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LncRNA FAL1 promotes carcinogenesis by regulation of miR-637/NUPR1 pathway in colorectal cancer

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ABSTRACT

LncRNA FAL1 has been demonstrated to play an important role in promoting carcinogenesis via the ceRNA mechanism in several types of cancer. However, the role and the mechanism of lncRNA FAL1 in CRC remain unclear. Here our results demonstrate that lncRNA FAL1 is markedly upregulated in CRC tissues and cells, and lncRNA FAL1 promotes proliferation ability, migration and invasion in CRC cells. Additionally, we demonstrate that lncRNA FAL1 acts as a sponge of miR-637, which functions as a suppressor via targeting and down-regulation of NUPR1 expression. Moreover, we demonstrate that lncRNA FAL1 promotes carcinogenesis of CRC cells via regulation of the miR-637/NUPR1 pathway. Taken together, our findings underscore the crucial roles of lncRNA FAL1 in CRC carcinogenesis and its potential prognostic and therapeutic value.

1. Introduction

Colorectal cancer (CRC) is still one of the most common cancers worldwide and a leading cause of cancer-related death, with a high yearly mortality rate (Siegel et al., 2017a,b). Although therapeutic advances have been made in CRC, the 5-year survival rate of CRC patients has still been poor in the past several decades due to recurrence, distant metastasis and drug resistance after treatment with surgery, chemotherapy and radiotherapy (Sasaki et al., 2008). Therefore, further investigation of the detailed molecular mechanisms underlying CRC proliferation and metastasis is urgently needed to develop specific biomarkers for early diagnostic or therapeutic strategies.

Recently, increasing evidence has proved that many long noncoding RNAs (lncRNAs) play important roles in modulating the carcinogenesis and progression of human CRC. LncRNAs are a newly discovered class of long RNA transcripts that are between 200 nucleotides and 100 kilobases (kb) in length and have limited or no protein-coding capacity (Dinger et al., 2008; Nagano and Fraser, 2011). As an important class of regulatory molecules, lncRNAs play critical roles in multiple biological processes, including cell proliferation, the cell cycle and cancer metastasis. Evidence suggests that lncRNAs could perform their function by (1) interacting with protein partners (Wang et al., 2018), (2) modulating alternative splicing (Tripathi et al., 2010), (3) regulating chromatin structure (Guttman et al., 2009) and (4) acting as decoys that titrate microRNAs (miRNAs) (Ma et al., 2012; Song et al., 2014; Spizzo et al., 2012). Recent reports suggest that the overwhelming majority of lncRNAs perform their functions by competing with endogenous RNA (ceRNA) for miRNA targets, thus indirectly regulating the downstream targets of miRNAs. For example, the role of lncRNA FAL1 as an oncogene through the ceRNA mechanism has been proved in some types of cancer, including papillary thyroid cancer (PTC) (Jeong et al., 2016), non-small cell lung cancer (NSCLC) (Pan et al., 2017), ovarian cancer (OC) (Hu et al., 2014) and hepatocellular carcinoma (HCC) (Li et al., 2018). However, the role and the mechanism of lncRNA FAL1 in CRC remain poorly understood.

In this study, we demonstrate that lncRNA FAL1 is upregulated in CRC tissues and cells. LncRNA FAL1 functions as an oncogene by promoting tumorigenic behaviour via a ceRNA mechanism that involves competitive binding with miR-637. In addition, the detailed role of miR-637 in CRC was investigated in this study. Upon bioinformatics analysis and luciferase assays, nuclear protein 1 (NUPR1) was identified as the direct target of miR-637, and NUPR1 was proved to function as an oncogene by regulating the HIF-1α/LASP1 pathway in CRC. Finally, we found that lncRNA FAL1 could regulate the expression level of NUPR1 and its downstream molecules in a miR-637-dependent manner. Our results may provide new insights into the mechanisms underlying tumorigenesis and potential diagnostic biomarkers for CRC.

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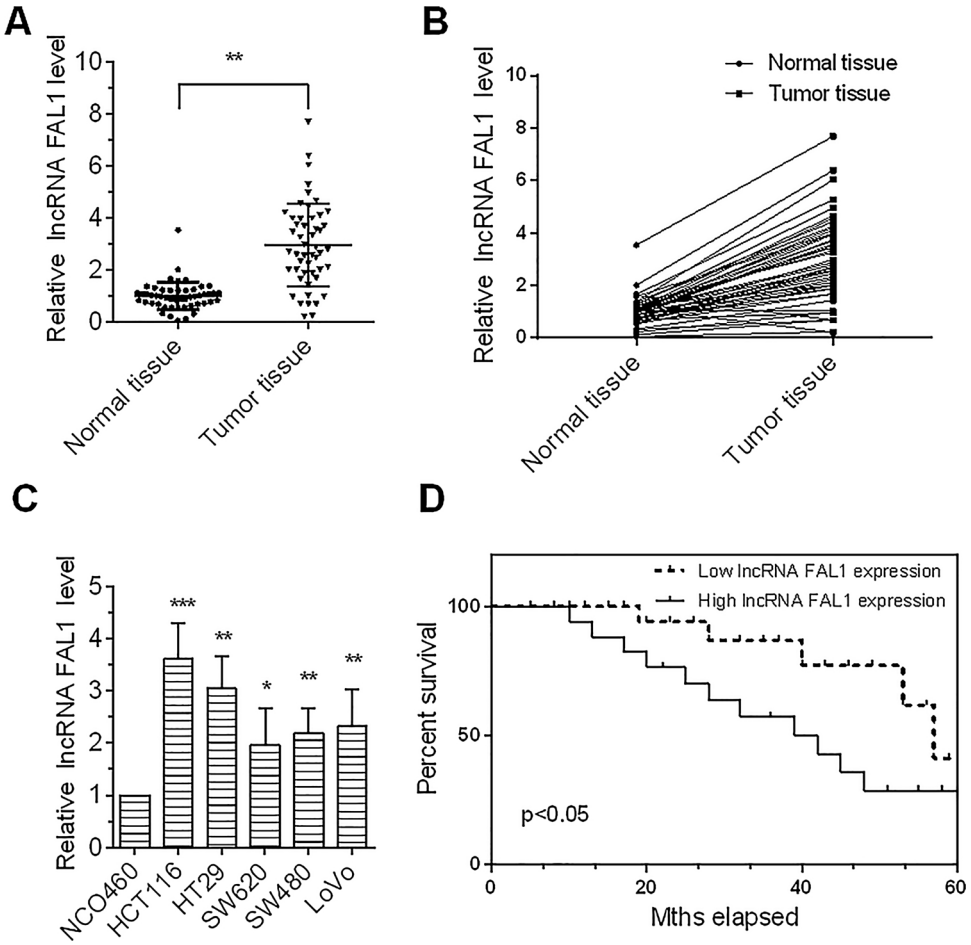

Fig. 1. LncRNA FAL1 is upregulated in CRC tissues and cell lines. (A and B) The lncRNA FAL1 expression level in 50 paired CRC tissues and adjacent normal tissues. (C) The lncRNA FAL1 expression level in normal colonic epithelial cell HCoEpiC and 5 CRC cell lines. (D) Kaplan-Meier survival curves is performed to assess the prognosis of CRC patients with high- and low- lncRNA FAL1 expression. *P < 0.05; **P < 0.01; ***P < 0.001.

Table 1
Clinical and pathologic characteristics of CRC patients.

Vairables	No. cases (50)	lncRNA FAL1 expression		*p*-value
		Low (%)	High (%)	
Age				
< 60 years (%)	34	15	19	0.225
≥60 years (%)	16	10	6	
Gender				
Male	42	20	22	0.440
Female	8	5	3	
Tumor size				
≥5 cm	23	8	15	0.047
< 5 cm	27	17	10	
TNM stage				
I–II	35	21	14	0.031
III–IV	15	4	11	
LNM				
No	20	14	6	0.021
Yes	30	11	19	
Histological type				
Squamous	38	18	20	0.508
Adenocarcinoma	12	7	5	

aχ^2 test. P-values in bold print indicate statistically significant differences. TNM, Tumor Node Metastasis.

2. Materials and methods

2.1. Tissue samples

50 paired CRC issues and correlatively adjacent normal tissues were obtained from Endoscopy Center, China-Japan Union Hospital of Jilin University (Changchun, China). All the tissue specimens were confirmed by pathologically and histologically examination. The Ethics Committee of the China-Japan Union Hospital of Jilin University reviewed and approved the use of clinical tissues samples.

2.2. Cell culture

The human CRC cell lines (HCT116, HT29, SW620, SW480 and LoVo) were purchased from the American Type Culture Collection (ATCC) and cultured in RPMI1640 medium (Gibco, Grand Island, NY, USA) containing 10% (v/v) fetal bovine serum (FBS) and 1% antibiotics (100 μg/ml streptomycin, and 100 U/ml penicillin). The NCM460, a normal human colon mucosal epithelial cell line, was provided by INCELL (San Antonio, TX) and cultured in DMEM (Life Technology™, 1645798) containing 10% (v/v) FBS and 1% antibiotics (100 μg/ml streptomycin, and 100 U/ml penicillin). They were maintained at 37 °C under a humidified atmosphere containg 5% CO_2.

2.3. RNA isolation and real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from the tissues and culturing cells using

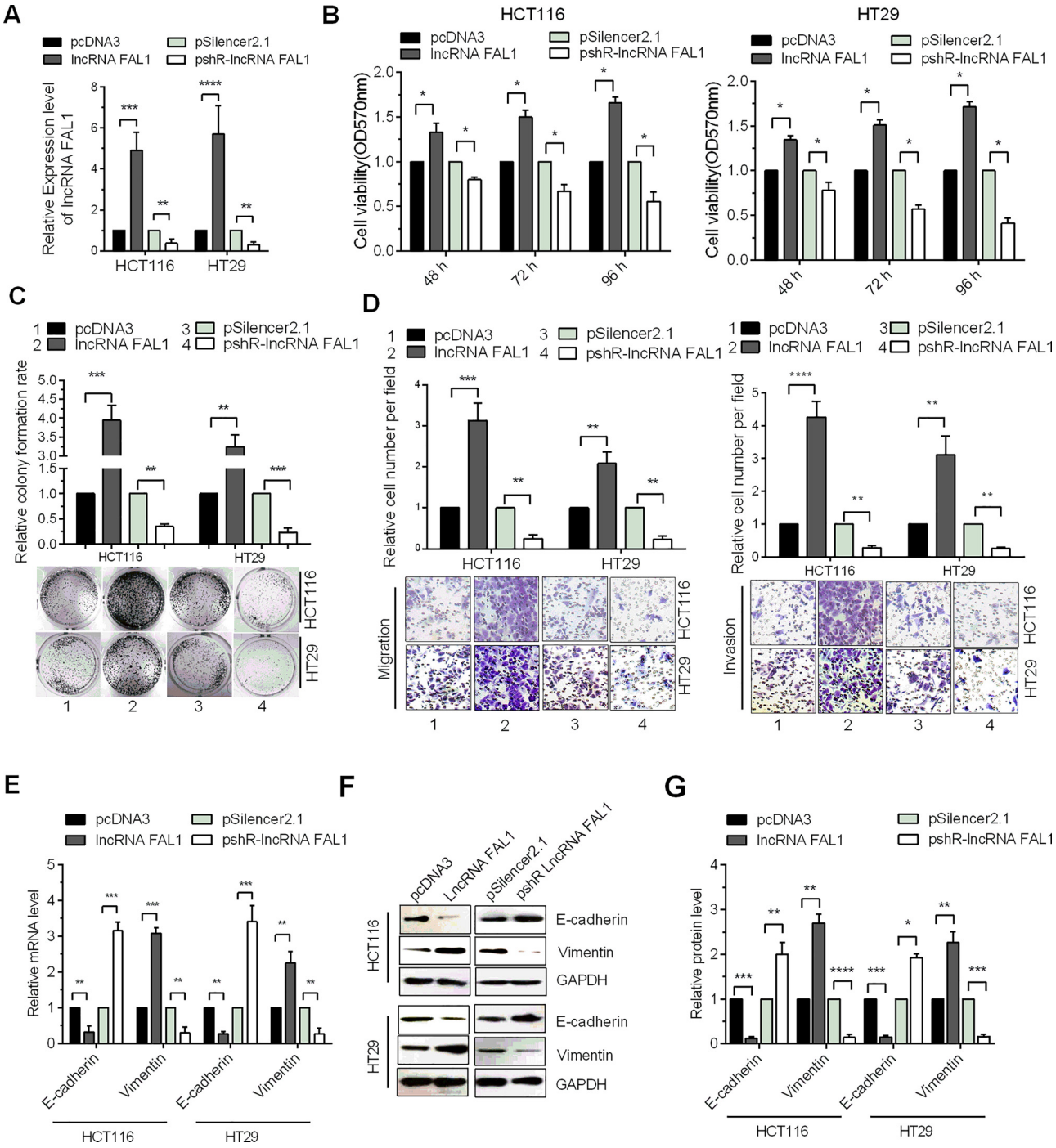

Fig. 2. LncRNA FAL1 promotes carcinogenesis in CRC cell lines. (A) CRC cells are transfected with lncRNA FAL1 overexpression or knockdown plasmids, and lncRNA FAL1 expression level is detected by RT-qPCR. (B and C) MTT and colony formation assays were used to detect the viability and proliferation ability of CRC cells after transfection with lncRNA FAL1 overexpression or knockdown plasmids. (D) Transwell migration and invasion assays are used to detect cell migration ability and invasion ability induced by lncRNA FAL1. (E) The mRNA levels of E-cadherin and vimentin are detected by RT-qPCR. (F) The protein levels of E-cadherin and Vimentin are measured by western blot in CRC cells after transfection with lncRNA FAL1 overexpression or knockdown plasmids. (G) Quantification of western blotting results. All of the experiments are repeated at least three times. *, $p < 0.05$; **, $p < 0.01$;***, $p < 0.001$; ****, $p < 0.0001$.

the TRIzol reagent (Sigma-Aldrich) according to the manufacturer's instructions. First strand complementary DNA (cDNA) was synthesized using the AMV Reverse Transcriptase following the manufacturer's instructions of First Strand cDNA Synthesis Kit (Sigma-Aldrich). Quantitative PCR was performed by using KiCqStart® SYBR® Green qPCR ReadyMix™ (Sigma-Aldrich) according to the manufacturer's instructions. GAPDH was used as an internal reference for normalization of lncRNA-FAL1 and mRNAs. The specific primers for qPCR were as follows: lncRNA-FAL1-forward: 5'-CCTGGCCAAGAAGCTCATAC-3' and lncRNA-FAL1-reverse: 5'- TGAGGACACCGACTACTGAGAA -3'; Vimentin-forward: 5'-GCTGAATGACCGCTTCGCCAACT-3' and Vimentin-reverse: 5'-GCTCCCGCATCTCCTCCTCGTA-3'; E-cadherin-forward: 5'-TTGCTCACATTTCCCAACTCC-3' and E-cadherin-reverse: 5'-CTCTGTCACCTTCAGCCATCCT-3'; NUPR1-forward: 5'-ATGGCCACC TTCCCACCAGCAACC-3' and NUPR1-reverse:5'-CAGCGCCGTGCCCCT CGC-3'; GAPDH-forward: 5'-GTGGACCTGACCTGCCGTCT-3' and

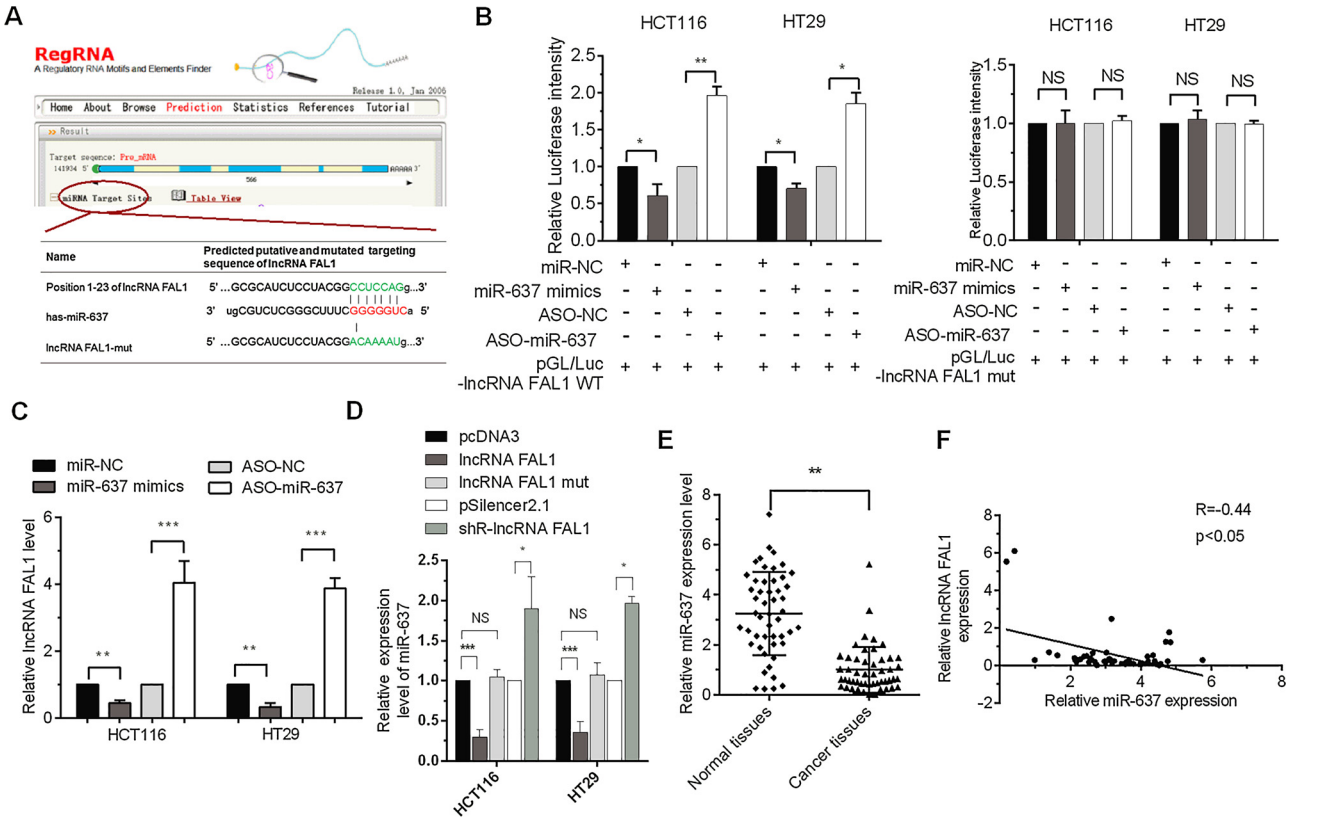

Fig. 3. LncRNA FAL1 is targeted by miR-637. (A) The miRNA target sites of lncRNA FAL1 is predicted by using RegRNA, the miR-637 binding site and the mutational lncRNA FAL1 sequence are shown. (B) Luciferase reporter assay results are shown that miR-637 directly targeted lncRNA FAL1. (C) RT-qPCR method is used to detect the expression level of lncRNA FAL1 after transfection with miR-637 mimics or its inhibitor. (D) Luciferase reporter assay results are shown the effect of lncRNA FAL1 on endogenic miR-637 level. (E) RT-qPCR method is used to detect the expression level of miR-637 in CRC tissues and normal tissues. (F) Pearson's correlation analysis indicates the negative correlation between the expression of lncRNA FAL1 and miR-637. All of the experiments are repeated at least three times. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.

GAPDH-reverse: 5'-GGAGGAGTGGGTGTCGCTGT-3'; LASP1-forward: 5'-ATGAACCCCAACTGCGCC-3' and LASP1-reverse: 5'-TCAGATGGCCT CCACGTAGTT-3'; HIF-1α-forward: 5'-GGCGCGAACGACAAGAAAAA-3' and HIF-1α-reverse: 5'-GTGGCAACTGATGAGCAAGC-3'. To detect the expression levels of miR-637, commercial TaqMan microRNA assay of miR-637 (Life Technologies; ID 4464084) was used in this study, and U6 snRNA TaqMan microRNA assay (ID 001973) was used as an internal reference for normalization of miR-637 according to the manufacturer's instructions. Comparative Ct method was introduced to calculate the relative expression of RNAs.

2.4. Vectors construction

To construct lncRNA FAL1 overexpression plasmid pcDNA3-lnRNA FAL1, the total lncRNA FAL1 fragment was amplified from the HCT116 cDNA by using the primers lncRNA FAL1-F (5'-CCCAAGCTTGCGCATC TCCTACGGCCTCCAGGACAGAG-3') and lncRNAFAL1-R (5'-CCGGAAT TCAGACATCCAAGTGTCCTGTGTAATAGGC-3') and cloned to pcDNA3.1 vector between the restriction enzyme sites of *Hin*dIII and *Eco*RI. To construct NUPR1 overexpression plasmid pcDNA3-NUPR1, the primers NUPR1-exp-F (5'-GACGGTACCGCCACCATGGCCACCTTCC CAC-3') and NUPR1-exp-R (5'-CCGTCTAGATTCAGCGCCGTGCCCCTC GCTT-3') were used to amplify the whole NUPR1 sequence from the HCT116 cDNA and cloned to the restriction enzyme sites of *Kpn*I and *Xba*I in pcDNA3.1 vector. To construct shRNAs specifically targeting lncRNA FAL1, NUPR1 and HIF-1α, the shRNA sequences were designed as follows: shRNA-FAL1 sense: 5'-GATCCGACTTCTCAGTAGTCGGTGT CCTCGAGGACACCGACTACTGAGAAGTCTTTTTTGA-3' and shRNA-FAL1 antisense: 5'- AGCTTCAAAAAAGACTTCTCAGTAGTCGGTGTCCT CGAGGACACCGACTACTGAGAAGTCG-3'; shRNA-NUPR1 sense: 5'-GATCCGGATGAATCTGACCTCTATAGCTCGAGCTATAGAGGTCAGATT CATCCTTTTTGA-3' and shRNA-NUPR1 antisense: 5'- AGCTTCAAAAA GGATGAATCTGACCTCTATAGCTCGAGCTATAGAGGTCAGATTCAT CCG-3'; shRNA-HIF-1α sense: 5'-GATCCGCACAGGCCACATTCACGTAT CTCGAGATACGTGAATGTGGCCTGTGCTTTTTGA-3' and shRNA-HIF-1α antisense: 5'- AGCTTCAAAAAGCACAGGCCACATTCACGTATCTCG AGATACGTGAATGTGGCCTGTGCG-3'. The paired shRNAs oligonucleotides were annealed and cloned to the restriction enzyme sites of *Bam*HI and *Hin*dIII in pSilencer 2.1-U6 Neo vector.

2.5. Cell transfection

Cells were transfected with different plasmids, miRNA negative control (miR-NC), miR-637 mimics, negative control antisense oligonucleotides (ASO-NC) and antisense oligonucleotides against miR-637 (ASO-miR-637) by using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions.

2.6. MTT assay

3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was employed to detect cell viabilities. Briefly, 3×10^3 cells that transfected with related plasmids or oligonucleotides were seeded into 96-well plates and cultured at 37 °C under a humidified atmosphere containg 5% CO_2. At the indicated time points (48, 72 and 96 h), 10 μl MTT was added to each well with the final concentration of 0.5 mg/ml, and the cells were reincubatedat 37 °C for 4 h. Then, the supernatant liquid was removed, and 100 μl DMSO was added to each well to

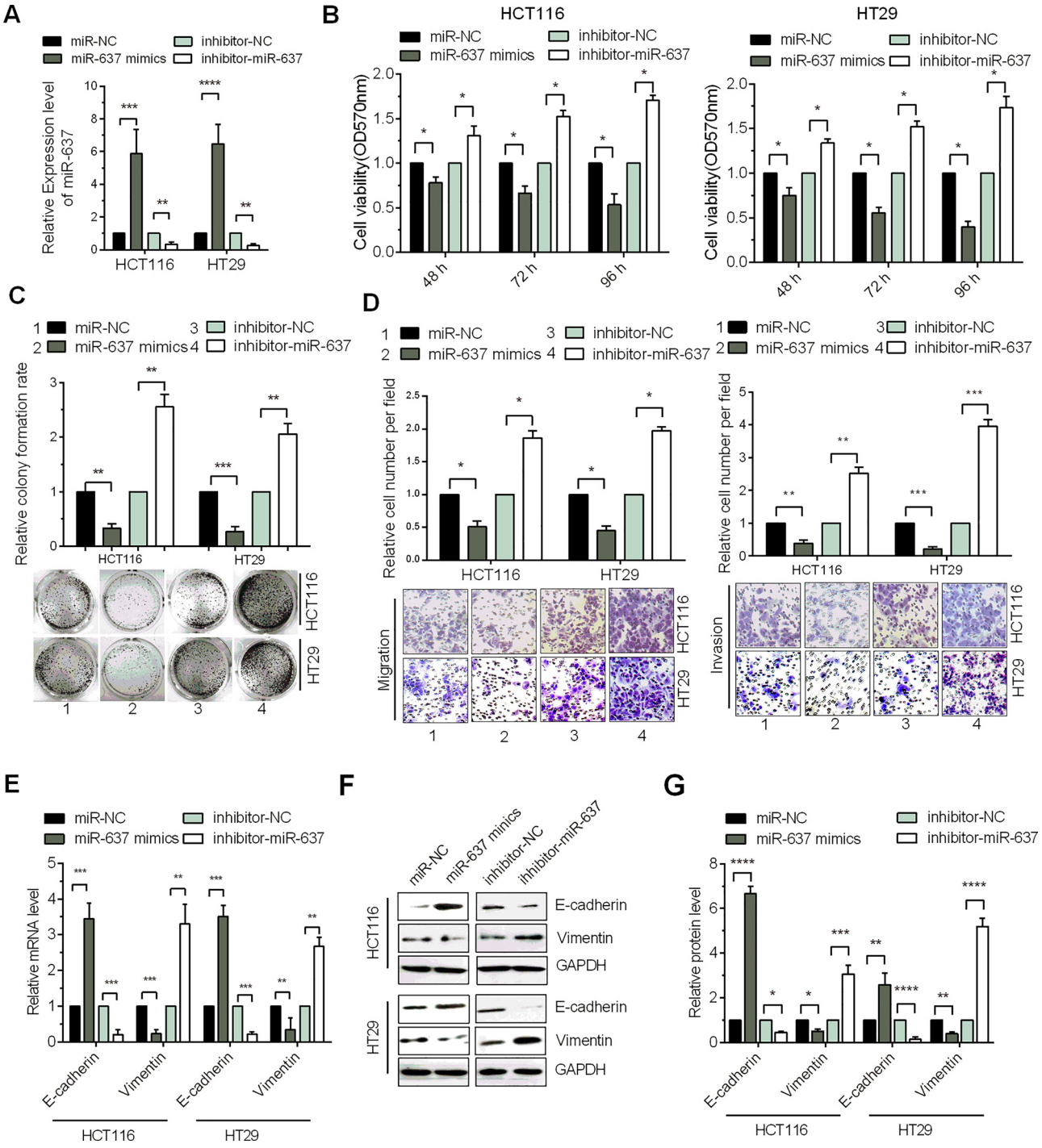

Fig. 4. MiR-637 inhibits carcinogenesis of CRC cell lines. (A) CRC cells are transfected with miR-637 mimics or its inhibitor, and the expression level of miR-637 is detected by RT-qPCR. (B and C) MTT and colony formation assays show the viability and proliferation ability of CRC cells after transfection with miR-637 mimics or its inhibitor. (D) Transwell migration and invasion assays show the cell migration ability and invasion ability of CRC cells induced by miR-637 mimics or its inhibitor. (E) RT-qPCR results show the mRNA levels of E-cadherin and vimentin. (F) Western blot shows the protein levels of E-cadherin and Vimentin. (G) Quantification of western blotting results. All of the experiments are repeated at least three times. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

dissolve the precipitated formazan. Quant Universal Microplate Spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) was used to measure the absorbance at 490 nm (A490).

2.7. Colony formation assay

CRC cells that transfected with related plasmids or oligonucleotides were seeded into 12-well plates at density of 5×10^2 per well and cultured for 14 days. The supernatant liquid was removed, the cells were washed with 1 × PBS cells for three times and fixed with 4% paraformaldehyde for 30 min. After that, crystal violet was used to stain the cells for 10 min and washed with 1 × PBS. Colonies were examined and counted using the following formula: colony formation rate (100%) = (number of colonies formed/number of seeded cells) × 100%.

2.8. Transwell assay

Transwell migration and invasion assays were carried out using

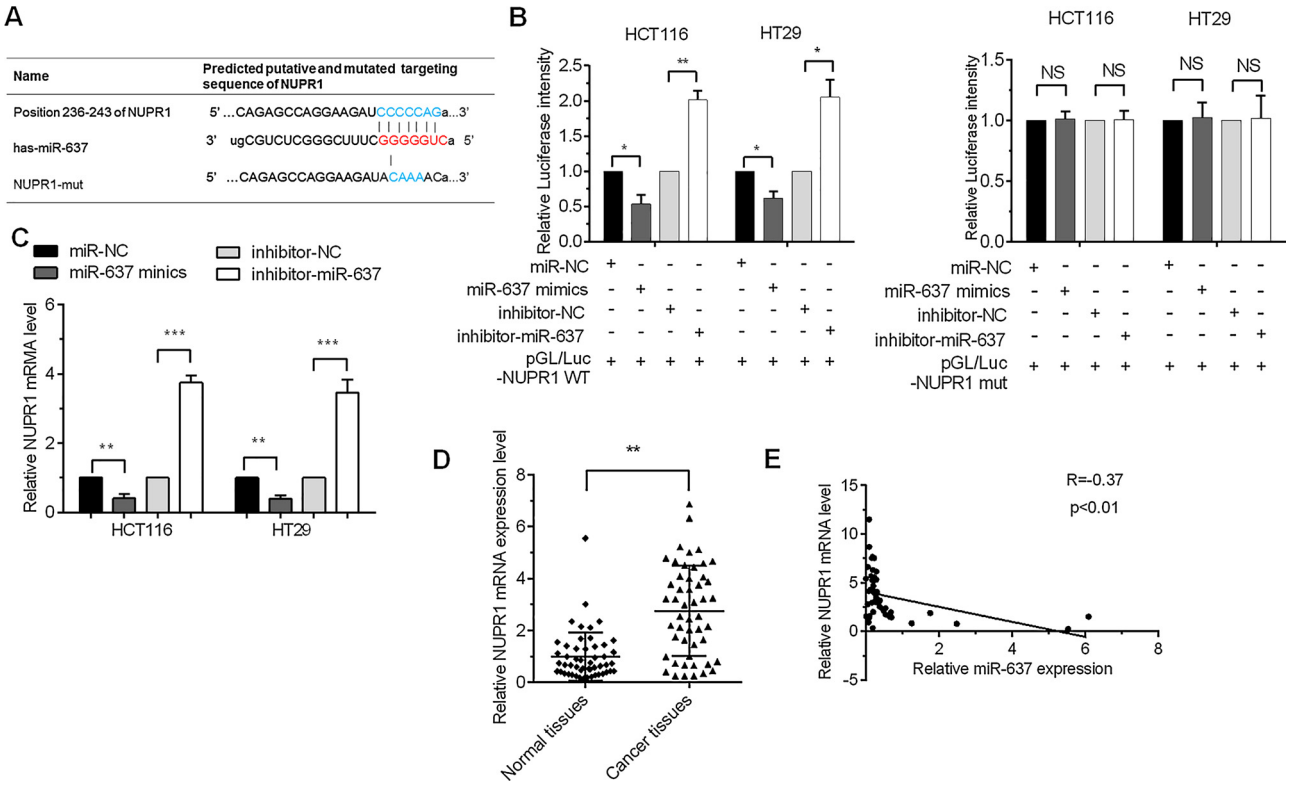

Fig. 5. NUPR1 is the direct target of miR-637. (A) TargetScan 7.1 is used to predict that NUPR1 mRNA 3'UTR contains miR-637 binding site, and the mutational 3'UTR of NUPR1 mRNA are indicated. (B) Luciferase reporter assay shows that NUPR1 is a direct target of miR-637. (C) NUPR1 mRNA level is measured by RT-qPCR. (D) RT-qPCR is used to detect the mRNA level of NUPR1 in CRC tissues and normal tissues. (F) The correlation analysis of experiments data shows negative correlation between the expression level of miR-637 and NUPR1 mRNA level. All of the experiments are repeated at least three times. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS, not significant.

chamber inserts (8-μm-pore size Millipore, MA, USA). Experiments were repeated in three times independently. Details of the procedure were carried out according to the literature as described previously (Liu et al., 2017; Zhao et al., 2015a,b).

2.9. Luciferase assay

To confirm the target relationship between lncRNA FAL1 and miR-637, pGL/Luc-lncRNA FAL1 or pGL/Luc-lncRNA FAL1 mut vectors were co-transfected into the CRC cells with miR-637 mimics or ASO-miR-637. Then, the cells were grown for 48 h and harvest, cell pellet was lysed with RIPA. Dual-Luciferase Reporter System (Promega, Madison, WI, USA) was employed to detect the fluorescence intensity. To confirm the target relationship between NUPR1 3'UTR and miR-637, pGL/Luc-NUPR1 WT or pGL/Luc-NUPR1 mut vectors were co-transfected into the CRC cells with miR-637 mimics or ASO-miR-637. Then, the cells were grown for 48 h and harvest, cell pellet was lysed with RIPA. Dual-Luciferase Reporter System (Promega, Madison, WI, USA) was employed to detect the fluorescence intensity.

2.10. Western blot

Total protein was extracted from cells using RIPA Lysis and Extraction Buffer (Thermo Scientific™) and quantified using BCA method (Beyotime Biotechnology, China). Protein lysates (30 μg/lane) were separated on a 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride membranes (PVDF, Millipore, MA, USA). Primary antibodies were added to the PVDF membranes and incubated at 4 °C overnight. After washing the PVDF membranes, HRP-linked secondary antibody (Jackson, USA) was added and incubated at room temperature for two hours. Western blotting data was quantify by Alpha Innotech (San Leandro, CA, USA) imaging software. Primary antibodies sunch as E-cadherin, Vimentin, NUPR1, HIF-1α, LASP1 and GAPDH were obtained from Abcam Trading (Shanghai) Company Ltd.

2.11. Statistical analysis

Each experiment was carried out triplicate. The data are presented as the means ± SD, and the comparisons between two groups were determined using two-tailed Student's t-tests. Pearson's correlation analysis was performed to clarify the association between two factors. $P \leq 0.05$ was considered to be statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

3. Results

3.1. LncRNA FAL1 is upregulated in CRC tissues and cells

To investigate the role of lncRNA FAL1 in CRC, we first detected the level of lncRNA FAL1 in 50 paired CRC tissues and adjacent normal tissues using an RT-qPCR assay. The result showed that the expression level of lncRNA FAL1 was markedly upregulated in 90% (45 of 50) of tumour tissues, indicating that lncRNA FAL1 could be involved in CRC carcinogenesis (Fig. 1A and B). Then, we also measured the expression level of lncRNA FAL1 in CRC cell lines and the normal human colon mucosal epithelial cell line NCM460. The results showed that the expression of lncRNA FAL1 was higher in CRC cell lines than in the normal cell line, and of these, the HCT116 and HT-29 cell lines had the highest expression levels of lncRNA FAL1 (Fig. 1C). Moreover, these 50 paired CRC patients were divided into high and low expression groups according to the median lncRNA FAL1 expression level to obtain

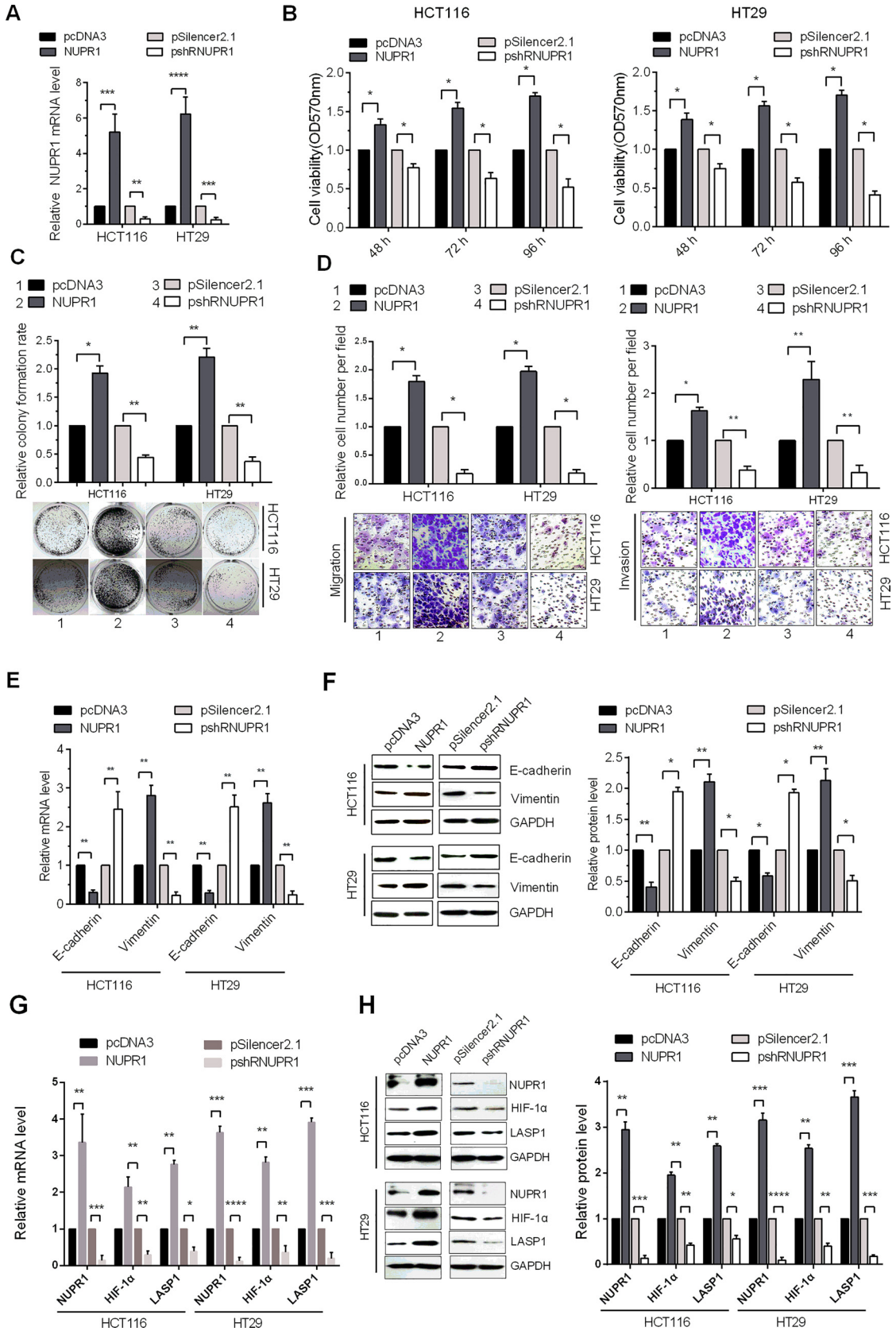

Fig. 6. NUPR1 promotes carcinogenesis of CRC cell lines. (A) CRC cells are transfected with NUPR1 overexpression or knockdown plasmids, and NUPR1 mRNA level is detected by RT-qPCR. (B and C) MTT and colony formation assays show the viability and proliferation ability of CRC cells after transfection with NUPR1 overexpression or knockdown plasmids. (D) Transwell migration and invasion assays show cell migration ability and invasion ability of CRC cells induced NUPR1. (E) RT-qPCR results show the mRNA levels of E-cadherin and vimentin. (F) Western blot shows the protein levels of E-cadherin and Vimentin. (G) Quantification of western blotting results. All of the experiments are repeated at least three times. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

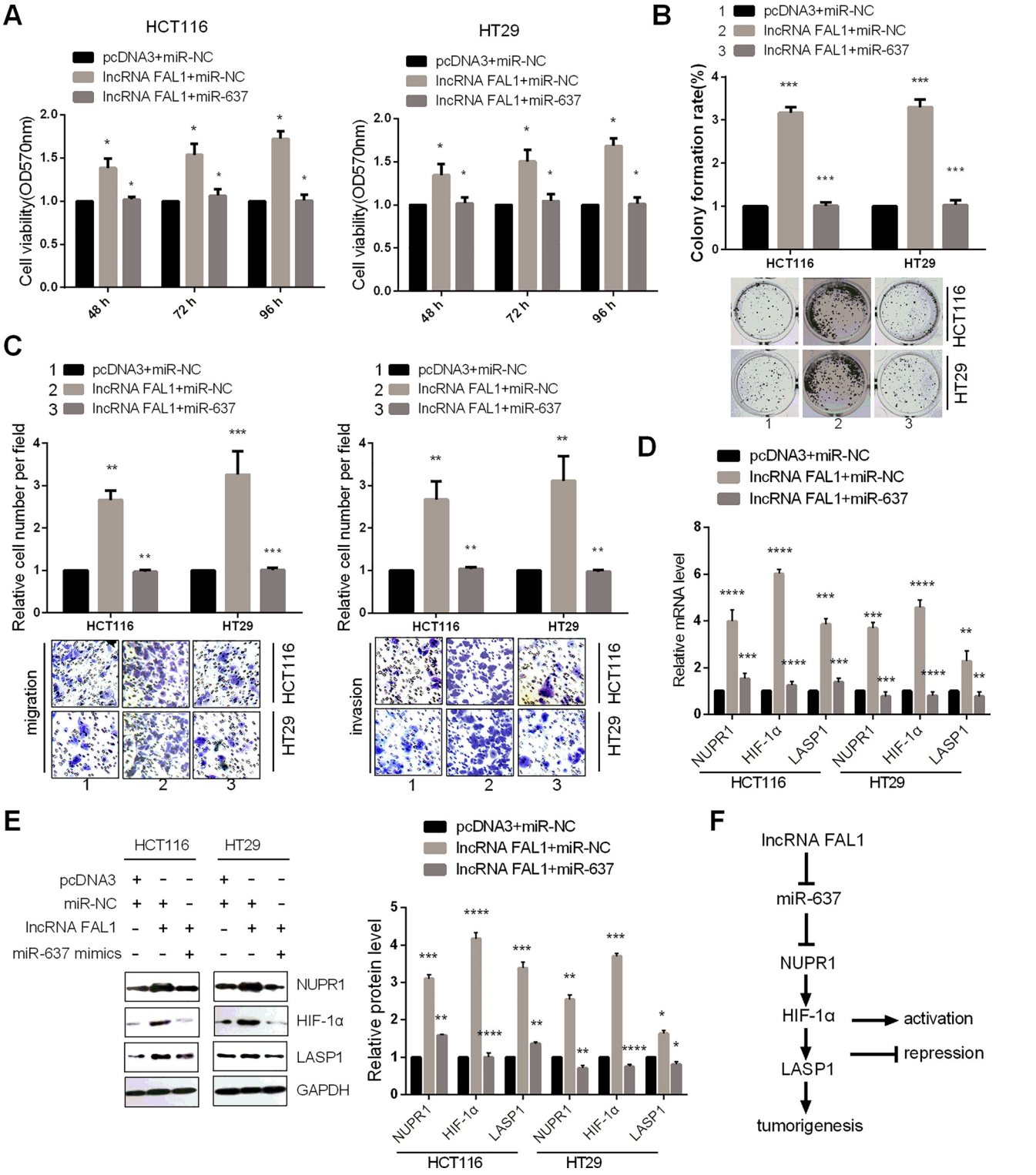

Fig. 7. LncRNA FAL1 promotes carcinogenesis of CRC cell lines via regulation of miR-637/NUPR1 and downstream signal molecules. (A and B) MTT and colony formation assays are used to assess the viability and proliferation ability of CRC cells. (C) Transwell migration and invasion assays are used to assess cell migration ability and invasion ability. (D) RT-qPCR method is used to assess the mRNA levels of E-cadherin and vimentin. (E) Western blot is used to assess the protein levels of E-cadherin and Vimentin. (F) Quantification of western blotting results. All of the experiments are repeated at least three times. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

correlations between the expression of lncRNA FAL1 and clinicopathological characteristics. The expression level of lncRNA FAL1 was markedly correlated with tumour size, TNM stage and lymph node metastasis, while no associations were shown with age, gender, or histological type (Table 1). In addition, high expression of lncRNA FAL1 in CRC patients is markedly associated with worse overall survival (Fig. 1D). These results indicate that lncRNA FAL1 may act as an oncogene in CRC.

3.2. LncRNA FAL1 functions as an oncogene in CRC cells

To further investigate the role of lncRNA FAL1 in CRC, gain- or loss-of-function experiments were performed with transfected cell lines, and the effectiveness of the plasmids was verified by RT-qPCR (Fig. 2A). MTT analysis showed that upregulation of lncRNA FAL1 significantly increased cell viability of CRC cells, while downregulation of lncRNA FAL1 inhibited cell viability (Fig. 2B). Additionally, the colony forming ability of CRC cells was increased after overexpression of lncRNA FAL1; conversely, cells with downregulation of lncRNA FAL1 formed fewer colonies than the controls (Fig. 2C). To confirm whether lncRNA FAL1 has an effect on cell migration and invasion, transwell migration and invasion experiments showed that overregulation of lncRNA FAL1 promoted the migration and invasion ability of CRC cells, while knockdown of lncRNA FAL1 inhibited it (Fig. 2D). As epithelial-mesenchymal transition (EMT) is closely related to cancer cell invasion and metastasis, EMT markers (E-cadherin and Vimentin) were also further evaluated by RT-qPCR and western blot analysis. Results showed that overexpression of lncRNA FAL1 significantly reduced the level of the epithelial marker E-cadherin but increased the level of the mesenchymal marker Vimentin (Fig. 2E–G); however, downregulation of lncRNA FAL1 markedly increased the level of the epithelial marker E-cadherin but decreased the level of the mesenchymal marker Vimentin (Fig. 2E–G). Taken together, these results indicated that lncRNA FAL1 promotes proliferation ability, migration and invasion in CRC cells.

3.3. LncRNA FAL1 is targeted by miR-637

Usually, lncRNAs might act as ceRNAs to indirectly regulate miRNAs and their downstream targets. To assess whether lncRNA FAL1 also regulates gene expression through a ceRNA mechanism, potential miRNA binding sites in lncRNA FAL1 were predicted the using RegRNA 2.0 (http://regrna2.mbc.nctu.edu.tw/), and greater than 100 potential miRNA binding sites were predicted in lncRNA FAL1. Considering its known function in other cancers, miR-637 was chosen for further investigation (Fig. 3A). To ascertain whether miR-637 could bind to lncRNA FAL1, luciferase reporter plasmids containing miR-637 binding sequences or mutant sequences downstream of the luciferase gene were constructed. Luciferase assays showed that miR-637 could directly bind to lncRNA FAL1 according to the changes in luciferase activity (Fig. 3B). To assess whether miR-637 also affects the level of lncRNA FAL1, we detected the level of lncRNA FAL1 after transfection with miR-637 mimics or its inhibitor. Results showed that transfection with miR-637 mimics dramatically inhibited the endogenous levels of lncRNA FAL1, while blockage of miR-637 could increase the levels of lncRNA FAL1 in CRC cells (Fig. 3C). Similarly, overexpression of lncRNA FAL1 also suppressed endogenous levels of miR-637, and the contrary result was observed after knockdown of endogenous lncRNA FAL1 (Fig. 3D). Additionally, the expression level of miR-637 was downregulated in CRC tissues compared with normal tissues (Fig. 3E). Furthermore, a negative correlation was observed between lncRNA FAL1 and miR-637 (Fig. 3F). Collectively, these results suggest that lncRNA FAL1 could be directly bound by miR-637 in CRC cells.

3.4. MiR-637 functions as a suppressor in CRC cells

According to the above results, we surmised that miR-637 could function as a suppressor in CRC cells. Evidence has proved that miR-637 is downregulated and inhibits tumorigenesis in many cancers, including PTC (Yuan et al., 2018), pancreatic ductal adenocarcinoma (PDAC) (Xu et al., 2018), gliomas (Que et al., 2015), and HCC (Zhang et al., 2011). However, the role and the mechanism of miR-637 in CRC remain unclear. To determine the role of miR-637, we first detected the expression level of miR-637 after transfection with miR-637 mimics and its inhibitor by RT-qPCR (Fig. 4A). Then, MTT analysis demonstrated that transfection with miR-637 mimics significantly inhibited the cell viability of CRC cells, whereas blockage of miR-637 using its inhibitor increased cell viability (Fig. 4B). In addition, the colony forming ability of CRC cells was inhibited after transfection with miR-637 mimics; conversely, blockage of miR-637 increased colony formation relative to the control group (Fig. 4C). Moreover, transwell migration and invasion experiments indicated that upregulation of miR-637 inhibited, whereas downregulation of miR-637 promoted the migration and invasion ability of CRC cells (Fig. 4D). Thereafter, RT-qPCR and western blot analysis showed that overexpression of miR-637 significantly increased that of E-cadherin but suppressed the level of Vimentin (Fig. 4E–G), while downregulation of endogenous miR-637 markedly decreased the level of E-cadherin but increased that of Vimentin (Fig. 4E–G). Collectively, these results suggest a tumour suppressive role of miR-637 in CRC cells.

3.5. MiR-637 targets and downregulates NUPR1 expression

As we know, miRNAs usually exert their effects through transcriptional regulation of downstream target genes (Bartel, 2009). Therefore, the targets of miR-637 were predicted using TargetScan 7.1 (http://www.targetscan.org/vert_70/), and NUPR1 was selected as a candidate target for further investigation because of the role of NUPR1 in tumorigenesis in many cancers (Fig. 5A). Then, luciferase assays showed that miR-637 could decrease the luciferase activity of the wildtype 3′-UTR of NUPR1; however, luciferase activity was increased after blockage of endogenous miR-637 (Fig. 5B). In addition, the luciferase activity was nearly unaffected by mutation of the miR-637 binding site of the NUPR1 3′-UTR (Fig. 5B). Then, we demonstrated that the mRNA level of NUPR1 was decreased after transfection with miR-637 mimics, and the opposite behaviour was observed after transfection with miR-637 inhibitor (Fig. 5C). Moreover, we further demonstrated that NUPR1 was highly expressed in CRC cancer tissues (Fig. 5D), and a negative correlation was observed between NUPR1 and miR-637 (Fig. 5E). These results suggest that NUPR1 is the direct target of miR-637.

3.6. NUPR1 functions as an oncogene in CRC cells

To our best knowledge, NUPR1 upregulated and promotes tumorigenesis in many cancers, however, no report has been found on CRC (Emma et al., 2016; Li et al., 2016). To further investigate the role of NUPR1 in CRC, the effectiveness of plasmids was verified by RT-qPCR (Fig. 6A). Then, MTT, colony formation, transwell migration and invasion assays were performed to demonstrate the role of NUPR1 in tumorigenesis in CRC cells (Fig. 6B–D). Additionally, we observed that overexpression of NUPR1 significantly inhibited the level of E-cadherin but increased the level of Vimentin (Fig. 6E and F), whereas downregulation of NUPR1 markedly increased the level of E-cadherin but decreased the level of Vimentin (Fig. 6E and F). Taken together, these results indicated that NUPR1 functions as an oncogene in CRC cells. Previous evidence showed that NUPR1 and hypoxia inducible factor-1 alpha (HIF-1α) are co-expressed in human PDAC tissues, and HIF-1α accumulation under hypoxic conditions in vitro seems to be partially dependent on NUPR1 expression (Hamidi et al., 2012). Additionally, LASP1 is a target gene of HIF-1α and is critical for metastasis of pancreatic cancer (Zhao et al., 2015a,b). To further investigate the molecular mechanisms of NUPR1 in CRC, we detected the mRNA and protein levels of HIF-1α and LASP1 after overexpression or knockdown of NUPR1 in CRC cells. The results showed that overexpression of NUPR1 could markedly increase the mRNA and protein levels of HIF-1α and LASP1, whereas knockdown of NUPR1 decreased them (Fig. 6). Moreover, rescue experiments showed that downregulation of HIF-1α partly rescued the tumour phenotypes induced by NUPR1 (Fig. S1A–C). Collectively, these results suggest that NUPR1 promotes tumorigenesis of CRC through the HIF-1α/LASP1 pathway.

3.7. LncRNA FAL1 promotes carcinogenesis of CRC cells via regulation of the miR-637/NUPR1 pathway

To investigate the role of the miR-637/NUPR1 pathway in lncRNA FAL1-induced carcinogenesis of CRC cells, we first verified whether the effects of lncRNA FAL1 on malignant characteristics in CRC cells are mediated by miR-637. Rescue experiments showed that individual overexpression of lncRNA FAL1 increased cell viability, colony formation ability, migration and invasion; however, co-transfection with miR-637 mimics partially rescued the phenotypes caused by lncRNA FAL1 in CRC cells (Fig. 7A–C). Moreover, the mRNA and protein levels of NUPR1 and downstream molecules were detected by RT-qPCR and western blot. Results showed that individual overexpression of lncRNA FAL1 markedly increased the mRNA and protein levels of NUPR1 and downstream molecules HIF-1α and LASP1, while co-transfection with miR-637 mimics partially decreased the mRNA and protein levels of NUPR1 and downstream molecules HIF-1α and LASP1 in CRC cells (Fig. 7D and E). Taken together, these results demonstrate that the carcinogenesis of CRC cells induced by lncRNA FAL1 is mediated by the miR-637/NUPR1 pathway.

4. Discussion

Increasing evidence suggest that lncRNAs, which may function as oncogenes or tumour suppressors, play important roles in the tumorigenesis of many malignant tumours. However, exploration of the detailed molecular mechanisms by which many lncRNAs are involved in tumorigenesis is urgently needed. For instance, the function of lncRNA FAL1 has been investigated in several cancers. However, the role of lncRNA FAL1 in CRC tumorigenesis, recurrence, and metastasis has not been reported. In this study, we first demonstrated that lncRNA FAL1 was upregulated in CRC tissues and cells, and high expression of lncRNA FAL1 was correlated negatively with overall patient survival. Our study first demonstrated that lncRNA FAL1 promoted the proliferation ability, migration and invasion of CRC cells.

Additionally, our research demonstrated that lncRNA FAL1 promotes CRC tumorigenesis as the target ceRNA of miR-637, thus indirectly regulating the downstream targets of miR-637. Studies have demonstrated that miR-637 was downregulated and functioned as a tumour suppressor in several cancers (Que et al., 2015; Xu et al., 2018; Yuan et al., 2018; Zhang et al., 2011). In this study, we also identified that miR-637 inhibited tumorigenesis of CRC cells, and the expression level of miR-637 presented a negative correlation with lncRNA FAL1, further suggested that lncRNA FAL1 could sponge miR-637 to promote cell proliferation and invasion in CRC. Moreover, luciferase assays further suggested that lncRNA FAL1 could be directly bound by miR-637 in CRC cells.

To our best knowledge, miRNAs usually exert their effects by regulating the transcriptional level of downstream target genes [22]. Therefore, NUPR1 was identified as a direct target of miR-637. NUPR1 was first identified as a product an inducible gene in injured pancreatic tissue (acute pancreatitis) (Mallo et al., 1997). Recent studies have demonstrated that NUPR1 is upregulated in several human cancers, such as breast cancer (Ito et al., 2005a,b), thyroid carcinoma (Ito et al., 2005a,b), pancreatic cancer (Su et al., 2001), and NSCLC (Mu et al., 2017). NUPR1 could inhibit cell proliferation by regulating the expression of ERK1/2, p38 MAPK and caspase-3 in glioblastoma cells (Li et al., 2016). NUPR1 is also demonstrated to regulate autolysosomal efflux by activating SNAP25 (Mu et al., 2017). Additionally, previous evidence has demonstrated an intensive correlation between NUPR1 and HIF-1α expression in human PDAC tissues (Hamidi et al., 2012). To further investigate the molecular mechanism of NUPR1, we demonstrated that the expression of HIF-1α was regulated by NUPR1 in CRC cells, which is in line with the viewpoint that HIF-1α accumulation seems to be partially dependent on NUPR1 expression (Hamidi et al., 2012). HIF-1α is an important transcription factor of the HIF transcription factor family, which was first shown to have a central role in mediating O_2-dependent transcriptional responses (Keith et al., 2012). HIF-1α is overexpressed in many cancers, most of which are associated with worse prognoses (Keith et al., 2012). Previous studies showed that HIF-1α could induce the expression of JMJD2B to promote the malignant phenotype of colorectal cancer cells (Fu et al., 2012). Recently, it was reported that HIF-1α could directly bind the promoter of LASP1 to regulate metastasis of pancreatic cancer (Zhao et al., 2015a,b). Our study demonstrated that NUPR1 could positively regulate the mRNA and protein levels of LASP1. It was previously reported that LASP-1 was upregulated and acted as an oncogene in many cancers, including CRC (Zhao et al., 2010). Our study showed that NUPR1 acted as an oncogene by regulating HIF-1α/LASP1 signalling in CRC cells. Moreover, upregulation of miR-637 could partially attenuate the increase in cell viability, proliferation, migration and invasion capacity caused by lncRNA FAL1 overexpression. In addition, the mRNA and protein levels of NUPR1 and downstream molecules HIF-1α and LASP1 were decreased by upregulation of miR-637 in CRC cells, providing sufficient evidence that lncRNA FAL1 promoted the malignant tumor phenotypes of CRC cells through miR-637/NUPR1/ IF-1α/LASP1 pathway (Fig. 7F).

In summary, our findings demonstrate the oncogene role of lncRNA FAL1 in CRC. We found that lncRNA FAL1 promoted cell viability, proliferation,migration and invasion capacity through the miR-637/NUPR1 signalling pathways in CRC. Our findings underscore the crucial roles of lncRNA FAL1 in CRC tumorigenesis and its potential prognostic and therapeutic value.

Conflicts of interest

No potential conflicts of interest were disclosed.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biocel.2018.09.015.

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