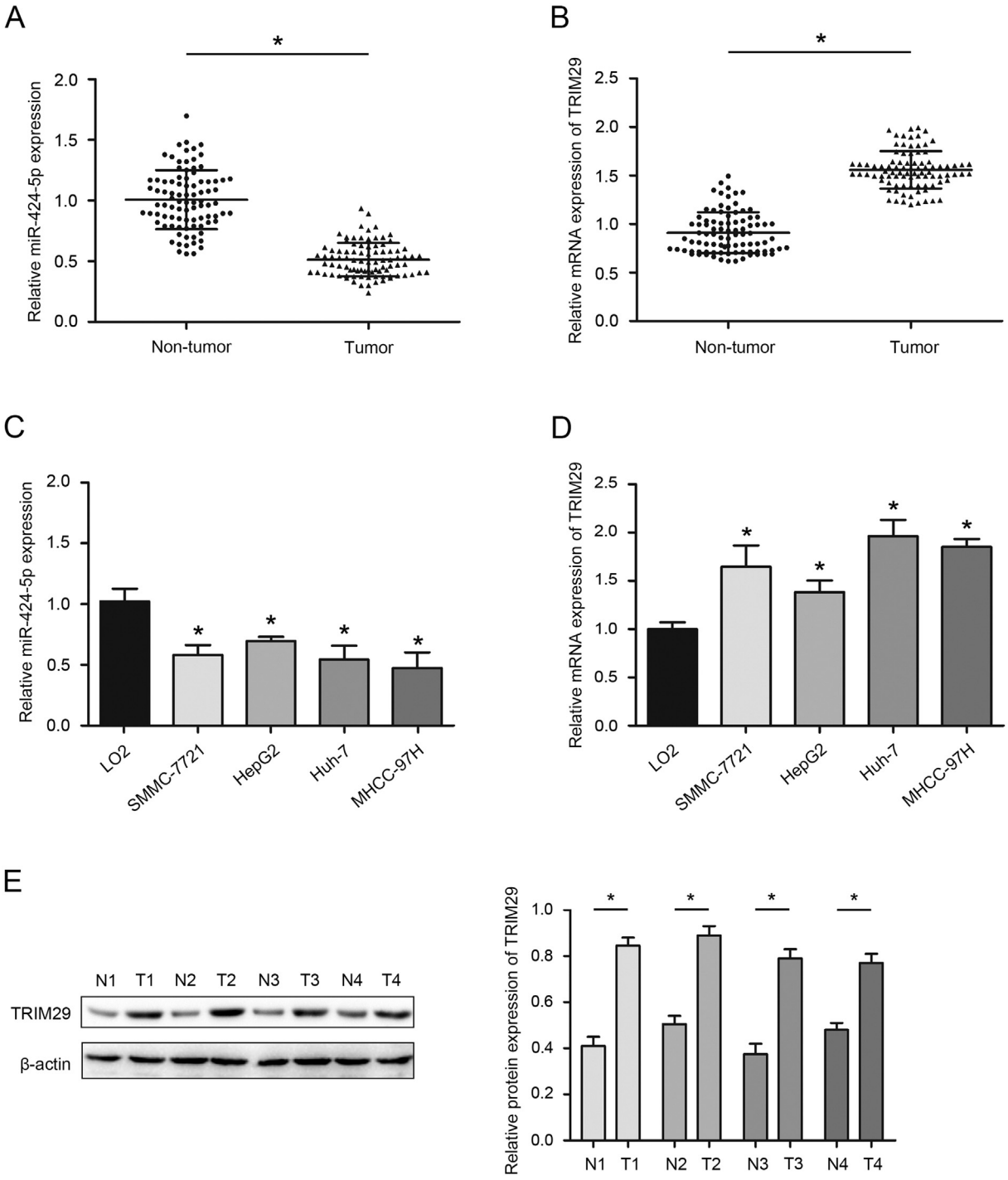


Fig. 1. miR-424-5p was downregulated and TRIM29 was upregulated in HCC tissues and cell lines. (A) qRT-PCR analysis of miR-424-5p expression in HCC and adjacent noncancerous tissues. (B) qRT-PCR analysis of TRIM29 expression in HCC and adjacent noncancerous tissues. (C) Relative expression of miR-424-5p in HCC cell lines (MHCC-97H, HepG2, SMMC-7721, and Huh-7) and the normal liver cell line LO2 was detected by qRT-PCR. (D) Relative expression of TRIM29 in HCC cell lines (MHCC-97H, HepG2, SMMC-7721, and Huh-7) and the normal liver cell line LO2 was detected by qRT-PCR. (E) Western blot analysis of TRIM29 expression in HCC and adjacent noncancerous tissues.*P $<$ 0.05 compared to control.

cancer cells and inhibits drug-induced apoptosis [9]. In thyroid cancer, the downregulation of TRIM29 blocks the activation of the P13K/AKT signaling pathway, and reduces the degree of the malignant progression of tumor cells [10]. In addition, TRIM29 was found to bind to many molecular components of the DNA damage response, leading to DNA virus infections [11]. However, the regulatory mechanism of TRIM29 in the malignant progression of HCC is not yet clear.

A microRNA (miRNA) is a small noncoding RNA molecule, that contains approximately 21–24 nucleotides, and functions in RNA silencing and the posttranscriptional regulation of genes [12,13]. The dysregulation of miRNA is associated with the progression of various tumors, and several miRNAs act as biomarkers for tumor diagnosis [14,15]. Recent studies have shown that miR-424-5p is involved in the development of multiple malignancies [16,17]. In ovarian cancer, miR-424-5p inhibits CCNE1 expression, and then regulates the cell cycle by inhibiting the E2F1-pRb pathway and promoting apoptosis [18].

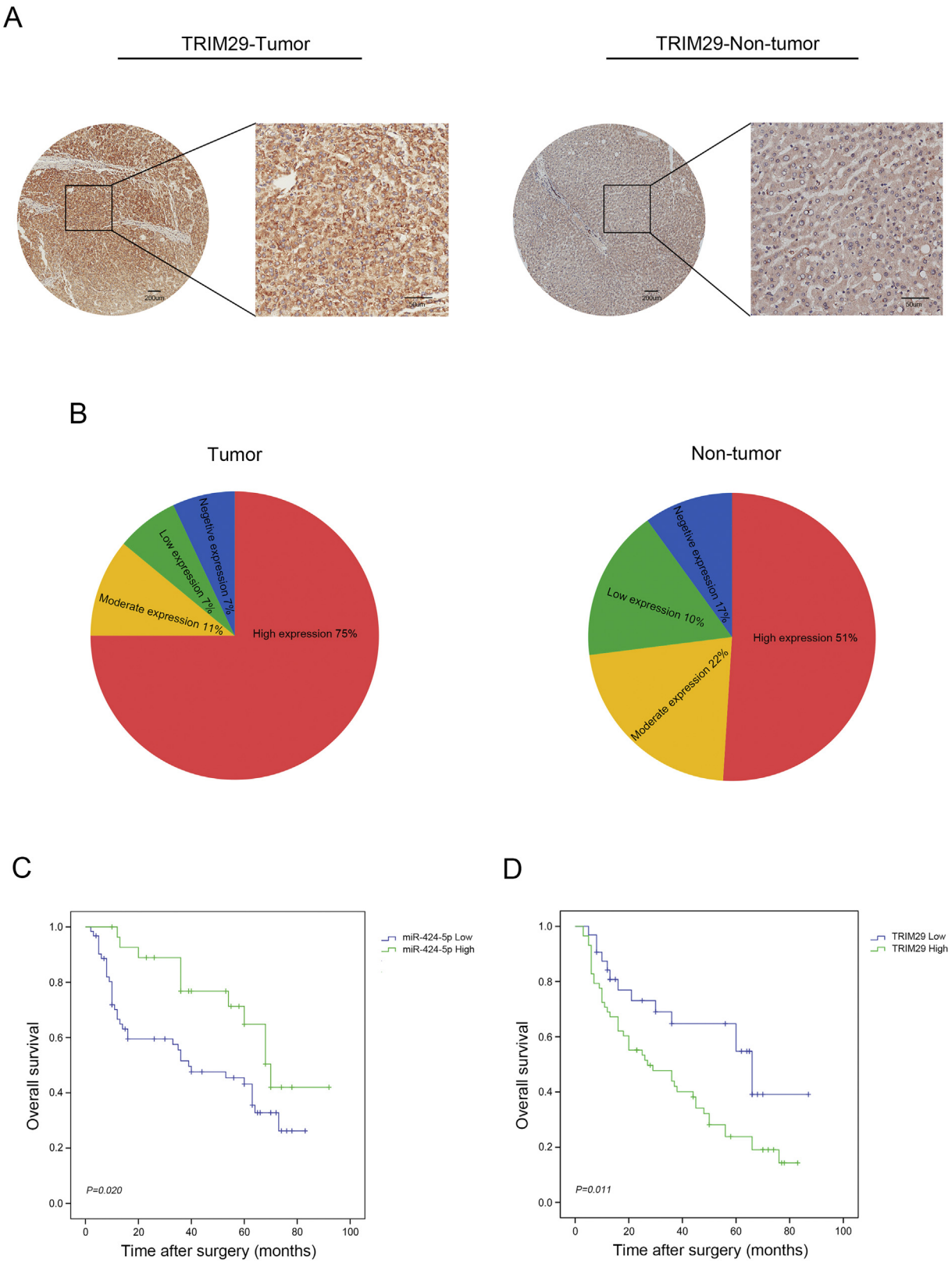


Fig. 2. The effect of miR-424-5p and TRIM29 on the prognosis of HCC patients. (A) Immunohistochemical staining analysis of TRIM29 expression in HCC and adjacent noncancerous tissues. (B) Statistical analysis of the expression of TRIM29 in HCC and adjacent noncancerous tissues. (C) Kaplan-Meier analysis of overall survival between in HCC patients with high and low miR-424-5p expression. (D) Kaplan-Meier analysis of overall survival in HCC patients with high and low TRIM29 expression.

Table 1
Correlations between miR-424-5p with clinicopathological features of HCC patients.

Variables	Cases	miR-424-5p expression		P
		Low (n = 57)	High (n = 33)	
Age (yr)				
< 50	43	25	18	0.384
≥ 50	47	32	15	
Gender				
Male	50	31	19	0.828
Female	40	26	14	
Tumor size(cm)				
≤ 5	47	27	20	0.276
> 5	43	30	13	
AFP (ng/ml)				
≤ 20	39	20	19	**0.048**
> 20	51	37	14	
Liver cirrhosis				
Presence	58	35	23	0.497
Absence	32	22	10	
HBsAg				
Positive	66	41	25	0.809
Negative	24	16	8	
TNM stage				
I/II	42	17	25	**0.001**
III/IV	48	40	8	
Vascular invasion				
Presence	49	32	17	0.826
Absence	41	25	16	
Multiplicity				
Single	50	29	21	0.276
Multiple (≥2)	40	28	12	
Intrahepatic metastasis				
Presence	42	19	23	**0.001**
Absence	48	38	10	

Bold data indicates statistically significant.

Recently, miR-424-5p was identified as a tumor suppressor microRNA, that regulates tumor cell proliferation, migration and invasion by targeting DCLK1 in basal-like breast cancer [19]. However, the underlying functional mechanism of the miR-424-5p regulation of HCC malignant progression remains unknown.

2. Materials and methods

2.1. Patients and specimens

All fresh HCC specimens, including 90 HCC tissues and paired normal adjacent tissues, were obtained for analysis. All HCC specimens were collected from February 2006 to March 2011 in the Second Hospital Affiliated of Chongqing Medical University. The samples were snap-frozen for mRNA and protein assessment. The histopathological diagnosis of HCC samples was independently diagnosed by two experienced pathologists. Our present study was approved by the Institutional Review Board of the Second Hospital Affiliated of Chongqing Medical University and informed consent was obtained from all patients according to the Declaration of Helsinki.

2.2. Cell culture and transfection

HCC cell lines (MHCC-97H, HepG2, SMMC-7721, and Huh-7) and a normal liver cell line (LO2) were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China). All cell lines were cultured in high-glucose DMEM. Cells were transfected with pcDNA-TRIM29 based on the manufacturer's instructions. The pre-miR-424-5p (miR-424-5p) and miR-424-5p inhibitor (anti-miR-424-5p) lentiviral vectors were obtained from GenePharma (Shanghai, China). Cells were transfected using Lipofectamine 3000 reagent (Invitrogen, USA) according to the manufacturer's instructions.

Table 2
Univariate and multivariate analysis of different prognostic variables influencing overall survival in HCC patients.

Variables	n	Univariate analysis		Multivariate analysis model	
		HR (95% CI)	P	HR (95% CI)	P
Sex		0.689 (0.807–1.765)	0.504		
Female	40				
Male	50				
Age (year)		0.485 (0.642–1.596)	0.547		
< 50	43				
≥ 50	47				
AFP(ng/ml)		1.704 (1.098–2.693)	**0.027**	1.079 (1.078–2.869)	**0.031**
≤ 20	39				
> 20	51				
HBsAg		1.178 (1.078–2.468)	0.715	1.006 (0.463–1.896)	0.796
Positive	66				
Negative	24				
Liver Cirrhosis		0.864 (0.485–1.763)	0.676	1.483 (0.789–2.394)	0.782
Presence	58				
Absence	32				
TNM stage		1.247 (0.946–2.573)	**0.024**	1.094 (1.048–3.166)	**0.011**
I/II	42				
III/IV	48				
Tumor size (cm)		1.098 (1.237–2.987)	0.331		
≤ 5	47				
> 5	43				
Multiplicity		0.697 (0.882–1.830)	0.546		
Single	50				
Multiple (≥2)	40				
Intrahepatic Metastasis		0.896 (1.204–2.864)	0.283		
Presence	42				
Absence	48				
Vascular Invasion		1.134 (1.364–2.841)	0.783		
Presence	49				
Absence	41				
miR-424-5p expression		1.309 (0.489–1.465)	**0.022**	1.023 (1.220–2.039)	**0.010**
Low	57				
High	33				

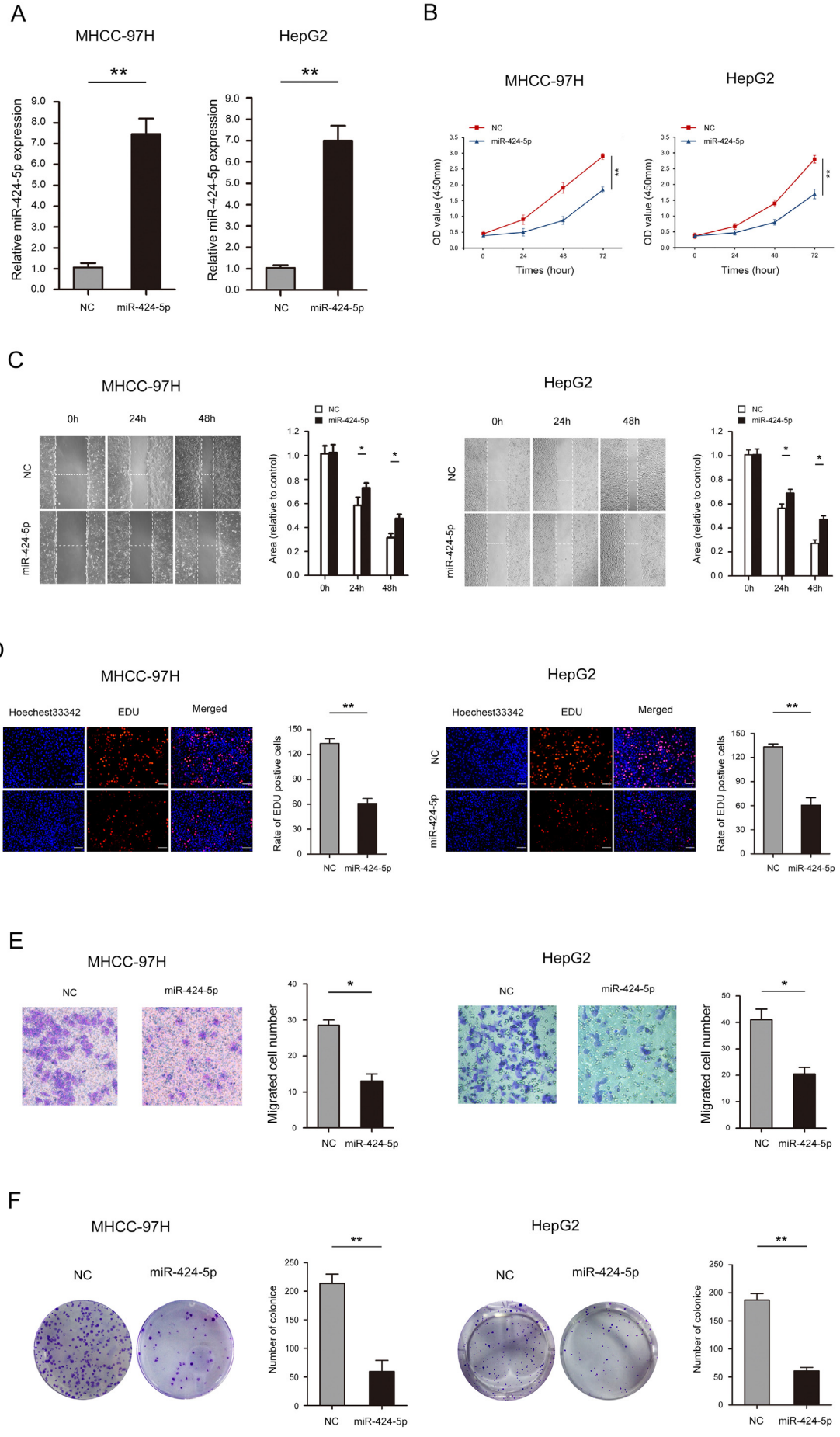
HR hazard rate, *CI* confidence interval.
Bold data indicates statistically significant.

2.3. Real-time PCR

Total RNA from frozen patient samples and cell lines was extracted using TRIzol reagent (TaKaRa, Japan) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using PrimeScript RT Reagent (TaKaRa), and qRT-PCR was performed with SYBR Premix Ex Taq II (TaKaRa) using a LightCycler system (Roche).

2.4. Western blot analysis

Total protein was extracted with RIPA lysis buffer, resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). After blocking with 5% nonfat powdered milk at room temperature for 1 h, the membranes were probed at 4 °C overnight with the following specific primary antibodies:TRIM29 (1:1000; Abcam, USA), BAX (1:5000, Abcam, USA), BCL-2 (1:1000, Abcam, USA), KI67 (1:1000, Abcam, USA) and β-actin (1:5000, Abcam, USA).



(caption on next page)

Fig. 3. miR-424-5p overexpression regulates proliferation and invasion in HCC cell lines. (A) MHCC-97H and HepG2 cells, which were transfected with pre-miR-424-5p, were assessed for miR-424-5p expression by qRT-PCR. (B) CCK-8 assays were carried out to examine the effects of miR-424-5p overexpression on the proliferative ability of HCC cells. (C) Wound-healing assays were performed to determine the effects of miR-424-5p overexpression on the migration of HCC cells. (D) Cell proliferation was detected with EdU in HCC cells after miR-424-5p overexpression. (E) The invasion assay was used to examine the effects miR-424-5p overexpression on HCC cell invasion. (F) Colony formation assays in HCC cells after the overexpression of miR-424-5p. *P < 0.05 vs control, **P < 0.01 vs control. NC, negative control; OD, optical density.

2.5. Immunohistochemistry

All paraffin-embedded tumor tissues collected from 90 consecutive patients with HCC were used for tissue microarray (TMA) construction and immunohistochemistry (IHC). After antigen retrieval in citrate buffer (pH 6.0) for 2 min in an autoclave, 0.3% hydrogen peroxide was applied for 15 min, and then the sections were incubated with goat serum. The sections were incubated with anti-TRIM29 rabbit polyclonal antibodies (Abcam, USA) at a 1:200 dilution.

2.6. Cell-counting Kit-8 assay

Cells were grown in a 96-well plate for 24 h, 48 h and 72 h. Cell proliferation analysis was performed using the Cell Counting Kit-8 assay (CCK-8, Dojindo, Japan) according to the manufacturer's instructions.

2.7. Colony-forming assay

Cells were grown in a 6-well plate for 14 days. Colonies formed by cell proliferation were stained with 20% methanol and 0.1% crystal violet, and the colonies were counted.

2.8. Wound healing assay

Cells were grown in a 6-well plate, and after reaching confluence, non-adherent cells were washed away twice with PBS. The cell monolayer was scratched with a pipette tip (10 ml) to generate 3 scratch wounds and then rinsed twice with PBS to remove non-adherent cells. After 0 h, 24 h and 48 h, the distance between the wound sides was measured.

2.9. Cell invasion assay

After transfection for 48 h, a cell suspension was added to the upper chamber (Corning, USA) and cultured in 200 μl of serum-free medium. In the lower chambers, 600 μl of medium containing 10% FBS was added. After 24 h, the upper unmigrated cells were gently wiped off with a cotton swab, washed 3 times with PBS, stained with 0.1% crystal violet for 20 min and counted.

2.10. 5-Ethynyl-2′-deoxyuridine (EdU) assay

Cells were seeded into 96-well plates (5×10^3 cells/well) and cultured for 24 h. Cells were incubated with EdU (50 μM) for 2 h at 37 °C, fixed in 4% formaldehyde for 30 min and permeabilized with 0.5% TritonX-100 solution for 10 min at room temperature. After washing with PBS, 1 × ApolloR reaction cocktail (100 μl) was added, and the reaction proceeded for 30 min at room temperature in the dark. Cell nuclei were stained by adding 1 × Hoechst 33342 (100 μl) for 30 min. Cell proliferation was analyzed using the mean number of cells in three fields for each sample.

2.11. Luciferase reporter assay

The Wild-type (WT) TRIM29-3'UTR and mutant (MUT) TRIM29-3'UTR containing the putative binding site of miR-424-5p were cloned and established in the firefly luciferase-expressing pMIR-REPORT vector (Obio Technology, China). After transfection for 24 h, luciferase assays were performed using the Luciferase Reporter Assay System (GloMax) according to the manufacturer's protocol.

2.12. miRNA target prediction

Four prediction databases, TargetScan (http://www.targetscan.org), Oncomir(http://www.oncomir.org/), MiRanda(http://www.microrna.org/microrna/home.do) and miRWalk(http://mirwalk.umm.uni-heidelberg.de/) were used to predict miRNA targets and conserved sites bound to miR-424-5p.

2.13. Mouse xenograft tumor model

Five-week-old male BALB/c-nu mice were purchased from the Shanghai Experimental Animal Center (Shanghai, China) and fed in the Experimental Animal Center of Chongqing Medical University (Chongqing, China). miR-424-5p and its associated negative control MHCC-97H cells and HepG2 cells (5×10^6) were subcutaneously injected into the left hip flanks of the mice. Then, tumor weight and volume were measured in each group.

2.14. Statistical analysis

All data were analyzed using GraphPad Prism version 6.0 (CA, USA) or SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation (SD). Statistical differences were analyzed by one-way analysis of variance, followed by the Newman-Keuls test, and repeated measures analysis of variance was used to evaluate the differences between groups. The Kaplan–Meier method was used to assess OS, and the log-rank test was used to analyze the differences between the curves. The prognostic significance of miR-424-5p and TRIM29 expression was calculated by univariate and multivariate Cox regression analyses. $P < 0.05$ was defined as having statistical significance.

3. Results

3.1. miR-424-5p is decreased, and TRIM29 is increased in HCC tissues and cell lines

To investigate the expression of miR-424-5p and TRIM29 in HCC tissues and cell lines, qRT-PCR was used. The results indicated that miR-424-5p was downregulated and that TRIM29 was upregulated in HCC tumors compared with paired normal samples ($P < 0.05$, Fig. 1A, B). As expected, miR-424-5p expression was reduced and TRIM29 expression was increased in HCC cell lines compared with normal liver cell lines ($P < 0.01$, Fig. 1C, D). Western blot data indicated that TRIM29 was significantly upregulated in HCC compared with paired normal samples ($P < 0.05$, Fig. 1E).

3.2. Downregulated miR-424-5p and upregulated TRIM29 are significantly correlated with a poor clinical prognosis in HCC

We found that the expression of TRIM29 in HCC was significantly higher than that in paired normal samples (Fig. 2A, B). Kaplan-Meier survival analysis showed that HCC patients with low miR-424-5p expression had a significantly shorter OS than those with high miR-424-5p expression ($P = 0.020$, Fig. 2C). Moreover, HCC patients with high

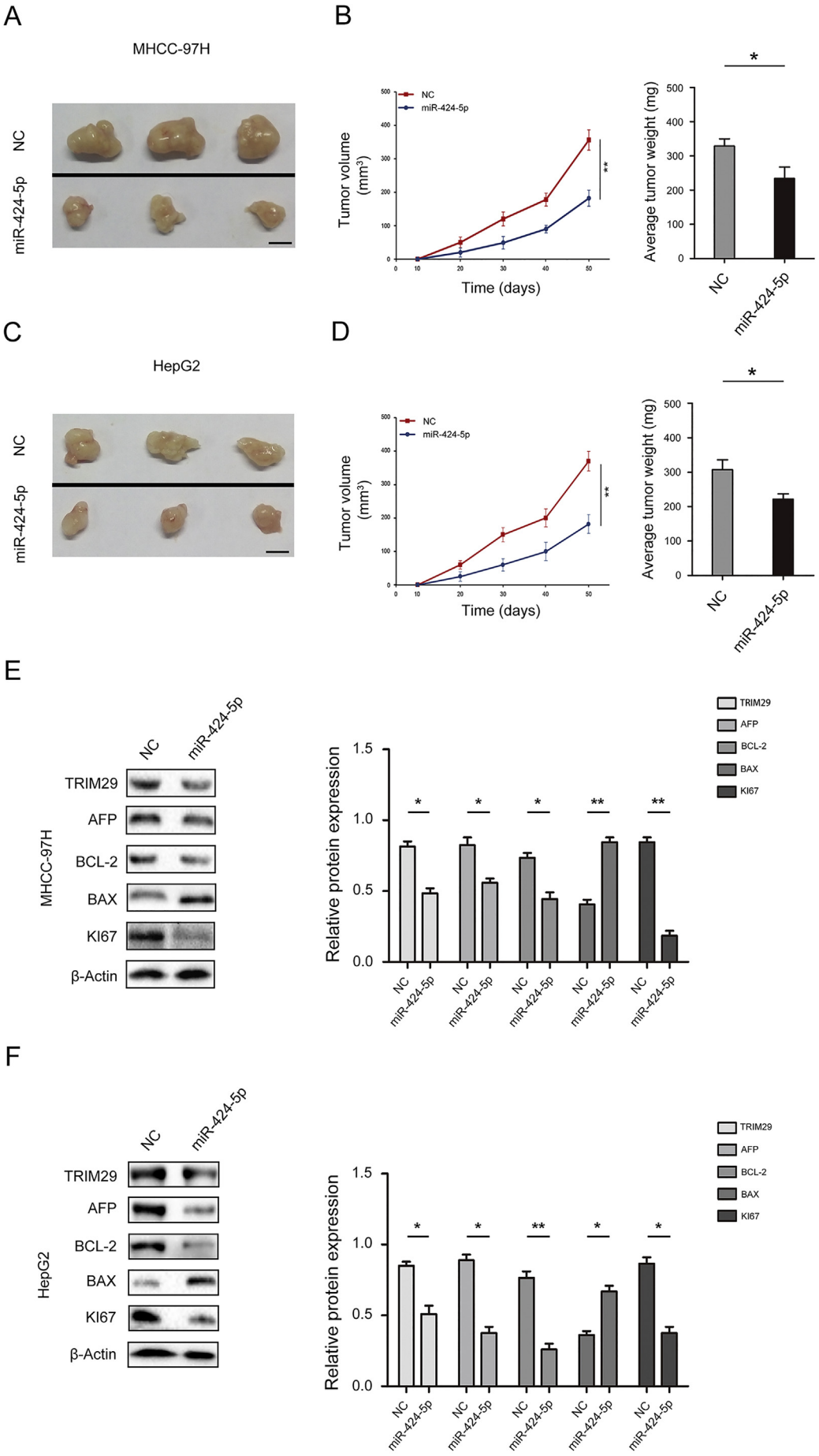
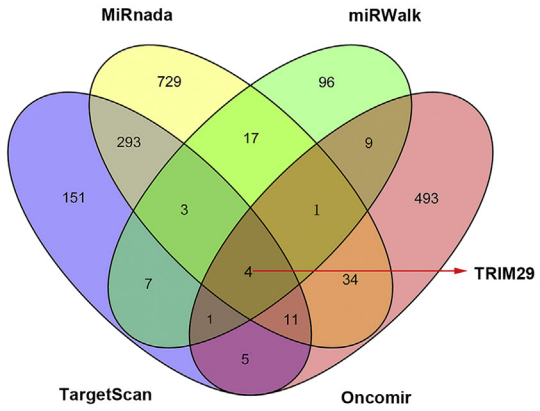
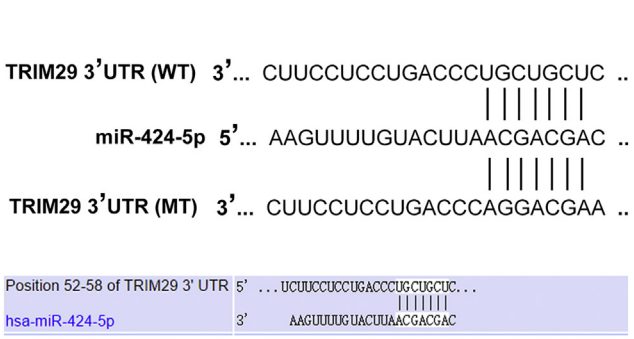
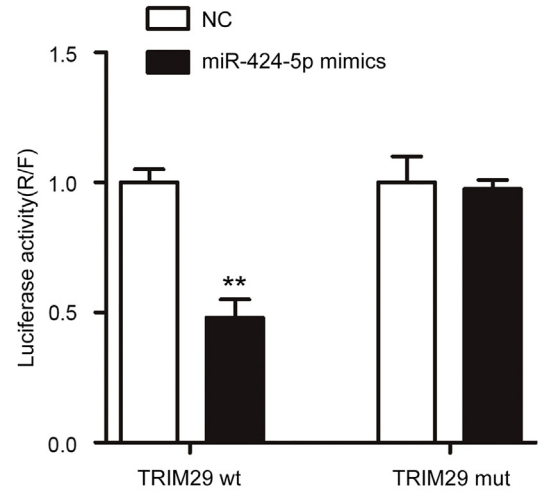
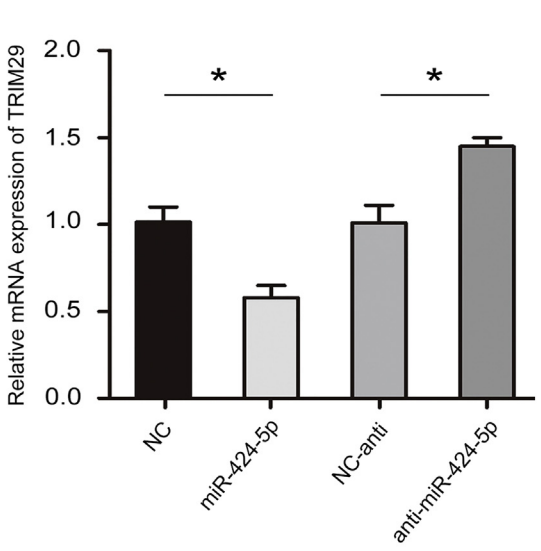


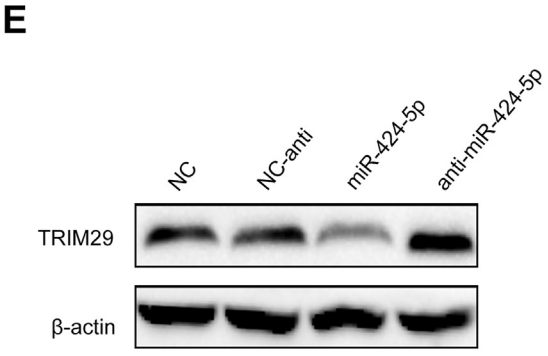
Fig. 4. miR-424-5p inhibits the tumorigenicity of HCC via TRIM29. (A) MHCC-97H cells transfected with pre-miR-424-5p were subcutaneously inoculated into nude mice to formed tumors. (B) Tumor weight and tumor volume were recorded in each group. (C) HepG2 cells transfected with pre-miR-424-5p were subcutaneously inoculated into nude mice to form tumors. (D) Tumor weight and tumor volume were recorded in each group. (E) Protein expression of proliferation- and apoptosis-related indicators, after transfection with pre-miR-424-5p in MHCC-97H cells. (F) Protein expression of proliferation- and apoptosis-related indicators, after transfection with pre-miR-424-5p in HepG2 cells. Data are shown as the mean ± SD. The time point of tumor sampling was 50 days after inoculation of the cells. *P < 0.05 vs control, **P < 0.01 vs control. NC, negative control.

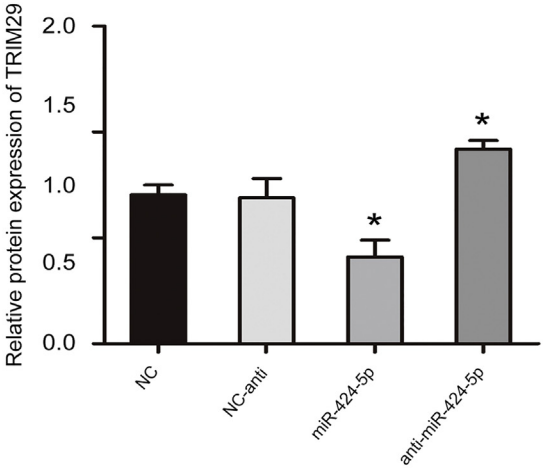












(caption on next page)

Fig. 5. miR-424-5p directly targeted TRIM29. (A) Four potential genes of miR-424-5p were predicted by four bioinformatics databases: TargetScan, MiRnada, Oncomir, and miRWalk. (B) miR-424-5p putative binding sites and the corresponding mutant sites of TRIM29. (C) The luciferase activity of wild-type (WT) TRIM29–3'UTR and mutant (MUT) TRIM29–3'UTR was assessed. (D-E) qRT-PCR and Western blotting analysis were used to examine the mRNA and protein levels of TRIM29 in MHCC-97H cells which were cotransfected with pre-miR-424-5p and miR-424-5p-inhibitor vectors. *P < 0.05 vs control, **P < 0.01 vs control. NC, negative control.

TRIM29 expression had a significantly shorter OS than those with low TRIM29 expression (P = 0.011, Fig. 2D).

Interestingly, miR-424-5p expression was positively correlated with AFP (P = 0.048), TNM stage (P = 0.001) and intrahepatic metastasis (P = 0.001) (Table 1). In univariate analysis, AFP (P = 0.027), TNM stage (P = 0.024) and miR-424-5p (P = 0.022) were significantly associated with OS. The multivariate model revealed that OS was significantly dependent on AFP (P = 0.031), TNM stage (P = 0.011) and miR-424-5p (P = 0.010) (Table 2), suggesting that miR-424-5p is an independent prognostic factor for OS in HCC.

3.3. miR-424-5p inhibits cell proliferation, migration and invasion in HCC cells

To further study the biological function of miR-424-5p in HCC cells, we conducted gain-of-function experiments of miR-424-5p in MHCC-97H and HepG2 cells. As detected by qRT-PCR, we confirmed that miR-424-5p was effectively overexpressed in both cell lines (P < 0.05, Fig. 3A). The CCK8 assay showed that increased miR-424-5p expression significantly inhibited cell proliferation in MHCC-97H and HepG2 cells compared with the NC groups (P < 0.01, Fig. 3B). Wound healing assays indicated that MHCC-97H and HepG2 cell mobility was significantly decreased with miR-424-5p overexpression (P < 0.05, Fig. 3C). As shown in Fig. 3D, the number of HCC cells incorporating EdU in the miR-424-5p overexpression group was less than that in the control group. In addition, a cell invasion assay showed that the upregulation of miR-424-5p inhibited MHCC-97H and HepG2 cell migration (P < 0.05, Fig. 3E). Moreover, colony formation assays showed that miR-424-5p showed overexpression resulted in remarkably decreased colony-formation abilities in MHCC-97H and HepG2 cells (P < 0.05, Fig. 3E).

3.4. miR-424-5p suppresses tumorigenicity in HCC

We also identified the influence of miR-424-5p on proliferation in vivo. As shown in Fig. 4A and B, the overexpression of miR-424-5p limited tumor growth significantly in MHCC-97H cells. Similar effects of miR-424-5p were observed in the HepG2 cell model (Fig. 4C, D). Western blot analysis showed that miR-424-5p overexpression decreased TRIM29, AFP, Bcl-2 and Ki67 expression and increased Bax expression (Fig. 4E). Similar effects of miR-424-5p were observed in the HepG2 cell model (Fig. 4F).

3.5. miR-424-5p directly interacts with TRIM29 in HCC

To further investigate the molecular mechanisms by which miR-424-5p exerts its functional effects on HCC, we searched miRNA databases, including TargetScan, miRWalk, Oncomir and miRNAda, and identified TRIM29 as the potential target gene of miR-424-5p (Fig. 5A). Moreover, the miRNA databases indicated that miR-424-5p could bind to the 3′-UTR of TRIM29 (Fig. 5B). The luciferase reporter assay suggested that cotransfection of miR-424-5p significantly inhibited luciferase activity in cells transfected with Wt-TRIM29 3'-UTR. However, the inhibition was not observed in cells cotransfected with Mut-TRIM29 3'-UTR (Fig. 5C). In addition, the overexpression of miR-424-5p significantly inhibited both the mRNA and protein expression of TRIM29 (Fig. 5D, E).

3.6. TRIM29 ameliorates the inhibitory effect of miR-424-5p on HCC proliferation, migration and invasion

To confirm whether miR-424-5p executed its functional effects by suppressing TRIM29, western blot analysis was performed. The results revealed that TRIM29 expression was restored by the overexpression of TRIM29 in MHCC-97H and HepG2 cells, which overexpress miR-424-5p (Fig. 6A). The restoration of TRIM29 expression significantly abolished the inhibitory effects of miR-424-5p on proliferation, migration and invasion (Fig. 6B–F). Moreover, the restoration of TRIM29 expression increased the expression of TRIM29, AFP, Bcl-2 and Ki67 and decreased Bax expression in MHCC-97H and HepG2 cells, which overexpress miR-424-5p (Fig. 6G).

4. Discussion

Hepatocellular carcinoma, as a malignant tumor with a high rate of metastasis and recurrence, has caused a large number of deaths and a huge burden on families and society [1]. The latest research shows that the ectopic expression of miRNA may lead to the occurrence and development of cancer [20]. Therefore, it has been found that oncogenic or tumor suppressor miRNAs are important for revealing the molecular mechanism of HCC progression. Recently, miR-424-5p was found to be involved in the progression of various malignant tumors, such as colon carcinoma, cervical cancer and epithelial ovarian cancer [21,22]. In this study, we discovered that miR-424-5p was decreased in both HCC tissues and cell lines and that miR-424-5p was significantly related to AFP, TNM stage, and intrahepatic metastasis. Meanwhile, HCC patients with low miR-424-5p expression had a significantly shorter OS than those with high miR-424-5p expression. Therefore, these results indicate that miR-424-5p is a potential biomarker and tumor suppressor gene for HCC.

Then, rescue experiments were conducted in HCC tissues and cell lines. The results showed that the overexpression of miR-424-5p inhibited proliferation, migration and invasion as well as tumor growth in vivo by inhibiting genes related to cell proliferation and apoptosis. New research has shown that the overexpression of miR-424-5p can regulate the malignant biological behavior of tumor cells by targeting KDM5B, which inhibits the notch signaling pathway in cervical cancer [16]. Studies have also identified that the upregulation of miR-424-5p can decrease invasion, metastasis and proliferation via the SMAD7 pathway mediated EMT in ESCC [17]. In contrast, miR-424-5p was upregulated and led to increased cell proliferation, migration and invasion by suppressing the expression of SOCS6 and regulating the ERK1/2 signaling pathway in pancreatic cancer [23]. This result further show that miR-424-5p plays different roles in malignant tumors, which may also be caused by different microenvironments of the tumor. A change in the tumor microenvironment is also one of the reasons for promoting tumor development. The role of miR-424-5p in different tumor microenvironments is our next direction of research.

miRNAs bind to a specific site in the 3′ noncoding region of the target mRNA and mediate degradation of the mRNA by incomplete complementation [24]. miRNA databases were used to identify the direct targets of miR-424-5p. TRIM29 was predicted as one of the target genes of miR-424-5p. In colorectal cancer, TRIM29 expression is upregulated expression and associated with a poor prognosis [25]. In addition, TRIM29 plays an important role in proliferation and invasion and increases chemosensitivity to cisplatin in human pancreatic cancer [26]. Furthermore, the knockdown of TRIM29 can increase

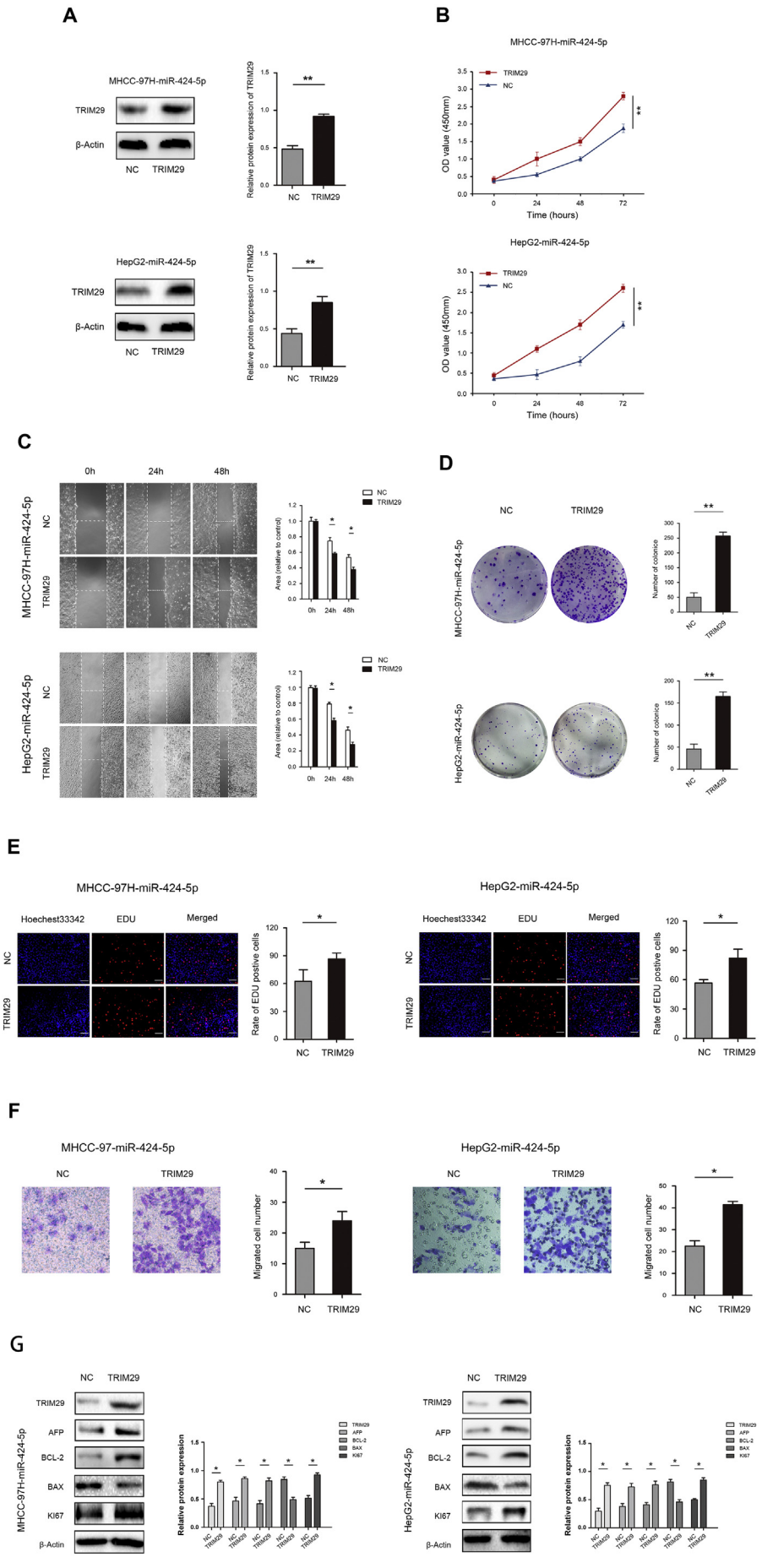


Fig. 6. Restoration of TRIM29 ameliorates the inhibitory effect of miR-424-5p in HCC cell lines. (A) Western blot showing the expression of TRIM29 in MHCC-97H-miR-424-5p and HepG2-miR-424-5p cells were transfected with the TRIM29 vector and the NC. (B) A CCK-8 assay was used to examine the effects of the overexpression of TRIM29 on proliferative ability in MHCC-97H-miR-424-5p and HepG2-miR-424-5p cells. (C) A wound-healing assay was performed to determine the effects of the overexpression of TRIM29 on the migration of MHCC-97H-miR-424-5p and HepG2-miR-424-5p cells. (D) Colony formation assays of the effects of the overexpression of TRIM29 on the migration of MHCC-97H-miR-424-5p and HepG2-miR-424-5p cells. (E) Cell proliferation was detected with EdU in MHCC-97H-miR-424-5p and HepG2-miR-424-5p cells after the overexpression of TRIM29. (F) The invasion assay was used to examine the effects of TRIM29 overexpression on MHCC-97H-miR-424-5p and HepG2-miR-424-5p cell invasion. (G) Western blot showing the proliferation- and apoptosis-related indicators in MHCC-97H-miR-424-5p and HepG2-miR-424-5p cells transfected with the TRIM29 vector and the NC. *P < 0.05 vs control, **P < 0.01 vs control. NC, negative control; OD, optical density.

chemosensitivity to cisplatin in human lung squamous cancer and promote tumor cell apoptosis [27]. In addition, the knockdown of TRIM29 results in decreased cell growth, motility, and invasiveness via inhibition of the EMT by TWIST1 in breast cancer [28]. In our research, we found that HCC patients with high TRIM29 expression had a worse prognosis. As expected, the restoration of TRIM29 reversed the effect of miR-424-5p overexpression on cell proliferation, migration and invasion in HCC.

In conclusion, our study demonstrated that the expression of miR-424-5p was reduced in HCC, and reduced miR-424-5p expression is correlated with AFP, TNM stage, and intrahepatic metastasis, moreover, HCC patients with low expression of miR-424-5p have a worse prognosis. The upregulation of miR-424-5p inhibited cell proliferation, migration and invasion in vitro, and suppressed HCC tumor growth in vivo via TRIM29 and its related proliferation and apoptosis genes. In summary, our findings provide a new direction for the molecular pathogenesis of HCC and identify the miR-424-5p/TRIM29 axis as a potential therapeutic target in HCC.

Conflict of interest

The authors have no conflicts of interest.

Authors' contributions

Huimin Du and Hao Wu conceived and designed the study. Qing Xu, Sheng Xiao and Zhenru Wu performed the experiments. Jianping Gong, Guosheng Ren, Hao Wu and Changan Liu collected and analyzed the clinical data. Huimin Du and Hao Wu analyzed and interpreted the data. Huimin Du and Hao Wu wrote the manuscript.

Competing interest

The authors have declared that no competing interest exists.

Acknowledgements

This work was supported by the Chongqing Graduate Research and Innovation Project (CYB18156).

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2017, CA Cancer J. Clin. 67 (2017) 7–30.
[2] W. Chen, et al., Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2016) 115–132.
[3] T.F. Greten, X.W. Wang, F. Korangy, Current concepts of immune based treatments for patients with HCC: from basic science to novel treatment approaches, Gut 64 (2015) 842–848.
[4] S. Hatakeyama, TRIM proteins and cancer, Nat. Rev. Cancer 11 (2011) 792–804.
[5] L.M. Napolitano, G. Meroni, TRIM family: pleiotropy and diversification through homomultimer and heteromultimer formation, IUBMB Life 64 (2012) 64–71.
[6] Z. Xiao, Q. Jiang, J. Willette. Brown, S. Xi, F. Zhu, S. Burkett, et al., The pivotal role of IKKα in the development of spontaneous lung squamous cell carcinoma, Cancer Cell 23 (2013) 527–540.
[7] N. Fristrup, K. Birkenkamp-Demtröder, T. Reinert, M. Sanchez. Carbayo, U. Segersten, et al., Multicenter validation of cyclin D1, MCM7, TRIM29, and UBE2C as prognosticprotein markers in non-muscle-invasive bladder cancer, Am. J. Pathol. 182 (2013) 339–349.
[8] C.A. Iacobuzio-Donahue, R. Ashfaq, A. Maitra, N.V. Adsay, G.L. Shen-Ong, K. Berg, et al., Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies, Cancer Res. 63 (2003) 8614–22.
[9] S.T. Tan, S.Y. Liu, B. Wu, TRIM29 overexpression promotes proliferation and survival of bladder cancer cells through NF-κB signaling, Cancer Res. Treat. 48 (2016) 1302–1312.
[10] J.K. Xu, Z.Y. Li, Q.H. Su, J. Zhao, J.C. Ma, TRIM29 promotes progression of thyroid carcinoma via activating P13K/AKT signaling pathway, Oncol. Rep. 37 (2017) 1555–1564.
[11] J.J. Xing, A. Zhang, H. Zhang, J. Wang, X.C. Li, M.S. Zeng, Z.Q. Zhang, TRIM29 promotes DNA virus infections by inhibiting innate immune response, Nat. Commun. 8 (2017) 945.
[12] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[13] D.P. Bartel, MicroRNAs, Cell 173 (2018) 20–51.
[14] C.N. Hayes, K. Chayama, MicroRNAs as biomarkers for liver disease and hepatocellular carcinoma, Int. J. Mol. Sci. 17 (2016) 2016.
[15] A. Cohen, M.A. Burgos-Aceves, Y. Smith, Estrogen repression of microRNA as a potential cause of cancer, Biomed. Pharmacother. 78 (2016) 234–238.
[16] Y. Zhou, Q. An, R.X. Guo, Y.H. Qiao, L.X. Li, X.Y. Zhang, X.L. Zhao, miR424-5p functions as an anti-oncogene in cervical cancer cell growth by targeting KDM5B via the notch signaling pathway, Life Sci. 171 (2017) 9–15.
[17] F. Wang, J. Wang, X. Yang, Chen, L. Wang, MiR-424-5p participates in esophageal squamous cell carcinoma invasion and metastasis via SMAD7 pathway mediated EMT, Diagn. Pathol. 11 (2016) 88.
[18] J. Liu, Z. Gu, Y. Tang, J. Hao, C. Zhang, Tumour-suppressive microRNA-424-5p directly targets CCNE1 as potential prognostic markers in epithelial ovarian cancer, Cell. Cycl. 17 (2017) 1–10.
[19] J.L. Wang, S.B. Wang, J.J. Zhou, Q. Qian, miR-424-5p regulates cell proliferation, migration and invasion by targeting doublecortin-like kinase 1 in basal-like breast cancer, Biomed. Pharmacother. 102 (2018) 147–152.
[20] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (2006) 857–866.
[21] J. Zhang, H. Liu, L. Hou, G. Wan, R. Zhang, Circular RNA_LARP4 inhibits cell proliferation and invasion of gastric cancer by sponging miR-424-5p and regulating LATS1 expression, Mol. Cancer 16 (2017) 151.
[22] J. Xu, Y. Li, F. Wang, X. Wang, B. Cheng, F. Ye, X. Xie, C. Zhou, W. Lu, Suppressed miR-424 expression via upregulation of target gene Chk1 contributes to the progression of cervical cancer, Oncogene 32 (2013) 976–987.
[23] Kemin. Wu, G.H. Hu, X. He, P. Zhou, J. Li, B. He, W.J. Sun, MicroRNA-424-5p suppresses the expression of SOCS6 in pancreatic cancer, Pathol. Oncol. Res. 19 (2013) 739–748.
[24] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233.
[25] T. Jiang, H.M. Tang, S. Lu, Y.X. Yang Yan, Z.H. Peng, Up-regulation of tripartite motif-containing 29 promotes cancer cell proliferation and predicts poor survival in colorectal cancer, Med. Oncol. 30 (2013) 715.
[26] L. Wang, H. Yang, P. L. Palmbos, G. Ney, T. A. Detzler, D. Coleman, J. Leflein, et al., ATDC/TRIM29 phosphorylation by ATM/MAPKAP kinase 2 mediates radioresistance in pancreatic cancer cells, Cancer Res. 74 (2014) 1778–1788.
[27] C. Liu, X. Huang, S. Hou, B. Hu, H. Li, Silencing of tripartite motif (TRIM) 29 inhibits proliferation and invasion and increases chemosensitivity to cisplatin in human lung squamous cancer NCI-H520 cells, Thorac. Cancer. 6 (2015) 31–37.
[28] L. Ai, W.J. Kim, M. Alpay, M. Tang, C.E. Pardo, TRIM29 suppresses TWIST1 and invasive breast cancer behavior, Cancer Res. 74 (2014) 4875–4887.