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MiR-372-3p promotes tumor progression by targeting LATS2 in colorectal cancer

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Abstract. – OBJECTIVE: Many studies suggest that microRNAs can promote the malignant development of tumors. MiRNA-372-3p (miR-372-3p) has been proved to be associated with a variety of cancers. However, the role of miR-372-3p in colorectal cancer (CRC) is unclear.

PATIENTS AND METHODS: We analyzed the expression of miR-372-3p in CRC tissues and several CRC cell lines by quantitative Real Time-PCR. The relationship between miR-372-3p and clinical pathology was also analyzed in CRC patients. Kaplan-Meier analysis and Cox multivariate analysis were used to evaluate the prognostic significance of miR-372-3p in CRC. Next, we investigated the biological function of miR-372-3p, including cell proliferation, migration, and invasion and analyzed its potential molecular mechanism *in vivo* and *in vitro*.

RESULTS: Our data showed that the expression of miR-372-3p was dramatically increased in CRC tissues compared with normal tissues. Moreover, the high expression of miR-372-3p was significantly correlated with tumor size and differentiation. Kaplan-Meier analysis showed that the high miR-372-3p expression group patients had a significantly shorter recurrence-free survival (RFS) and disease-specific survival (DSS) than those with the low miR-372-3p group. The analysis of the prognostic factors revealed that miR-372-3p was an independent prognostic factor for RFS and DSS in CRC patients. The knockdown of miR-372-3p inhibited the proliferation, migration, and invasion in HCT116 and SW480 cells. Interestingly, the overexpression of LATS2 partially reversed the miR-372-3p -mediated cell proliferation, migration, and invasion of CRC. Besides, the Hippo signaling pathway was demonstrated to be activated by decreasing of miR-372-3p in CRC. Thus, our study revealed that miR-372-3p is involved in CRC progression by inhibiting the Hippo signaling pathway through its target LATS2. MiR-372-3p and its target genes with signaling pathways are new hope for precise treatment of CRC.

CONCLUSIONS: The upregulation of miR-372-3p was involved in the process of CRC progression by inhibiting the Hippo signaling pathway through inhibition of LATS2. We showed that miR-372-3p and its target genes with signaling pathways are a novel hope for precise treatment of CRC.

Key Words:
Colorectal cancer, MiR-372-3p, Prognosis, Tumor progression, LATS2, Hippo signaling.

Abbreviations

CRC: Colorectal cancer; miRNAs: MicroRNAs; LATS2: Large tumor suppressor gene 2; YAP: Yes-associated protein; HE: Hematoxylin and eosin; NC: negative control; SDS-PAGE: sulfate polyacrylamide gel electrophoresis; RFS: recurrence-free survival; DSS: disease-specific survival.

Introduction

Colorectal cancer (CRC) is one of the most common cancers, which has a hidden incidence, rapid progress, and high mortality rate, CRC is responsible for approximately 600,000 deaths annually[1,2]. Early CRC may have no evident symptoms or only result in changes in the bowel habits[3,4]. Due to the lack of effective and timely diagnostic methods, most patients are already in an advanced stage at the time of diagnosis and have corresponding adverse outcomes. Therefore, it is very important and urgent to de-

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termine the molecular mechanisms that collaboratively regulate CRC progression, which can provide new insights and effective personalized treatments for CRC.

MicroRNAs (miRNAs) are single-stranded non-coding small RNAs with a length of 17-22 nt. MiRNAs are encoded by higher eukaryotic genomes and degrade mRNAs or hinder their translation by base-pairing with target gene mRNAs leading to a silencing complex[5]. Dysregulated miRNA expression is implicated in the development and progression of various human tumors[6,7]. There is a growing concern about mir-372-3p in the development of cancer. Wang et al[8] reported that miR-372-3p regulates FGF9 as oncogene and promotes cell growth and metastasis in lung squamous cell carcinoma. MiR-372-3p can reduce the expression of DKK1, thereby promoting the epithelial-mesenchymal transition by activating the Wnt pathway in breast carcinoma[9]. However, it is unknown whether the functional mechanism of miR-372-3p regulates the malignant progression of CRC and whether it can serve as a promising prognostic biomarker in CRC.

Large tumor suppressor gene 2 (LATS2) is an important core of the Hippo signaling pathway, which phosphorylates the downstream Yes-associated protein (YAP) and inhibits its function[10]. The Hippo signaling pathway regulates the dynamic balance between organ size, cell growth, and death, and it effectively controls the generation of tumors[11]. A lot of researches have shown that LATS2 plays an important role in regulating the malignant development of tumor cells[12,13]. However, the relationship between LATS2 and miR-372-3p remains unknown.

In the current study, we observed that miR-372-3p negatively regulated LATS2 in CRC. Additionally, the low expression of LATS2 can regulate the expression of YAP to monitor the transcription of the downstream target gene and promote the malignant progress of CRC. In conclusion, our results suggest that miR-372-3p promotes the malignant progress of CRC *via* regulating LATS2 and inactivating the Hippo signaling pathway.

Patients and Methods

Clinical Specimens

All CRC specimens from patients were collected from May 2007 to July 2012 at the Nan chong Central Hospital. The diagnosis of CRC was identified histologically by haematoxylin and eosin (HE) staining. A total of 79 fresh CRC tissues and paired normal adjacent tissues were obtained for analysis. This investigation was approved by the Institutional Review Board (IRB) of the Nanchong Central Hospital and informed consent document was signed by all patients. (IACUC-20070510015, Nanchong, China).

Cell Lines

HT29, SW480, LOVO, and HCT116 colorectal cancer cell lines were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). CRC cell lines were cultured in high-glucose DMEM (Gibco, Rockville, MD, USA), and the normal colon epithelial (FHC) cell lines were maintained in Dulbecco's Modified Eagle's Medium (Gibco, Rockville, MD, USA). All cell lines were supplemented with 10% fetal bovine serum (Gibco, Rockville, MD, USA) and 100 units/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in a humidified 5% CO_2 incubator.

Cell Transfection

HCT116 and SW480 cells were transfected with miR-372-3p mimics (miR-372-3p), miR-372-3p inhibitor or a negative control (NC; RiboBio, Guangzhou, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The PcDNA3.1-LATS2 plasmid was also constructed by GenePharma Co., Ltd. (Shanghai, China) which was used in the rescue assay to restore LATS2 expression. Forty-eight hours after transfection, the cells were collected for cell function experiment, qRT-PCR, and Western blotting.

RNA Preparation and Real Time-PCR

The total RNA from CRC tissue and cells was extracted using TRIzol reagent (TaKaRa, Otsu, Shiga, Japan). RNA was reverse-transcribed to cDNA using the PrimeScript RT Reagent (TaKaRa, Otsu, Shiga, Japan). The quantitative Real Time-PCR was performed using the SYBR Green Real-time PCR Master Mix (TaKaRa, Dalian, Liaoning, China). All primers were shown in the **Supplementary Table I**. Reaction Cycle Conditions of PCR: 30 s of denaturation at 94°C, at 96°C for 20 s and 60°C for 1 min (40 cycles). The fold change was calculated according to the $2^{-\Delta\Delta Ct}$ method. U6 or GAPDH was used as the control.

Western Blot Analysis

The proteins from CRC cells and tissues were extracted with RIPA lysis buffer. The extracted protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Millipore, Billerica, MA, USA). Then, the extracted proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Next, the membranes block with non-fat powdered milk for 1 hour at 37°C. The membranes were probed at 4°C overnight with anti-LATS2 (1:1000, Abcam, Cambridge, MA, USA), anti-YAP (1:1000, Abcam, Cambridge, MA, USA), anti-KI67 (1:500, Abcam, Cambridge, MA, USA), anti-BAX (1:2000, Abcam, Cambridge, MA, USA), anti-BCL-2 (1:1500, Abcam, Cambridge, MA, USA) and anti-GAPDH (1:5000, Abcam, Cambridge, MA, USA) and incubated with a suitable secondary antibody. The protein expression levels were visualized using an enhanced chemiluminescence (ECL) detection system (Promega, Madison, WI, USA).

Luciferase Assay

The wild-type LATS2-3'UTR (WT) and mutant LATS2-3'UTR (MUT) oligonucleotides containing the putative binding site of miR-372-3p were synthesized using the firefly Luciferase-expressing pMIR-REPORT vector (Promega, Madison, WI, USA). CRC cells were seeded into 24-well plates for 24 h and then transfected with either the WT or Mut LATS2-3'UTR construct along with the miR-372-3p mimic or miR-NC, using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for 48 h. Then, the cells were collected, and detected from the Luciferase activity by using the Dual-Luciferase Reporter Assay kit (Promega, Madison, WI, USA) according to the manufacturer's protocol.

CCK-8 Assay

CRC cells were seeded at a density of 2000 cells/well in 96-well plates for 1-3 days. Subsequently, we added 10 μL of CCK-8 (Dojindo, Laboratories, Tokyo, Japan) solution to each well. According to the manufacturer instructions, the plates were incubated for 1 h at 37°C. The absorbance of each hole was recorded at 450 nm.

Colony-Forming Assays

CRC cells were plated in 6-well plates (300 cells/well) containing 2.5 mL of medium and were cultured for 21 days. The colonies were fixed with 4% paraformaldehyde for 20 min and stained with 1% crystal violet for 10 min.

Cell Migration and Invasion Assays

All cells were seeded onto the upper chamber. The cell invasion and migration assays were performed using the transwell chambers (Corning Costar, Cambridge, MA, USA). Briefly, 500 ul of high-glucose DMEM containing 10% fetal bovine serum (FBS) was added to the matched lower chamber. After incubation for 48 h, the transwell chambers were fixed in methanol and stained with 0.1% crystal violet. For the invasion assay, the inserts were pre-coated with Matrigel (1 mg/mL).

Tumorigenesis Assay

Five-weeks-old male BALB/c-nu mice were purchased from the Shanghai Experimental Animal Center (Shanghai, China) and were fed at the Animal Center of North Sichuan Medical College. The study was approved by the Ethics Committee of Nanchong Central Hospital (IACUC-20160314007). All animal experiments were performed in accordance with the institutional guidelines, and the method of mice sacrificed was cervical dislocation. Xenograft tumors were generated by subcutaneously injecting CRC cells (3×10^6) into the mice (n = 3 for each group). Tumor volume calculation formula: volume = (length × width^2)/2.

Statistical Analysis

All data were presented as the mean ± SD and were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7 (La Jolla, CA, USA). The statistical differences were analyzed by One-way analysis of variance, followed by the Newman-Keuls test, and they were used to evaluate the differences between groups, and repeated measures analysis of variance. The correlation between miR-372-3p and LATS2 expression was evaluated using Spearman's correlation analysis. The Kaplan-Meier method was used to assess RFS and DSS, and the log-rank test was used to analyze the differences between the curves. The prognostic significance of miR-372-3p expression was calculated by univariate and multivariate Cox regression analysis. The values of $p<0.05$ indicated statistical significance.

Results

MiR-372-3p and LATS2 Expression in Colorectal Cancer and Colorectal Cancer Cell Lines

We detected the levels of miR-372-3p and LATS2 in CRC tissues and paired normal adjacent tissues. The qRT-PCR results indicated that miR-372-3p levels were significantly increased in CRC tissues compared with the levels in paired normal adjacent tissues (Figure 1A). The mRNA levels of LATS2 were significantly decreased in tumor tissues compared to the levels in the paired normal adjacent tissues (Figure 1B). As expected, miR-372-3p and LATS2 were significantly increased and decreased in the CRC cells, respectively (Figures 1C and 1D).

High Expression of MiR-372-3p Correlates With Aggressive Clinicopathological Features and Poor Prognosis of CRC

The miR-372-3p expression and clinicopathological characteristics of CRC patients were analyzed. High miR-372-3p expression was significantly correlated with tumor size (p=0.038) and differentiation (p=0.006, Table I). The Kaplan-Meier analysis revealed that patients with high miR-372-3p expression had a significantly shorter recurrence-free survival (RFS) and disease-specific survival (DSS) than those with low miR-372-3p expression (Figures 2A-2B). In addition, the Cox regression analysis results revealed that miR-372-3p expression (HR=0.652, p=0.010), tumor size (HR=1.646, p=0.035), and differentiation (HR=1.856, p=0.011) were independent prognostic factors for RFS in CRC patients (Table II). Moreover, miR-372-3p expression (HR=0.831, p=0.009) and tumor size (HR=1.078, p=0.017) were independent prognostic factors for DSS in CRC patients (Table III).

Downregulated MiR-372-3p Inhibits CRC Cell Proliferation, Migration and Invasion

We determined whether the downregulation of miR-372-3p affects SW480 and HCT116 cell proliferation. The CCK-8 assays and colony results

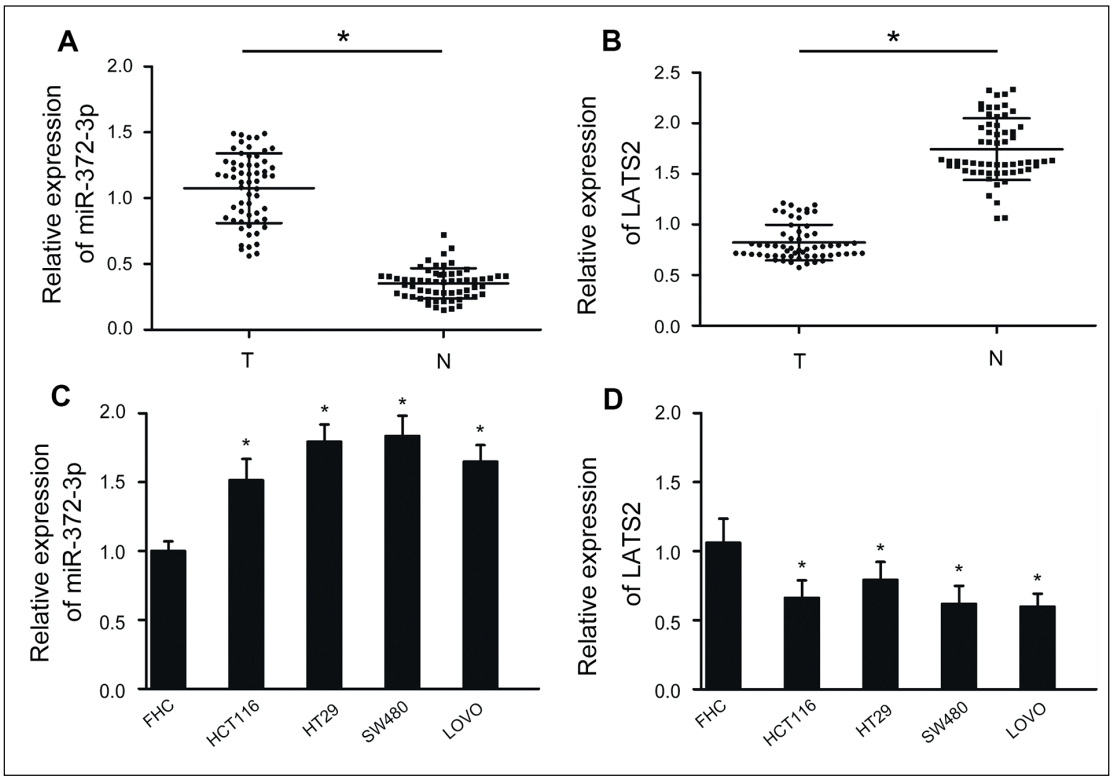

Figure 1. Upregulation of miR-372-3p and downregulation of LATS2 in CRC tissues and CRC cell lines. **A,** MiR-372-3p expression was evaluated in CRC tissues compared to paired adjacent normal tissues. **B,** LATS2 expression was evaluated in CRC tissues compared to paired adjacent normal tissues. **C,** MiR-372-3p expression was evaluated in CRC cell lines and FHC cells. **D,** LATS2 expression was evaluated in CRC cell lines and FHC cells. Data were expressed as mean ± SD, *p<0.05.

Table I. Correlations between miR-372-3p with clinicopathological features in CRC tissues.

Clinical parameter	Cases 79	miR-372-3p		*p*-value
		Low expression (n = 29)	High expression (n = 50)	
Gender				0.642
Male	39	13	26	
Female	40	16	24	
Age (year)				0.353
< 45	35	15	20	
≥ 45	44	14	30	
Tumor location				0.277
Colon	37	11	26	
Rectum	42	18	24	
Tumor size				0.038
≤ 5 cm	34	17	17	
> 5 cm	45	12	33	
Local invasion				0.101
T1-T2	41	19	22	
T3-T4	38	10	28	
Lymph node metastasis				0.149
Yes	46	12	34	
No	33	17	16	
TNM stage				0.209
I	20	9	11	
II	30	13	17	
III	29	7	22	
Differentiation				0.006
Well	17	10	7	
Moderate	25	12	13	
Poor	37	7	30	

r = right side; l = left side.

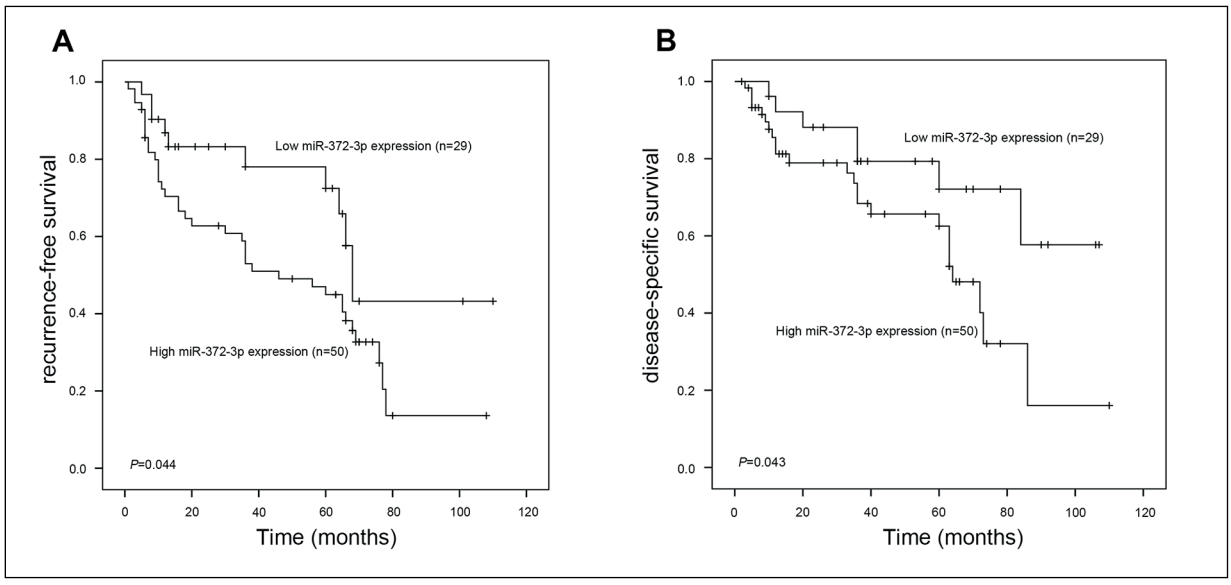

Figure 2. According to the miR-372-3p expression, the Kaplan-Meier curves for RFS and DSS in 79 colorectal cancer patients compared to paired adjacent normal tissues. **A,** Kaplan-Meier curve for RFS in CRC. **B,** Kaplan-Meier curve for DSS in CRC. RFS, recurrence-free survival; DSS, disease-specific survival.

Table II. Univariate and multivariate analyses of RFS in CRC patients.

Variables	Univariate analysis			Multivariate analysis model		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender Male Female	0.846	1.056-2.685	0.868			
Age (year) < 45 ≥ 45	1.281	0.793-1.907	0.775			
Tumor location Colon Rectum	1.424	0.284-1.558	0.631			
Tumor size ≤ 5 cm > 5 cm	1.307	0.657-1.864	0.019	1.646	1.536-3.951	0.035
Local invasion T1-T2 T3-T4	0.397	0.478-1.964	0.266			
Lymph node metastasis Ye No	1.308	1.007-2.844	0.593			
TNM stage I/II III	0.846	1.205-3.667	0.642			
Differentiation Well/Moderate Poor	1.039	0.587-2.884	0.026	1.856	1.590-3.334	0.011
miR-372-3p High Low	0.581	0.909-2.084	0.021	0.652	0.434-2.186	0.010

demonstrated that downregulation of miR-372-3p inhibited SW480 and HCT116 cell proliferation (Figures 3A-3F). Next, we examined the effect of miR-372-3p on SW480 and HCT116 cell migration and invasion through transwell assays. Our data showed that the downregulation of miR-372-3p inhibited SW480 and HCT116 cell migration and invasion (Figures 3G-3L). The level of miR-372-3p following SW480 and HCT116 cell transfection is shown in the **Supplementary Figures 1C-1D**.

MiR-372-3p Directly Targets the 3'-UTR of LATS2 in CRC

We searched for miRNAs data using databases, including TargetScan, miRWalk, Oncomir, and miRNAda, and identified that LATS2 was a potential target of miR-372-3p (Figure 4A). The complementary sequence of miR-372-3p was found in the 3'-UTR of LATS2 mRNA (Figure 4B). The Luciferase reporter vectors containing the wild-type (Wt) or mutant (Mt) LATS2 3'-UTR sequences revealed the combination of miR-372-3p on LATS2. We found that the co-transfection of miR-372-3p significantly inhibited the Luciferase activity in the cells transfected with Wt LATS2 3'-UTR (Figures 4C and 4D). By contrast, the inhibition was not found in the cells co-transfected with Mt LATS2 3'-UTR (Figures 4C and 4D). The upregulation of miR-372-3p reduced LATS2 expression (Figure 4E). In addition, our study showed that miR-372-3p is increased in CRC and LATS2 is decreased in CRC, which demonstrated that miR-372-3p expression is inversely associated with LATS2 expression (Figure 4F). We demonstrated that LATS2 is the direct target of miR-372-3p. The level of miR-372-3p following SW480 and HCT116 cell transfection is shown in the **Supplementary Figures 1A and 1B**.

MiR-372-3p Inhibits Tumorigenicity of CRC via LATS2

We explored whether miR-372-3p regulates CRC cell proliferation, migration, and invasion via LATS2. First, using a Western blot assay, we

Table III. Univariate and multivariate analyses of DSS in CRC patients.

Variables	Univariate analysis			Multivariate analysis model		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender Male Female	0.378	0.576-1.687	0.289			
Age (year) < 45 ≥ 45	1.071	0.580-1.867	0.604			
Tumor location Colon Rectum	1.286	0.644-1.750	0.743			
Tumor size ≤ 5 cm > 5 cm	1.630	1.308-2.860	0.024	1.078	1.267-3.003	0.017
Local invasion T1-T2 T3-T4	0.753	0.697-1.820	0.486			
Lymph node metastasis Ye No	1.841	1.457-2.960	0.403			
TNM stage I/II II	0.767	0.864-3.667	0.550			
Differentiation Well/ Moderate Poor	1.004	0.317-1.849	0.717			
miR-372-3p High Low	0.508	0.599-1.670	0.011	0.831	0.998-2.591	0.009

found that decreasing the expression of miR-372-3p increased the expression of LATS2 and Bax, and decreased the YAP, Bcl-2, and Ki67 expression (Figures 5A-5C), which activated the Hippo signaling pathway. To confirm this effect *in vivo*, we used tumorigenesis assays to measure the role of miR-372-3p in tumor xenografts in nude mice. Compared with the control group, the miR-372-3p inhibitor group showed a significant reduction in tumor volume and a slower tumor growth rate (Figures 5D-5I).

Overexpression of LATS2 Rescued the MiR-372-3p-Mediated Effect on CRC Cells

To further confirm that LATS2 is a functional target gene of miR-372-3p, we restored the expression of LATS2 using LATS2 plasmids. The expression of LATS2 was significantly elevated in the cells co-transfected with pcDNA-LATS2 and miR-372-3p mimics compared with the cells co-transfected miR-372-3p mimics group in HCT116 and SW480 cells (Figures 6A and 6B). Notably, the overexpression of LATS2 could reverse the effect of proliferation, migration, and invasion by miR-372-3p mimics (Figures 6C-6H). In conclusion, these results indicated that miR-372-3p affects the proliferation, invasion, and metastasis of CRC by regulating LATS2.

Discussion

Increasing evidence showed that miRNAs and their target genes are involved in the malignant progression of CRC[13-17]. We explored the role of miR-372-3p in the malignant progression of CRC. MiR-372-3p is an important member of miRNA-371-373 (miR-371-373) cluster. There are many reports evidencing that miR-372-3p may act as a carcinogen and plays an important role in tumorigenesis in various cancers, such as hepatoblastoma, testicular germ cell tumors, and oral carcinomas[18-20]. Carter et al[21] demonstrated that miR-372-3p contributes to CRC development and progression by enhancing the stemness of colorectal cancer cells. Recently, several researches[22,23] have found that miR-372-3p acts as an oncogenic microRNA, which can predict

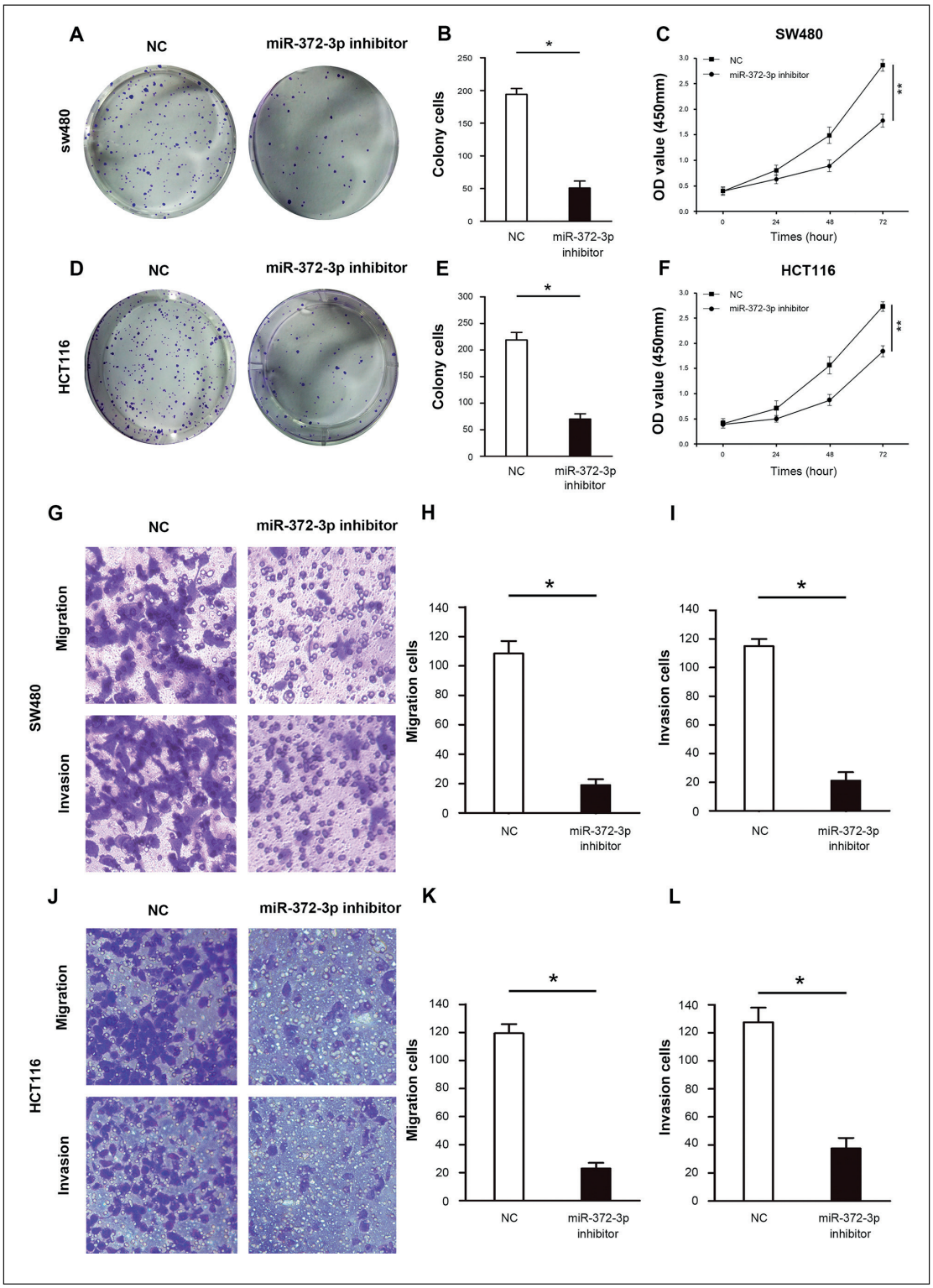

Figure 3. Downregulated miR-372-3p inhibits CRC cell proliferation, migration, and invasion. **A-B**, SW480 cells were subjected to colony forming assays and were quantitatively analyzed. **C**, SW480 cells were subjected to CCK-8 assays. **D-E**, HCT116 cells were subjected to colony forming assays and were quantitatively analyzed. **F**, HCT116 cells were subjected to CCK-8 assays. **G-I,** SW480 cells were subjected to transwell assays and relative migration and invasion capabilities were quantitatively analyzed. The magnification is 200x. **J-L**, HCT116 cells were subjected to transwell assays and relative migration and invasion capabilities were quantitatively analyzed. The magnification is 200x. Data were expressed as mean ± SD, $^{**}p<0.01$.

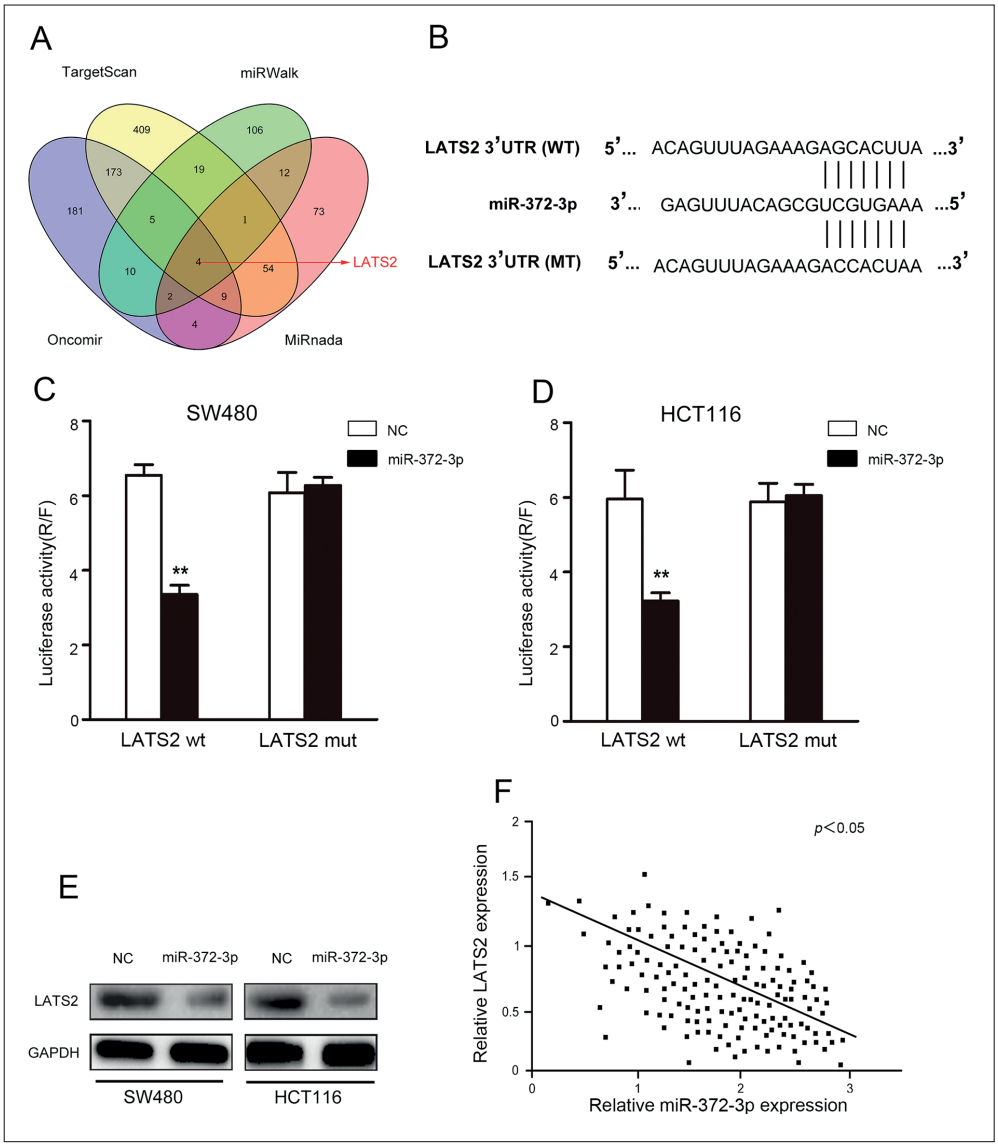

Figure 4. MiR-372-3p directly targets LATS2. **A,** Venn diagram demonstrating that miR-372-3p was computationally predicted to target LATS2 by four different prediction algorithms: TargetScan, MiRnada, Oncomir, and miRWalk. **B**, Diagrams show the putative binding sites of miR-372-3p and the corresponding mutant sites of LATS2. **C-D**, miR-372-3p directly targets LATS2. A Luciferase reporter vector containing wild type (wt) or mutated (mut) LATS2 3'-UTR was co-transfected with a miR-372-3p precursor or scramble control. **E,** Protein expression of LATS2 in SW480 and HCT116 cells was assessed by Western blotting after transfecting cells with miR-372-3p mimics. **F,** MiR-372-3p expression was negatively correlated with LATS2 expression in CRC tissues. Data were expressed as mean ± SD, ** $p<0.01$.

poor prognosis in patients with CRC. Our results demonstrated that miR-372-3p was upregulated in CRC tissues compared with the expression in paired normal adjacent tissues, which is consistent with the results of a previous study in CRC. Moreover, the expression of miR-372-3p was significantly associated with tumor size and differentiation in CRC. We also found that high expression of miR-372-3p is closely related to poor prognosis of patients, indicating that miR-372-3p was used as an independent factor for DSS and RFS and might participate in CRC progression.

The large tumor suppressor 2 (LATS2) is a tumor suppressor gene involved in the regulation of cell proliferation, migration, and apoptosis through various cellular signal transduction pathways, especially the Hippo pathway[24,25]. A large amount of evidence has shown that LATS2

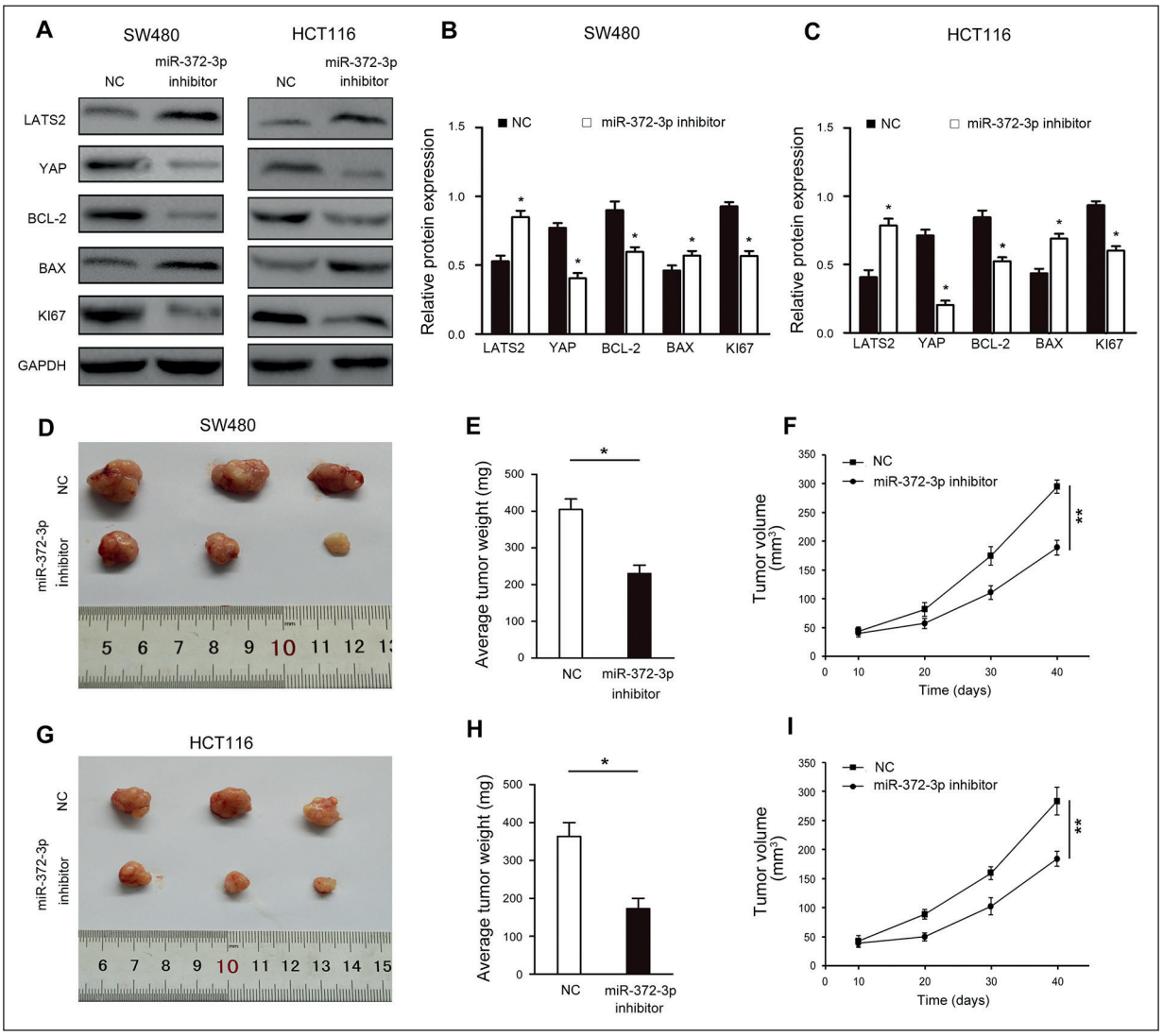

Figure 5. MiR-372-3p inhibits tumorigenicity of CRC via LATS2. **A-C**, The expression of LATS2, as proliferation-related and apoptosis-related indicators, after transfection with miR-372-3p inhibitor in SW480 and HCT116 cells. **D**, SW480 cells transfected with miR-372-3p inhibitor was subcutaneously inoculated into nude mice to form tumor. **E-F,** Tumor volume and weight growth curves were recorded. **G,** HCT116 cells transfected with miR-372-3p inhibitor was subcutaneously inoculated into nude mice to form tumor. **H-I,** Tumor volume and weight growth curves were recorded. Data were expressed as mean ± SD, *p<0.05; **p<0.01.

can inhibit the malignant development of tumors. After activation, LATS2 kinase phosphorylates YAP and blocks its nuclear translocation, which acts as a transcription factor to initiate the downstream gene expression involved in cell proliferation, invasion, and apoptosis[26]. LATS2 is often inactivated by methylation changes, resulting in the inability of the Hippo signaling pathway to regulate the malignant development of tumors[27]. In several types of cancer, a loss of LATS2 expression has been observed. This loss of expression has been well-established in breast cancer, in which the loss of LATS2 expression is associated with poor prognosis[28]. Loss of LATS2 expression has also been demonstrated in non-small-cell lung carcinoma, liver cancer, and prostate cancer[29,30]. Some investigations[31] report that LATS2 is involved in p53, including its role in mediating LATS2 in G1 checkpoints and in identifying overlapping transcriptional targets between LATS2 and p53, such as P21 and TP53 IMP. Notably, our studies indicated that LATS2 is significantly downregulated in CRC. Moreover, we observed that LATS2 is a

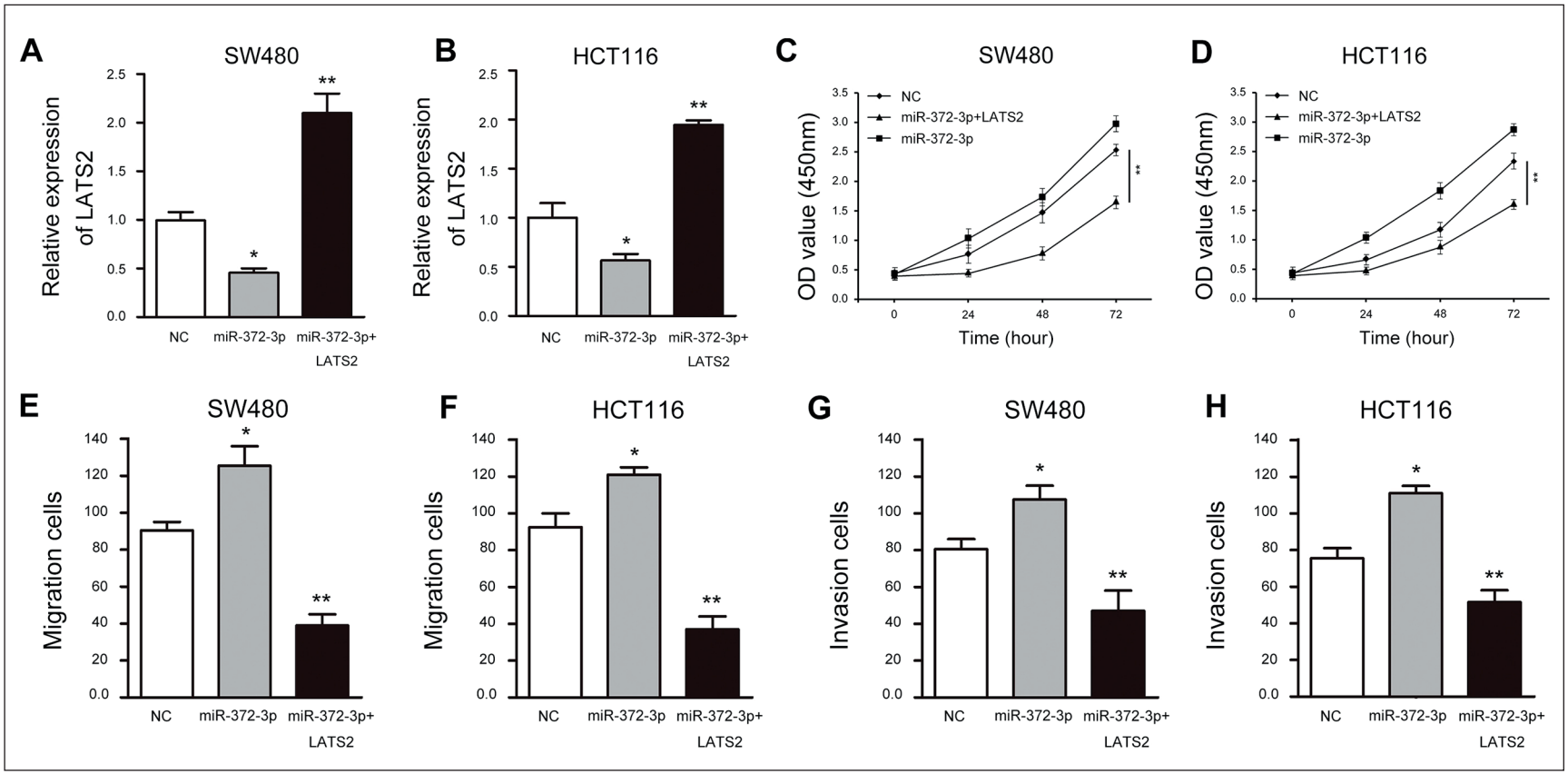

Figure 6. LATS2 is required for miR-372-3p-directed CRC cell proliferation, migration, and invasion. **A-B,** The expression of LATS2 was increased by transfection with LATS2 plasmids in miR-372-3p mimic-transfected SW480 and HCT116 cells. **C-D,** The CCK8 assay was used to detect co-transfection with miR-372-3p mimic and LATS2 plasmid in SW480 and HCT116 cells. **E-F,** The migration assay was used to detect the effects of SW480 and HCT116 cells treated as described on cell migration. **G-H,** The invasion assay was used to detect the effects of SW480 and HCT116 cells treated as described on cell invasion. Data were expressed as mean ± SD, *$p<0.05$; **$p<0.01$.

target of miR-372-3p. Functionally, miR-372-3p silencing inhibits CRC cell proliferation, promotes CRC cell apoptosis, and reduces cell tumorigenicity via LATS2, suggesting a crucial functional role of miR-372-3p in CRC.

Conclusions

We revealed that miR-372-3p promotes CRC development and progression by suppressing of LATS2 expression and inactivating the Hippo signaling pathway. Our findings provide new directions and ideas for the accurate diagnosis and treatment of CRC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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