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A long non-coding RNA, ELFN1-AS1, sponges miR-1250 to upregulate MTA1 to promote cell proliferation, migration and invasion, and induce apoptosis in colorectal cancer

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Abstract. – OBJECTIVE: Long non-coding RNA (lncRNA), is essential for the development and progression of cancers. LncRNA regulates target gene expression by sponging the corresponding microRNA (miRNA) during tumorigenesis. This work aimed to explore the role of one lncRNA, ELFN1–AS1, in colorectal cancer (CRC) development and elucidate the pertinent signaling pathway.

PATIENTS AND METHODS: First, we found that ELFN1–AS1 was highly abundant in the human CRC tissues and cell lines. Silence of ELFN1–AS1 expression reduced cell proliferation, colony formation, migration and invasion, while inducing apoptosis in vitro; moreover, knockdown of ELFN1–AS1 decreased the size and weight of tumor in vivo.

RESULTS: Luciferase reporter assay revealed that ELFN1–AS1 interacted with miR-1205 and suppressed its expression. In addition, miR-1205 could bind to the 3′ untranslated region (3′-UTR) of Metastasis Associated Protein1 (MTA1) and inhibited ELFN1–AS1 expression. More importantly, overexpression of MTA1 completely rescued the phenotype of ELFN1–AS1 knockdown.

CONCLUSIONS: In sum, our study demonstrated that ELFN1–AS1 sponges miR-1205 to upregulate MTA1, which is essential for CRC cell proliferation, migration, and invasion as well as apoptosis induction.

Key Words:
Colorectal cancer, ELFN1-AS1, MiR-1205, MTA1.

Abbreviations

CRC=colorectal cancer; ELFN1−AS1=lncRNA ELFN1−AS1; microRNA= miRNAs; MTA1=Metastasis Associated Protein1; Roswell Park Memorial Institute=RPMI 1640; Dulbecco's Modified Eagle Media=DMEM; EMT=epithelial-to-mesenchymal transformation; siRNAs=interfering RNAs; OD=optical density; PVDF=polyvinylidene difluoride.

Introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer death worldwide[1]. Although the treatments of CRC, such as surgery, chemotherapy, radiotherapy, and targeted drugs have been widely applied, severe side effects including bleeding and drug resistance have nevertheless developed[2]. The overall survival rate of CRC patients is still low[3]. Hence, it is imperative to further study the molecular mechanism underlying CRC carcinogenesis and explore new biomarkers and targets for the early diagnosis and treatment of CRC.

LncRNAs belong to a type of non-coding RNA with a length greater than 200 nucleotides[4,5]. LncRNAs typically act as ceRNA (competitive endogenous RNA) to sponge their target miRNAs through which lncRNAs modulate gene expression and function epigenetically, transcriptionally, and post-transcriptionally[6-9]. An increasing number of lncRNAs have been implicated in pathological processes such as proliferation, angiogenesis, migration and invasion of cells, epithelial-to-mesenchymal transition (EMT), apoptosis, and anti-tumor drug resistance[10,11]. As such, some lncRNAs are involved in carcinogenesis and are regarded as potential targets and biomarkers for the diagnosis and prognosis.

ELFN1−AS1 is located at 7p22.3 antisense to ELFN1[12]. Upregulation of ELFN1−AS1 is closely linked to poor prognosis in gastrointestinal cancers, such as colon adenocarcinoma, esophageal cancer, and colorectal cancer[13-16]. Therefore, ELFN1-AS1 is a crucial transcriptional regulator involved in tumorigenesis. However, how ELFNA-AS1 upregulation contributes to CRC progression is still unclear.

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MiRNAs are single-stranded RNAs and are generated from pre-miRNAs by RNA polymerase II. MiR-1205, a member of the PVT1 region, plays an important role in many cancer types, such as prostate cancer, lung cancer, gastric cancer, glioma, and CRC[17-21]. MiR-1205 could act as a tumor suppressor by sabotaging the synergy between KRAS and MDM4/E2F1 in non-small cell lung cancer[22]. The differences in the expression and functions of cell-specific target genes may result in the opposing functions of miR-1205 in human tumors.

MiRNAs promote tumorigenesis and cancer progression by directly binding to the 3′-UTRs of target genes in papillary thyroid cancer[23]. For instance, MTA1 is a transcriptional regulator of the progression and metastasis in a variety of solid tumors such as non-small cell lung cancer, gastric cancer, ovarian cancer, prostate cancer, esophageal squamous carcinoma and pancreatic cancer[24-27]. In particular, MTA1 promotes tumorigenic and metastatic behavior in hepatocellular carcinoma by inhibiting the phosphorylation-dependent activation of H1.2T146ph[28]. This work aimed to identify the role of ELFN1−AS1 on CRC progression and elucidate the underlying molecular mechanisms. To this end, we firstly proved that ELFN1−AS1 is highly abundant in both CRC cell lines and CRC tissues. Second, we demonstrated that ELFN1–AS1 sponges miRNA-1250 to upregulate MTA1, which ultimately promotes CRC progression. Collectively, our work substantiated the essential role of ELFN-AS1 and the importance of the miR-1250/MTA1 pathway in CRC progression, providing the potential targets for drug development to treat CRC.

Patients and Methods

Tissue Samples and Cell Culture

The tumor and adjacent noncancerous tissues were obtained from the International Institute of Advancement of Medicine (IIAM, Edison, NJ, USA) and collected from 40 pairs of patients who were diagnosed with CRC and received radical colectomy before other adjuvant therapies. The patients included 18 females and 22 males at the age of 45 ± 6 when diagnosed and the tumors were at stage I or II according to the American Joint Committee on Cancer (AJCC) staging system. The detailed clinicopathological features are described in Table I. Our study was approved by the Research Ethics Committee of Shanxi Provin-

Table I. The clinicopathological variables of CRC patients.

Clinicopathological characteristics	Total	High expression	Low expression	χ^2	*p*-value
Gender					
Male	22	12	10	0.404	0.525
Female	18	8	10		
Age					
<=60	26	14	12	0.440	0.507
>60	14	6	8		
Tumor size					
T1+T2	19	5	14	8.120	0.004*
T3+T4	21	15	6		
Distant metastasis					
Positive	17	12	5	5.013	0.025*
Negative	23	8	15		
Differentiation					
High	19	13	6	4.912	0.027*
Moderate+poor	21	7	14		
Lymph node metastasis					
Positive	21	15	6	8.120	0.004*
Negative	19	5	14		
TMN stages					
I+ II	17	6	11	2.558	0.110
III+ IV	23	14	9		

Table II. Primer Sequences for qRT-PCR.

Gene Name	Forward (5′ to 3′)	Reverse (5′ to 3′)
ELFN1-AS1	ACTCTCAGCCCCCACCTAGT	ATTCAACGGAAGAGGAAGCA
MiR-1205	AACAAGAGACGTCCCAAACGA	GTCGTATCCAGTGCAGGGT
MAT1	ACGCAACCCTGTCAGTCTG	TTCACGAATTTGCGTGTCAT
U6	CGCTTCGGCAGCACATATAC	TTCACGAATTTGCGTGTCAT
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

cial People's Hospital (Approval Number: [KY-E-2019-7-16]). Six colorectal cancer cell lines (HT29, HCT116, HCT8, LOVO, SW480, and SW620) and the normal human colon epithelial cell line (FHC) were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium or Dulbecco's Modified Eagle Media (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37ºC containing 5% CO_2.

Cell Transfection

The design and synthesis of small interfering RNAs (siRNAs) for ELFN1−AS1, the construction of pcDNA 3.1 (10 nM) miR-1205 mimics vector overexpressing miR-1205 mimics (20 nM), and the construction of a lentiviral vector overexpressing MTA1 were conducted by Genechem (Pudong, Shanghai, China). Cell transfection (1×10^5) was carried out by using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) for 48 h based on the guideline of manufacturer. After transfection, RT-qPCR was performed to investigate the transfection efficiency.

RNA Extract and Quantitative Real-Time PCR (qRT-PCR) Assay

TRIzol reagent (TaKaRa, Changping, Beijing, China) was used to extract total RNA from tissues or cultured cells according to the manufacturer's instructions. Following quality determination and concentration quantification, 500 ng of total RNA was reverse transcribed to cDNA (complementary DNA) by using the PrimeScript RT Master Mix (TaKaRa, Changping, Beijing, China). The SYBR Premix Ex Taq II Kit (TaKaRa, Changping, Beijing, China) was employed to examine the relative RNA expression. The primer sequences (Sigma-Aldrich, St. Louis, MO, USA) used in this study were shown in Table II. U6 and GAPDH were used as endogenous controls. The relative expression level was calculated through the $2^{-\Delta\Delta Ct}$ method.

Cell Viability and Colony Assay

The Cell Counting Kit-8 (CCK-8) experiment was used to measure cell viability. Cells were seeded into 96-well plates. Following transfection, cell proliferation was measured at 0, 24, 48, 72 and 96 h via CCK-8 assay (Dojindo, Tokyo, Japan) according to the manufacturer's instructions. Then, the optical density (OD) value was measured spectrophotometrically by a microplate reader at 450 nm. For colony formation assay, cells were seeded at a density of 2000 cells/well in 6-well plates and cultured for 2 weeks at 37°C with 5% CO_2. Then, the cells were washed twice with phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) and stained with crystal violet dye (Sigma-Aldrich, St. Louis, MO, USA) for 15 min. The number of clone spots was counted in 5 random view fields under a microscope (Olympus, Tokyo, Japan).

Transwell Migration and Invasion Assays

Transwell chambers with 8 mm pores were used to examine cell migration and invasion ability. For cell invasion assay, the transfected HT29 and HCT116 cells with a concentration of 1.0×10^6 cells in each well were seeded into the upper chamber of a 24-well insert (Corning Inc. Glendale, Arizona, USA) precoated with Matrigel. The upper chamber was filled with RPMI-1640 medium or DMEM without FBS while the lower chamber was filled with FBS contained medium. Cells were incubated for 48 h, then the invading cells were fixed with methanol, stained with crystal violet, and counted under a light microscope from 5 random fields. For the migration assay, the upper chambers were covered without Matrigel, and the following protocols were performed similarly as described above.

Plasmid and Luciferase Reporter Assay

The full-length of MTA1 3′-UTR containing either wild type (WT) or mutant (Mut) of miR-1205 binding sequence was inserted downstream of the firefly Luciferase gene in psiCHECK2 to generate the psiCHECK2-MTA1 3′-UTR-WT or ELFN1−AS1 WT plasmid and psiCHECK2-MTA1 3′-UTR-Mut plasmid or ELFN1−AS1 Mut, respectively. The WT and Mut plasmids subsequently were co-transfected into cells with the negative control, miR-1205 mimics along with control Renilla Luciferase expression plasmid (phRL-TK). After 36 h of transfection, the Dual-Luciferase reporter Assay System (Promega, Madison, WI, USA) was applied to detect Luciferase and Renilla signals.

Western Blotting

Protein was isolated using RIPA cell lysis buffer (Beyotime, Baoshan, Shanghai, China). Protein samples were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electroblotted to polyvinylidene difluoride (PVDF) membranes (Hybond, Herndon, VA, USA). Membranes were blocked in 5% milk for 2 h and then incubated with primary antibodies, including anti-cleaved caspase-3, anti-Bcl-2, anti-MMP2, anti-MMP9, and GAPDH (1:500, Abcam, Cambridge, UK) overnight at 4 °C. The next day, the membranes were treated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling Technology, Boston, USA) at 37°C for 1 h. Finally, the band intensity was quantified using a chemiluminescence reagent kit (Merck KGaA, Darmstadt, Germany).

Tumor Xenografts in Nude Mice

BALB/c nude mice (6-8 weeks) were purchased from Shanghai Animal Laboratory Center (Shanghai, China). The animal protocol was reviewed and approved by the Research Ethics Committee of Shanxi Provincial People's Hospital. Negative control cells or transfected cells with the indicated lentivirus vector were digested with 0.25% trypsin, diluted in PBS, counted by trypan blue staining and adjusted to a concentration of 1.0×10^7 HT29 cells. Subsequently, 0.1 mL of this solution was injected subcutaneously on the back flank of each mouse at day 0. Tumor size was measured with a caliper every 7 days until 42 days. The tumor weight was observed every 7 days until 42 days.

Bioinformatics Analysis

The Cancer Genome Atlas (TCGA) database from Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) was used to explore the clinical characteristics of ELFN1−AS1 in CRC patients. lncBASE database (http://carolina.imis.athenainnovation.gr/diana_tools/web/index.php?r=lncbasev2/index-predicted) was used to predict ELFN1−AS1 binding sites to miR-1205. The miRNA target prediction website TargetScan (http://www.targets can.org/) database was used to predict the potential miR-1205 binding sites to 3′UTR of MTA1.

Statistical Analysis

Results were presented as mean ± SEM (Standard error of mean). The data were visualized via the software GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA). Log rank test and Kaplan-Meyer method were performed for evaluating the prognosis and the overall survival in patients with CRC. All statistical analysis was conducted via SPSS 13.0 (IBM SPSS Statistics, Armonk, NY, USA) including two-tailed Student's *t*-test and analysis of variance. $p < 0.05$ was considered statistically significant.

Results

ELFN1−AS1 is Up-Expressed in CRC Tissue Samples and Cells and Associated with Poor Prognosis

To investigate the potential clinical significance of ELFN1−AS1 in CRC tissue samples, we analyzed data from TCGA database and found that ELFN1−AS1 expression was significantly upregulated in CRC compared with normal samples (Figure 1A). Meanwhile, ELFN1-AS1 expression in 40 pairs of human CRC tissues and their adjacent normal tissues was determined by qRT-PCR. Similarly, ELFN1−AS1 expression in CRC tissues was much higher than that in normal tissues (Figure 1B). In addition, ELFN1−AS1 expressions in six CRC cell lines (HT29, HCT116, HCT8, LOVO, SW480, SW620) were also higher than that in the normal human colon mucosal epithelial cell line (FHC) (Figure 1C). Furthermore, we also analyzed the correlations between ELFN1−AS1 and clinical characteristics and found that lELFN1−AS1 expression was negatively correlated with overall survival rate of colorectal cancer patients (Figure 1D). Considering the median expression value of ELFN1−AS1 in CRC tissue as a cut-off

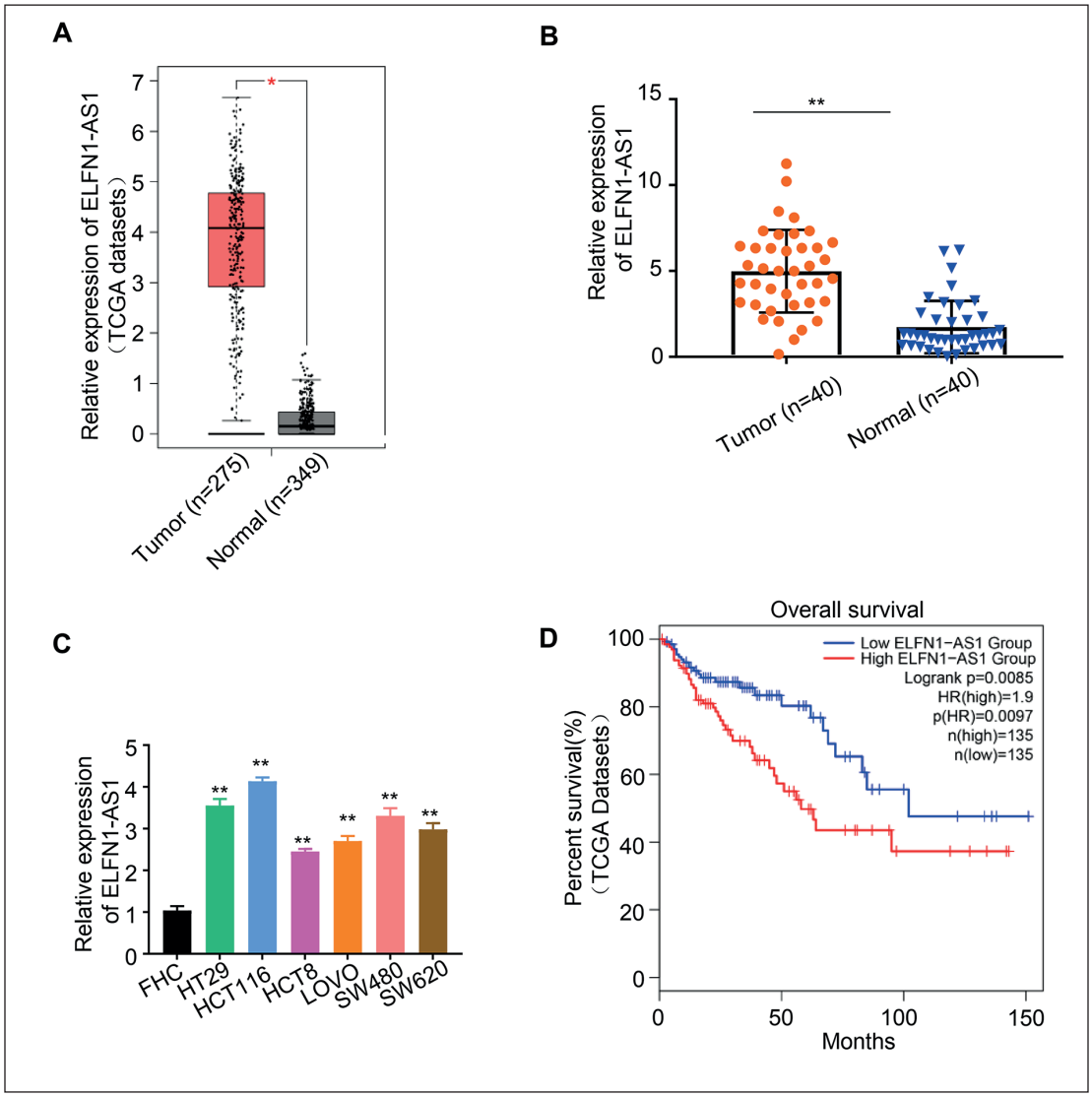

Figure 1. ELFN1−AS1 is upregulated in CRC tissue samples and cells and associated with poor prognosis. **A**, ELFN1−AS1 was over-expressed in CRC samples from TCGA database and associated with poor prognosis. **B**, Quantification of ELFN1−AS1 in 40 pairs of CRC tissue samples and adjacent normal tissues using qRT-PCR. **C**, Relative ELFN1−AS1 expression in different CRC cell lines (HT29, HCT116, HCT8, LOVO, SW480 and SW620) and normal human colon mucosal epithelial cell line FHC. **D**, The KM-Plotter was used to evaluate survival in CRC patients. Patients with high ELFN1−AS1 expression had worse overall survival than those with low ELFN1−AS1 expression. *$p < 0.05$; **$p < 0.01$.

value, 40 CRC patients were subdivided into two categories, one as high expression (n = 20) and the other low expression (n = 20) ELFN1−AS1 group. Results demonstrated the significant and direct correlation between tumor size and distant metastasis, lymph node metastasis, and tumor differentiation (Table I). Taken together, these data supported that ELFN1−AS1 was prominently expressed in CRC tissues and cells, and strongly associated with poor prognosis in CRC patients.

ELFN1−AS1 Knock-Down Inhibits Colorectal Cancer Proliferation, Migration, Invasion and Induced Apoptosis

To examine the role of ELFN1−AS1 in CRC progression, we knocked down ELFN1−AS1 in HT29 and HCT116 cell lines by two siRNAs specifically targeting ELFN1−AS1. ELFN1−AS1 expression was significantly reduced by si-ELFN1−AS1 pool transfection (Figure 2A). Next, CCK-8 assay revealed that in contrast to

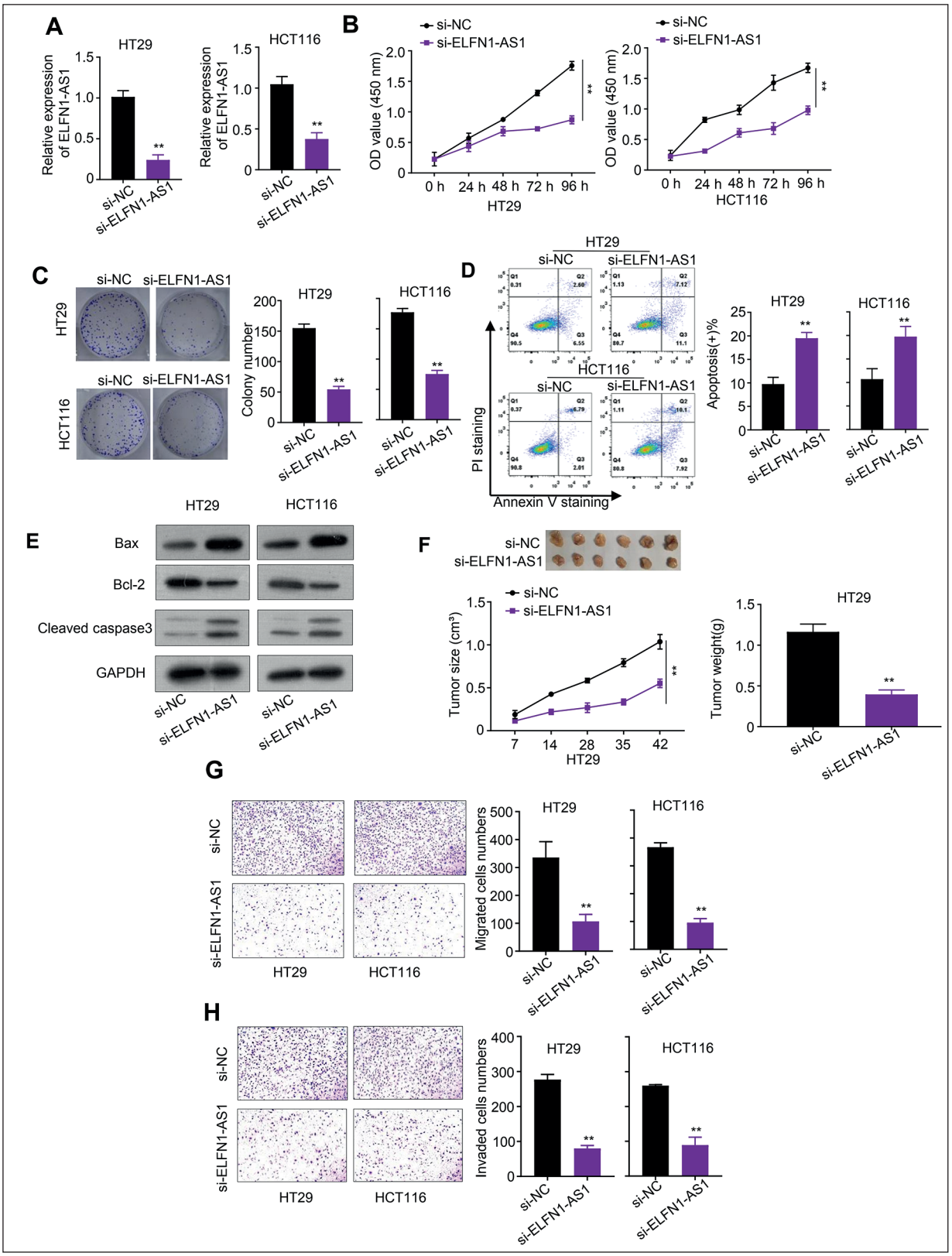

Figure 2. ELFN1−AS1 knock-down inhibits colorectal cancer proliferation, migration and invasion, and induces apoptosis. **A**, qRT-PCR analysis of knockdown efficiency of ELFN1−AS1 in HT29 and HCT116 cells transfected with si-NC and si-ELFN1−AS1. **B**, CCK-8 assay results of cell viability in HT29 and HCT116 cells transfected with si-NC and si-ELFN1−AS1. **C**, Colony formation in HT29 and HCT116 cells transfected with si-NC and si-ELFN1−AS1. **D**, Flow cytometric analysis showing the effects of si-ELFN1−AS1 on the cell apoptosis in HT29 and HCT116 cells. **E**, Apoptosis-related protein cleaved caspase-3 and Bcl-2 expression in HT29 and HCT116 cells transfected with si-NC and si-ELFN1−AS1. GAPDH was used as a loading control. **F**, Tumor size and weight in nude mice implanted subcutaneously with 1×10^6 of HT29 cells transfected with si-NC and si-ELFN1−AS1. **G**, Transwell assays showing migration ability in HT29 and HCT116 cells transfected with si-NC and si-ELFN1−AS1, the magnification is 200X. **H**, Transwell assays showing invasion ability in HT29 and HCT116 cells transfected with si-NC and si-ELFN1−AS1, the magnification is 200X. $*p < 0.05$; $**p < 0.01$.**Abbreviations:** si-NC, Scramble siRNA; si-ELFN1−AS1, siRNA of ELFN1−AS1.

the cells transfected with control scramble RNA (si-NC), the HT29 and HCT116 cells transfected with si-ELFNA-AS1showed impaired proliferative ability (Figure 2B). Similarly, knock-down of ELFN1−AS1 reduced colony numbers of HT29 and HCT116 cell lines (Figure 2C). Furthermore, we also studied whether ELFN1−AS1 affected apoptosis in CRC cell by flow cytometry. We found that the proportion of cells showing apoptosis was significantly increased when ELFN1−AS1 expression was downregulated (Figure 2D). Next, the apoptosis marker, Bax that specifically cleaves caspase-3, was assessed. In the context of knocking down, both Bax expression and the amount of cleaved caspase-3 were significantly increased. Conversely, another apoptosis marker, Bcl-2 (B-cell lymphoma-2) was downregulated when ELFN1−AS1 was knocked down (Figure 2E). Then, tumor subcutaneous xenografts were performed in BALB/c nude mice to evaluate the effect of ELFN1−AS1 *in vivo*. Figure 2F shows that subcutaneous tumor size and tumor weight were smaller in si-ELFN1−AS1 group compared with si-NC group. Moreover, we used transwell assays to test migration and invasion. and found that ELFN1−AS1 knock-down significantly inhibited migration and invasion in CRC cells (Figure 2G and 2H). Taken together, our results demonstrated that ELFN1−AS1 facilitates CRC cell proliferation, migration, invasion, while inhibiting apoptosis.

ELFN1ÐAS1 Sponges miR-1205 in Colorectal Cancer Cells

We further demonstrated that ELFN1−AS1 6 was mainly localized in cytoplasm (Figure 3A). Since mounting evidence[6-9] has shown that lncRNA can serve as a ceRNA to sponge miRNA to regulate the expression of mRNA. We used an online database lncBASE to bioinformatically screen for the potential miRNA that could interact with ELFN1-AS1. The screen revealed that ELFN1 had a potential complementary sequence for miR-1205 (Figure 3B). Luciferase reporter assay was then performed to validate the interaction between ELFN1−AS1 and miR-1205. MiR-1205 expression was reduced in ELFN1−AS1-WT transfected cells while the Luciferase activity in ELFN1−AS1-Mut groups did not change (Figure 3B). Furthermore, RNA pull-down assay demonstrated that ELFN1−AS1 was enriched in bio- miR-1205 group (Figure 3C). Knockdown of ELFN1−AS1 upregulated miR-1205 (Figure 3D). Moreover, qRT-PCR demonstrated that miR-1205 was decreased in 40 pairs of human CRC tissue compared with adjacent normal tissue (Figure 3E). Meanwhile, the expressions of ELFN1−AS1 and miR-1205 were negatively correlated in CRC tissues (Figure 3F). Collectively, these results explicitly indicated that miR-1205 is the miRNA that is sponged by ELFN1-AS1 in CRC cells.

MiR-1205 Inhibits Cell Proliferation, Migration and Invasion, and Induces Apoptosis in CRC Cells

To further confirm that ELFN1−AS1 promotes CRC progression through sponging miR-1205, we overexpressed miR-1205 in CRC cell lines. Transfection of miR-1205 mimics significantly increased miR-1205 expression (Figure 4A). Subsequently, CCK-8 assay revealed miR-1205 overexpression remarkably decreased the cell viability compared with the control groups (Figure 4B). Similarly, colony formation in HT29 and HCT116 cells transfected with miR-1205 mimics was diminished (Figure 4C). Additionally, miR-1205 up-regulation increased the cell apoptosis rate revealed by flow cytometry (Figure 4D). Meanwhile, the amount of cleaved caspase-3 was significantly increased in the group of miR-1205 mimics transfection while Bcl-2 was relatively lower in cells with miR-1205 mimics transfection (Figure 4E). Cell migration and invasion assays indicated that miR-1205 upregulation decreased both cell migration and invasion ability (Figure 4F and 4G).

MTA1 Directly Interacts with MiR-1205

To discover the direct target mRNA of miR-1205, we firstly screened for the mRNA that contains a complementary sequence of miR-1250 using TargetScan. The screen identified a potential miR-1205 binding site in the 3′-untranslated region (3′UTR) of MTA1 (Figure 5A). Luciferase reporter assay was then used to validate the interaction. MiR-1205 upregulation reduced the relative Luciferase activity of MTA1-WT transfected cells, while no significant change was observed in the MTA1-Mut group (Figure 5A). Both mRNA and protein expression levels of MTA1 were decreased in HT29 and HCT116 cells transfected with miR-1205 mimics (Figure 5B and 5C). Furthermore, MTA1 was up-regulated in 40 pairs of human CRC tissues compared with adjacent normal tissues (Figure 5D). A negative correlation between the expressions of MTA1 and miR-1205 and a positive correlation between MTA1 expression and ELFN1-AS1 were observed in human

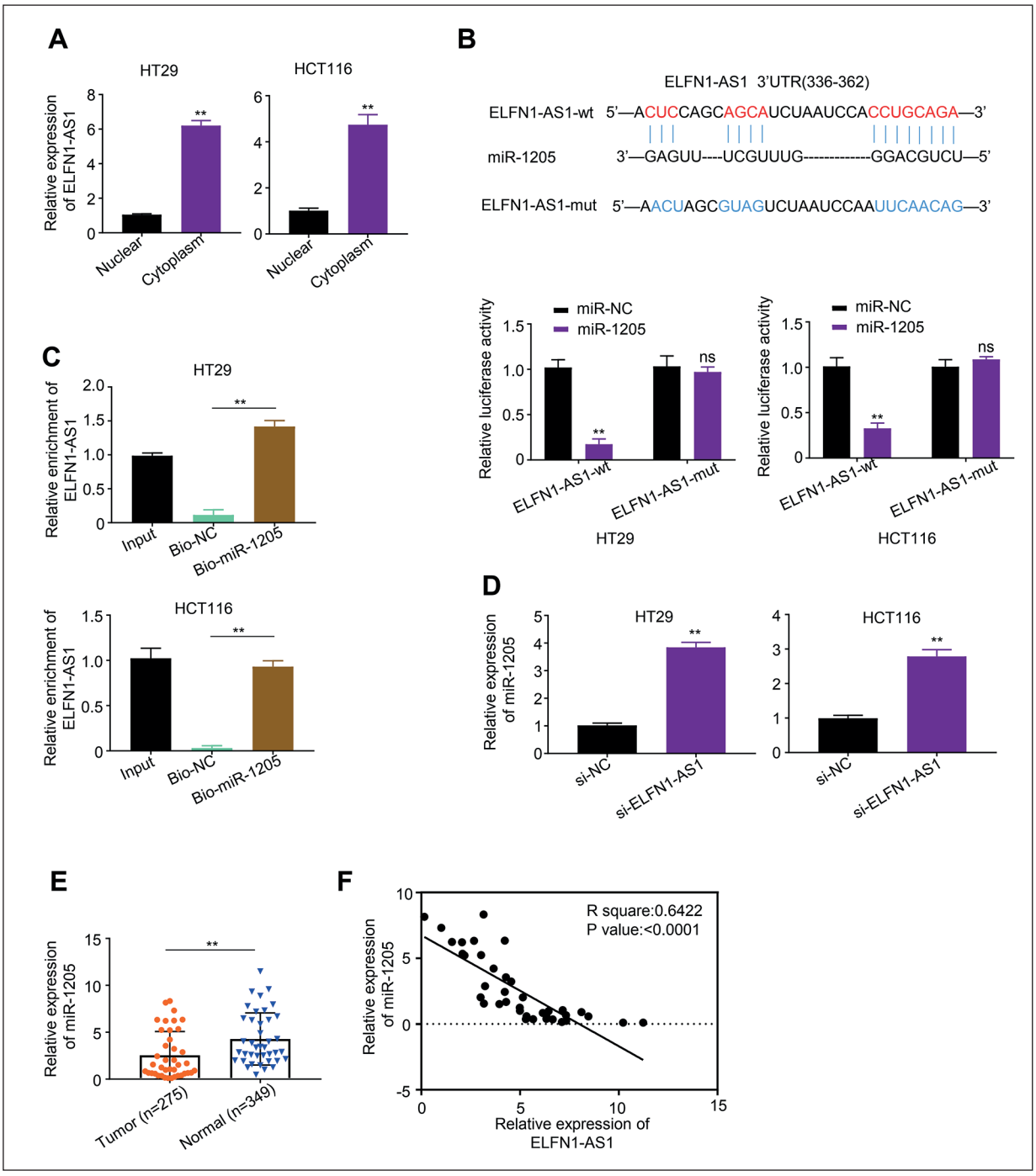

Figure 3. ELFN1−AS1 sponges miR-1205 in colorectal cancer cells. **A**, Localization of ELFN1−AS1 in HT 29 and HCT116 cells using nuclear cytoplasm separation experiment. **B**, The putative binding sites between ELFN1−AS1 and miR-1205. Relative miR-1205 expression in cells co-transfected with wt or mut ELFN1−AS1 and miR-1205 using Luciferase reporter assay. **C**, Enrichment of ELFN1−AS1 using RNA pull-down experiments. **D**, Relative miR-1205 expression in HT29 and HCT116 cells transfected with si-NC and si-ELFN1−AS1. **E**, Relative miR-1205 expression in 40 pairs of CRC tissue and their adjacent normal tissue. **F**, Spearman's rank-order correlation between miR-1205 and ELFN1−AS1.**$p < 0.01$. **Abbreviations:** ns, no significance. miR-NC, negative control of miR-1205; wt, wild type; mut, mutant.

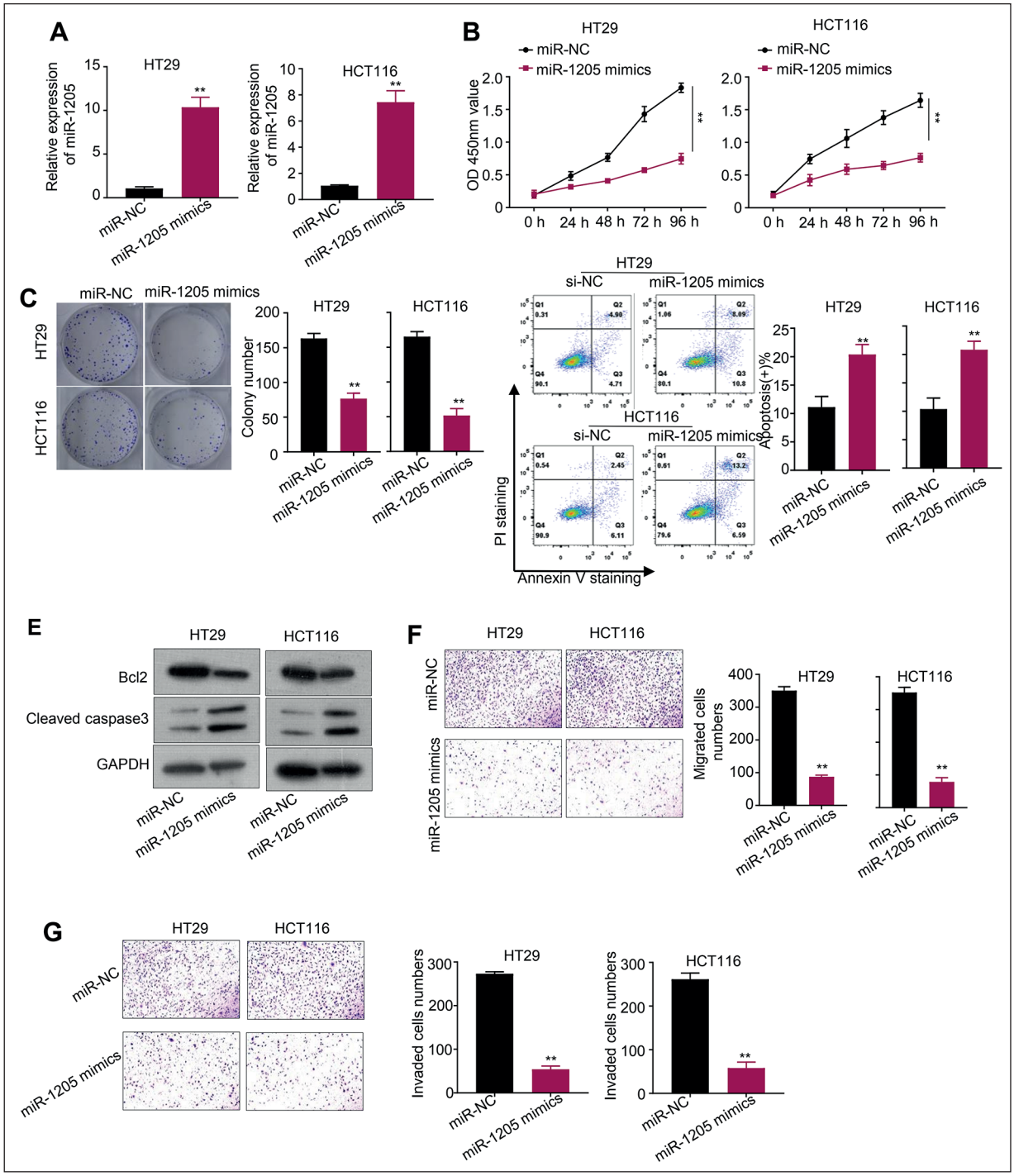

Figure 4. MiR-1205 inhibits cell proliferation, migration and invasion, and induces apoptosis in CRC cells. **A**, qRT-PCR analysis of relative miR-1205 expression in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics. **B**, CCK-8 assay of cell viability in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics. **C**, Colony formation in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics. **D**, Flow cytometric analysis showing the conditions of the cell apoptosis in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics. **E**, Apoptosis-related protein cleaved caspase-3 and Bcl-2 expression in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics. GAPDH was used as a loading control. **F**, Transwell assays showing migration ability in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics, the magnification is 200X. **G**, transwell assays showing invasion ability in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics, the magnification is 200X. $^{**}p < 0.01$

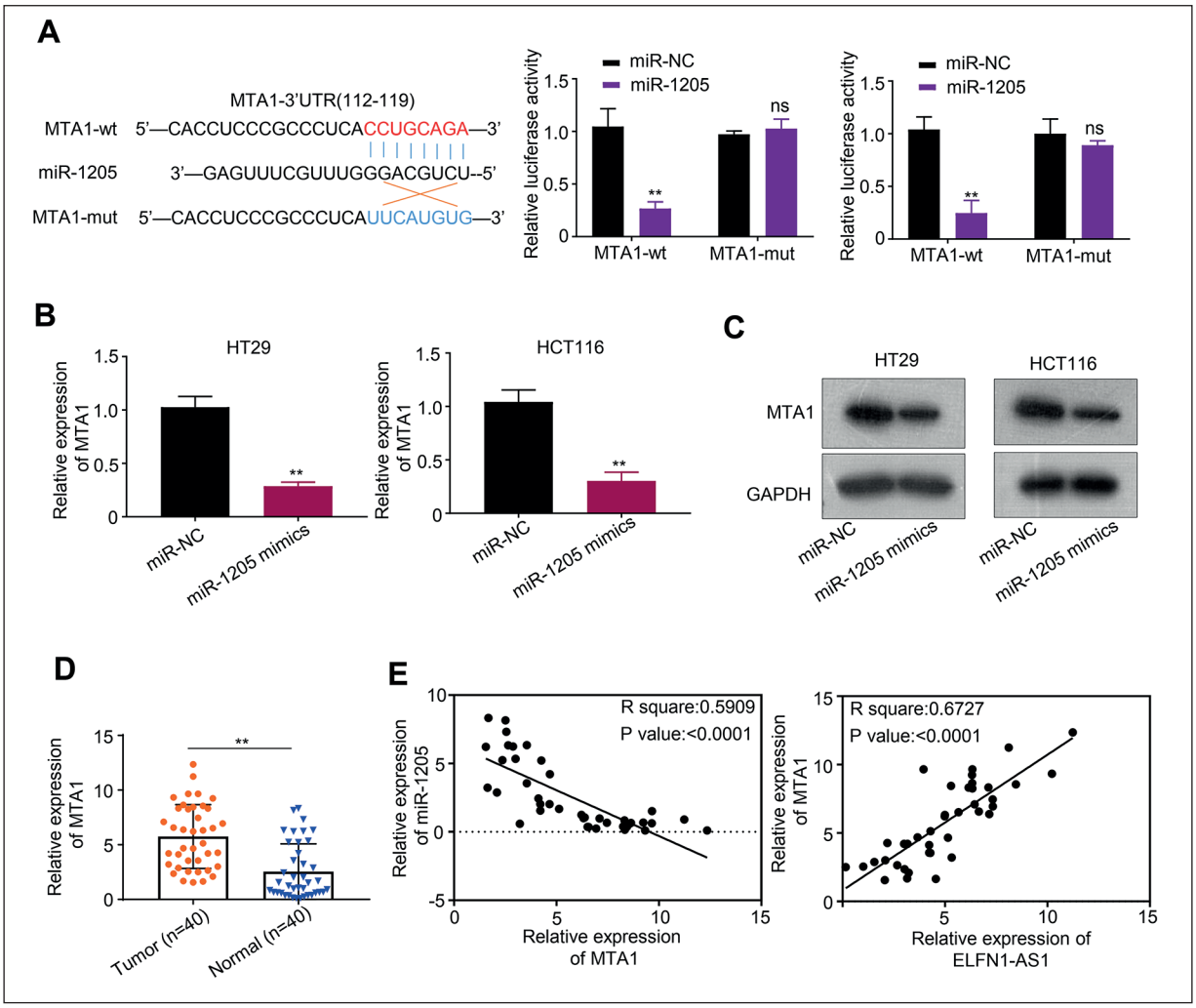

Figure 5. MTA1 directly interacts with miR-1205. **A**, The putative binding sites between MTA1 and miR-1205. Relative MTA1 expression in cells transfected with wt and mut MTA1 using Luciferase reporter assay. **B**, Relative MTA1 mRNA expression in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics. **C**, Relative MTA1 protein expression in HT29 and HCT116 cells transfected with miR-NC and miR-1205 mimics. **D**, Relative MTA1 expression in 40 pairs of CRC tissue and their adjacent normal tissue. **E**, Spearman's rank order correlation between miR-1205 and MTA1, and between MTA1 and ELFN1−AS1. $^{**}p < 0.01$. **Abbreviations:** ns, no significance. miR-NC, miRNA negative control.

CRC tissues via Spearman's rank-order analysis (Figure 5E). Together, these data suggested that MTA1 directly interacts with miR-1205.

MTA1 Restoration Completely Rescues the Inhibitory Effect of ELFN1ÐAS1 Knock-Down in CRC Cells

Both mRNA and protein levels of MTA1 were reduced by transfection with si-ELFN1−AS1, while these defects were restored in the cells by co-transfection with si-ELFN1−AS1 and MTA1 (Figure 6A). Both cell proliferation and colony formation were impaired in the si-ELFN1−AS1 transfected cells compared with si-NC group, but did not change in si-ELFN1−AS1 + MTA1 group compared with si-ELFN1−AS1 group (Figure 6B and 6C). In addition, flow cytometry assays showed that MTA1 overexpression reversed the promoting effect of ELFN1−AS1 in apoptosis (Figure 6D). Furthermore, transwell assay results indicated that both cell migration and invasion were inhibited by transfection of si-ELFN1−AS1 and increased by co-transfection of si-ELFN1−AS1 and MTA1 (Figure 6E and 6F). These results indicated that ELFN1−AS1 sponges miR-1205 that targets MTA1.

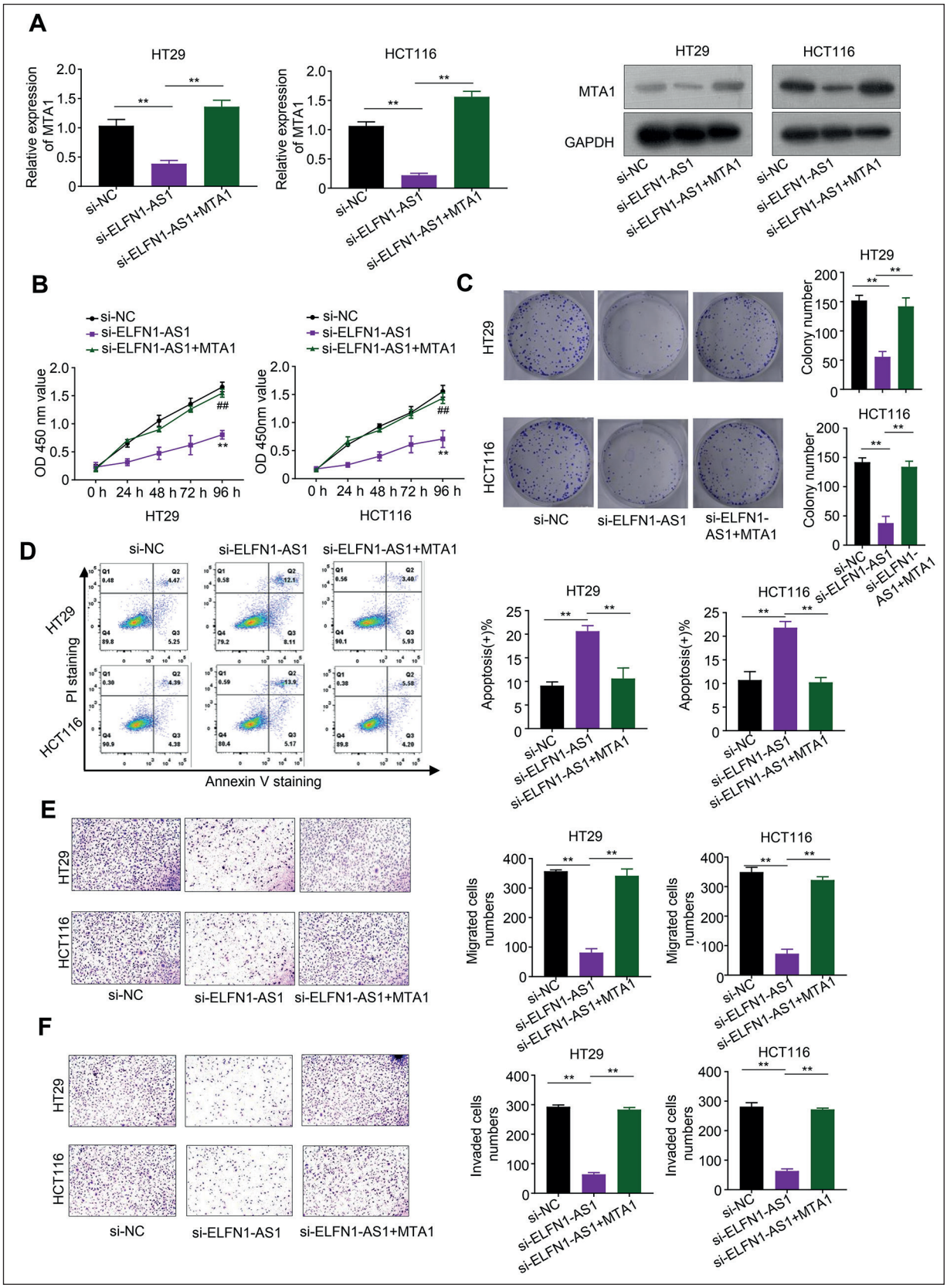

Figure 6. MTA1 restoration completely rescues the inhibitory effect of ELFN1−AS1 knock-down in CRC cells. **A**, MTA1 mRNA and protein expression in HT29 and HCT116 cells transfected with si-NC, si-ELFN1−AS1 and si-ELFN1−AS1 + MAT1. **B,** CCK-8 assay of cell viability in HT29 and HCT116 cells transfected with si-NC, si-ELFN1−AS1 and si-ELFN1−AS1 + MAT1. **C**, Colony formation in HT29 and HCT116 cells transfected with si-NC, si-ELFN1−AS1 and si-ELFN1−AS1 + MAT1. **D**, Flow cytometric analysis showing the conditions of cell apoptosis in HT29 and HCT116 cells transfected with si-NC, si-ELFN1−AS1 and si-ELFN1−AS1 + MAT1. **E**, Transwell assays showing migration ability in HT29 and HCT116 cells transfected with si-NC, si-ELFN1−AS1 and si-ELFN1−AS1 + MAT1, the magnification is 200X. **F**, Transwell assays showing invasion ability in HT29 and HCT116 cells transfected with si-NC, si-ELFN1−AS1 and si-ELFN1−AS1 + MAT1, the magnification is 200X. $^{**}p < 0.01$. **Abbreviations:** ns, no significance. miR-NC, miRNA negative control.

Discussion

Recent evidence[13] has proved that ELFN1−AS1 has an oncogenic effect and was closely associated with poor prognosis in CRC patients. ELFN1−AS1 is involved in the pathological activities of CRC cells via sponging miR-4644 to modulate TRIM44 expression. However, the contribution of ELFN1−AS1 to CRC has not been completely explored yet. This study demonstrated that ELFN1−AS1 contributes to poor prognosis of CRC. Inhibition of ELFN1−AS1 reduced cell proliferation, colony formation, migration and invasion, and induced apoptosis, which demonstrated that ELFN1−AS1 might be a therapeutic target of CRC.

Zhou et al[29] have shown that lncRNA acts as a sponge of miRNAs to regulate tumorigenesis. For example, lncRNA-ROR promotes the growth, migration and invasion of colon cancer cells through inhibiting the expression level of miR-145. SNHG6 accelerates CRC progression by regulating miR-26ab/ EZH2 and miR-214/ EZH2 axis[30]. Previously, Dai et al[31] showed that miR-1205 plays an oncogenic role in lung adenocarcinoma, promoting the growth, migration, and invasion of lung adenocarcinoma cells by targeting APC2[31]. In our study, we proved that ELFN1−AS1 sponges miR-1205. In addition, miR-1205 inhibited cell proliferation, migration and invasion, and induced apoptosis in CRC cells by regulating the expression of MTA1, which echoes the role of miR-1205 in papillary thyroid cancer. Data mentioned above demonstrated that miR-1205 plays a key role in tumor progression by targeting MTA1 in CRC. Co-transfection of MiR-1205 mimics with MTA1 3′-UTR WT markedly inhibited the Luciferase intensities of SW579 and TPC-1 cells, suggesting that MTA1 is the direct target of miR-1205. Additionally, MTA1 was over-expressed in CRC tissues and NTA1 expression negatively correlated with miR-1205 expression and MTA1 restoration completely rescued the inhibitory effect of ELFN1−AS1 knockdown in HT29 and HCT116 cells. These findings revealed that MTA1 is directly regulated by miR-1205 and plays an important role in CRC. In conclusion, although ELFN1−AS1 has been implicated in CRC as an oncogene, the molecular mechanism underlying its oncogenic role and the related signal pathway have never been studied.

Conclusions

Our data, for the first time, have demonstrated that ELFN1−AS1 regulation promotes cell proliferation, migration and invasion and induces apoptosis by sponging miR-1205 to regulate MTA1 expression in CRC, indicating ELFN1−AS1 may be a novel prognostic marker and therapeutic biomarker in CRC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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