MicroRNA-27a-3p promotes epithelial-mesenchymal transition by targeting NOVA alternative splicing regulator 1 in gastric cancer

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Abstract. NOVA alternative splicing regulator 1 (NOVA1) dysregulation has been detected in the gastric cancer microenvironment. Decreased NOVA1 expression has been linked to the progression and poor prognosis of gastric cancer; however, the role of NOVA1 in regulating epithelial-mesenchymal transition (EMT) remains unclear in this disease. Experimental evidence has shown that miR-27a-3p is a potential oncogene in gastric cancer. In the present study, we observed that miR-27a-3p expression was increased in gastric cancer and was inversely associated with overall survival. Overexpression of miR-27a-3p promoted EMT in AGS gastric cancer cells. Additionally, overexpression of miR-27a-3p inhibited NOVA1 expression, while silencing of NOVA1 promoted EMT in AGS cells. A total of 108 gastric cancer samples were examined for NOVA1 expression by immunohistochemistry. Decreased NOVA1 expression was linked to lymph node metastasis, tumor-node-metastasis stage and shorter overall survival. Therefore, these results indicated that NOVA1 could be a potential tumor suppressive gene and that miR-27a-3p promotes EMT by targeting NOVA1 in gastric cancer.

Introduction

NOVA alternative splicing regulator 1 (NOVA1) is a member of the NOVA family (1-6). The role of NOVA1 was first reported in neurological diseases (6). For example, NOVA1 regulates neuronal microRNA (miRNA/miR) expression via a variety of mechanisms (6). Decreased NOVA1 expression was observed in the gastric cancer microenvironment, and in tumor cells was correlated with the progression and poor prognosis of gastric cancer (4); however, the role of NOVA1 in regulating the epithelial to mesenchymal transition (EMT) in gastric cancer remains unknown.

miRs represent a class of small non-coding RNA molecules and are important post-transcriptional regulators that target key genes, such as NOVA1, which has been associated with the progression of gastric cancer (7). Numerous miRs are dysregulated in gastric cancer (8). For example, the expression of miR-27a-3p was increased in gastric cancer tissues and cell lines (8). Experimental evidence has demonstrated that miR-27a-3p is a potential oncogene (9-15). It was reported that miR-27a-3p can contribute to progression of gastric cancer by regulating different genes, such as BTG antiproliferation factor 2 (9,10). Circulating miR-27a-3p has been proposed as a biomarker for the early diagnosis and prognostic evaluation of patients with gastric cancer (11); high expression levels of miR-27a-3p were associated with poor overall survival (12). Additionally, miR-27a-3p expression within tumors has been proposed as a potential biomarker for predicting chemosensitivity and chemotherapy resistance in metastatic gastric cancer (12-15). EMT serves critical roles in the regulation of chemosensitivity (16); however, the mechanism underlying the effects of miR-27a-3p on EMT in gastric cancer requires further investigation.

Materials and methods

Clinical specimens. The present study was approved by the Ethics Committees of Renmin Hospital of Wuhan University (Wuhan, China) and Cixi People's Hospital (Cixi, China). Written informed consent was obtained from patients prior to the collection of tissues. Gastric cancer samples were obtained from those diagnosed with primary gastric cancer. Patients did not receive preoperative chemotherapy or radiation at Renmin Hospital of Wuhan University and Cixi People's Hospital between January 1990 and January 2002. In total, 108 gastric cancer samples were immediately frozen in liquid nitrogen following surgical resection and were then stored at -80˚C. The patients, (75 males and 33 females; age range, 35-90 years). Samples of the corresponding adjacent normal tissue were

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obtained >3 cm away from the site at which the primary tumor was sampled (17). All tumor tissues and adjacent normal tissues were reviewed in a blind manner by two pathologists. The duration of the follow-up was 9 years. The AJCC Cancer Staging system was used for tumor classification (18).

Cell culture. The AGS gastric cancer cell line obtained from Changhai Hospital, Second Military Medical University (Shanghai, China) (19) were cultured with RPMI-1640 medium (Sigma-Aldrich; Merck KGaA) containing 10% fetal bovine serum (Shanghai ExCell Biology, Inc.) and 100 mg/ml penicillin and 100 mg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere with 5% CO_2 at 37˚C. Cellular morphologies were observed using an optical microscopy (Leica Microsystems, Inc.).

Scramble- and NOVA1-short hairpin RNA-expressing plasmids, control miR (mock)/Pre-miR-27a-3p. Mock and NOVA1-shRNA expressing plasmids (Sequence, 5'-CCAGAT AAGCTTGCCACCATGGAAGGGGCCC-3') were obtained from Tiangen Biotech Co., Ltd. Mock (5'-CACUCGGACCCU GAGCUGCCG-3') and Pre-miR-27a-3p (5'-UUCACAGUG GCUAAGUUCCGC-3') were obtained from Ambion, Inc. (Ambion; Thermo Fisher Scientific, Inc.).

Transfection experiments. Transfection experiments were performed as described previously (20). Cells ($8x10^2$ cells/well) were seeded in six-well plates prior to transfection for each experiment. For transfection experiments, cells were cultured in serum-free medium without antibiotics at 60% confluence for 24 h. Subsequently, cells were using FuGENE HD transfection reagent (Promega Corporation) according to the manufacturer's protocol. In total, 5 μg Pre-miR-27a-3p or control miR mimics and 10 μg NOVA1 or mock-shRNA expressing plasmids were used. After incubation for 6 h, the medium was removed and replaced with serum-free medium without antibiotics, and cells were incubated for 24 h. Then, the western blot analysis, Immunofluorescence assay and luciferase reporter assay and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were performed as described below.

Western blot analysis. Western blot analysis was performed as previously described (20). Total protein was prepared using extraction buffer comprising NaCl/P_i containing 0.5% Triton X-100, 1 mM EDTA, 1 mM PMSF and 1X complete protease inhibitors (Roche Diagnostics). The concentration of each protein lysate was determined using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.). Total protein (50 μg/lane) was separated by 12% SDS-PAGE. Subsequently, samples were transferred to nitrocellulose membranes and blocked for 60 min at room temperature in 5% skimmed milk. The membranes were immunoblotted using the following primary antibodies: Anti-fibronectin (cat. no. ab2413; 1:500; Abcam), anti-N-cadherin (cat. no. ab18203;1:500; Abcam), anti-E-cadherin (cat. no. ab40772; 1:500; Abcam), anti-keratin 8 (cat. no. ab53280; 1:500; Abcam), anti-NOVA1 (cat. no. ab183024; 1:500; Abcam) and anti-β-actin (cat. no. ab5694; 1:500; Abcam) overnight at 4˚C. Subsequently, anti-rabbit secondary antibody (cat. no. ab6940; 1:10,000; Abcam) was used to incubate membranes for 30 min at room temperature. The specific protein bands were visualized by Odyssey Infrared Imaging system (LI-COR Biosciences). β-actin expression was used as an internal control to confirm equal loading of the protein samples.

Bioinformatics analysis. Bioinformatics analysis was performed as described previously (21). miRanda (release date, August 2010; http://www.microrna.org/microrna/home.do) was used to identify the target genes of miR-27a-3p. *Immunofluorescence assay*. *Immunofluorescence* assay was performed as described previously (20). After transfection with plasmids, the cells were fixed in 4% paraformaldehyde for 15 min at room temperature, and then blocked with 20% goat serum (Gibco; Thermo Fisher Scientific, Inc.) blocking solution for 20 min at room temperature.

Antibody staining. Rabbit antibody against NOVA1 (cat. no. ab183024; 1:200 dilution; Abcam) were added, and the mixtures were incubated overnight at 4˚C. After washing three times with NaCl/P_i, cells were incubated with appropriate Cy2-conjugated secondary antibodies (cat. no. ab6940; 1:10,000; Abcam) for 30 min at 37˚C. After washing with NaCl/Pi, the samples were observed under a laser scanning confocal microscope (Leica Microsystems). DAPI staining was used to stain nuclei for 30 min at 37˚C. Representative images (magnifications, x100) are presented. Fluorescence intensities were analyzed using ImageJ software (version 1.37v; http://rsb.info.nih.gov/ij/index.html).

Immunocytochemistry analysis. Immunocytochemistry was performed as described previously (22). Immunohistochemistry was performed on 10% formalin-fixed for 24 h, paraffin-embedded tissue sections using the envision peroxidase detection method. Sections (thickness, 4-μm) were deparaffinized in xylene for 10 min at 4˚C, dehydrated through graded ethanol series and subsequently treated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature to eliminate the endogenous peroxidase activity. For antigen retrieval, the sections were microwaved in 10 mM citrate buffer (pH 6.0) for 10 min. The primary antibody anti-CRP (cat. no. ab211631; 1:200, Abcam) was incubated for 12 h at 4˚C after blocking non-specific binding with 10% normal goat serum (Gibco; Thermo Fisher Scientific, Inc.) in PBS for 30 min at room temperature. Anti-rabbit secondary antibody (cat. no. ab211631; ab150077; 1:10,000, Abcam) was used for 60 min at room temperature. Sections were incubated with envision peroxidase complex (Dako; Agilent Technologies, Inc.) for 20 min at room temperature. Sections were counterstained with Mayer's hematoxylin for 10 min at room temperature after immunostaining prior to mounting. If ≥10% cells were stained, the expression levels of CRP were defined as 'High' expression. Otherwise, the expression levels of CRP were defined as 'low' expression.

Luciferase reporter assay. Luciferase reporter plasmids containing the 3'-untranslated region (3'-UTR) of human NOVA1 mRNA were obtained from Tiangen Biotech Co., Ltd. Luciferase reporter assay was performed as described previously (20). For reporter assays, cells was transiently transfected with reporter plasmids (Tiangen Biotech Co.) and

miRNA using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Reporter assays were performed 36 h post-transfection using the Dual-luciferase assay-system (Promega Corporation), normalized for transfection efficiency by cotransfected *Renilla*-luciferase.

Real-time PCR for miRNA expression. The analysis of miR expression via Real-time PCR was performed as described (23). Total RNA was isolated from the cells or tissues using the mirVana miRNA Isolation kit (cat. no. AM1561; Ambion; Thermo Fisher Scientific, Inc.). The detection of the mature form of miRNAs was performed using the mirVana RT-qPCR miRNA Detection kit (SYBR Green) and RT-qPCR Primer Sets, according to the manufacturer's instructions (Ambion; Thermo Fisher Scientific, Inc.). For the qPCR of miR-27a-3p, the forward primer was: 5'-CAGTTCACAGTGGCTAAG A-3'; and the reverse primer was: 5'-CAGTTTTTTTTTTTT TTTGCGGAA-3'. The quantification was performed using the $2^{-\Delta\Delta Cq}$ method (24). The U6 small nuclear RNA was used as an internal control. The thermocycling conditions were as follows: One cycle at 50˚C for 2 min, one cycle at 95˚C 10 min and 40 cycles of 95˚C for 15 sec and 60˚C for 30 sec. The primers used were as follows: U6 forward, 5'-GCTTCGGCA GCACATATACTAAAAT-3'; reverse, 5'-CGCTTCACGAAT TTGCGTGTCAT-3'.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The analysis of mRNA via RT-qPCR was performed as described previously (20). Briefly, total cellular RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc.) and 2 μg total RNA was reverse-transcribed using M-MLV reverse transcriptase (Promega Corporation) according to the manufacturer's protocol. The thermocycling conditions were as follows: Denaturation for 30 sec at 95˚C, annealing for 45 sec at 52-58˚C depending on the primers used, and extension for 45 sec at 72˚C. Each PCR reaction was performed for 28-32 cycles. qRT-PCR was performed using a StepOne™ real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Fast SYBR Green Master Mix was obtained from Applied Biosystems (Thermo Fisher Scientific, Inc.). Data are shown as a relative expression level after normalization to GAPDH. The primers employed were as follows: NOVA1 forward, 5'-TAC TGAGCGAGTGTGCTTGAT-3', reverse, 5'-GTCTGGGGT TGTAGAATGCTG-3'; E-cadherin forward, 5'-TCAACGATC CTGACCAGCAGTTCG-3', reverse, 5'-GGTGAACCATCA TCTGTGGCGATG-3'; keratin 8 forward, 5'-GAAGACCAC CAGCGGCTATG-3', reverse, 5'-GTCAGAGGACTCAGA CACCAG-3'; fibronectin forward, 5'-TTTTGACAACGGGAA GCATTATCAGATAA-3'; reverse, 5'-TGATCAAAACAT TTCTCAGCTATTGG-3'; N-cadherin forward, 5'-CATCCC TCCAATCAACTTGC-3', reverse 5'-ATGTGCCCTCAAATG AAA CC-3'; and GAPDH forward, 5'-CGGAGTCAACGG ATTTGGTCGTAT-3' and reverse, 5'-AGCCTTCTCCATGGT GGTGAAGAC-3'. The quantification of PCR performed was performed using the $2^{-\Delta\Delta Cq}$ method (24).

Statistical analysis. The results were analyzed using SAS software (version 9.4; SAS Institute, Inc.). Samples were analyzed with a Student's t-test for the comparison of two groups (two-tailed), unless otherwise indicated, such as a χ^2 test. Data are presented as the mean ± SEM from experiments performed in triplicate. Overall survival was analyzed via a Kaplan-Meier test. The log-rank test was used to examine the statistical significance of the difference between groups. Survival was investigated based on NOVA1 expression (two-tailed). $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-27a-3p expression is inversely associated with overall survival and overexpression of miR-27a-3p promotes EMT in AGS cells. To detect miR-27a-3p expression in adjacent normal tissues and gastric cancer tissues, we performed RT-qPCR. We isolated microRNA from 10 pairs of normal tissues and gastric cancer tissues. The results revealed that miR-27a-3p expression levels were significantly upregulated in gastric cancer tissues compared with normal tissues (Fig. 1A). To determine whether miR-27a-3p expression was associated with the overall survival of patients with gastric cancer, we analyzed tumor miR-27a-3p expression in patients with an overall survival ≥40 months and those with an overall survival <40 months. We observed that miR-27a-3p expression was significantly reduced in those with an overall survival ≥40 months than those with an overall survival <40 months (Fig. 1B). The results indicated that miR-27a-3p could be associated with progression of gastric cancer.

To investigate the role of miR-27a-3p *in vitro*, we transfected AGS cells with control miR and pre-miR-27a-3p, we performed RT-qPCR. We observed that miR-27a-3p expression was significantly increased in the cells transfected with pre-miR-27a-3p than in the control group (Fig. 1C); overexpression of miR-27a-3p revealed visible changes in the morphology of AGS cells (Fig. 1D). The phenotype was changed from a cobblestone-like to a spindle-like morphology. To confirm that these morphological changes in AGS cells were promoted by EMT, we performed western blotting and RT-qPCR to examine the expression of epithelial and mesenchymal markers in AGS cells. We observed that the expression of E-cadherin and keratin 8 (epithelial markers) was markedly suppressed, whereas that of N-cadherin and fibronectin (mesenchymal markers) were promoted by pre-miR-27a-3p compared with in the control group (Fig. 1E and F).

miR-27a-3p inhibits NOVA1 expression in AGS cells. miR-27a-3p expression was determined to be inversely associated with overall survival and overexpression of miR-27a-3p promoted EMT in AGS cells. We sought to determine the mechanism underlying the effects of miR-27a-3p. miRNAs are a novel class of small (~22 nucleotides) noncoding RNAs, and negatively regulate protein-coding gene expression by targeting mRNA degradation or inhibit translation (9-11). Thus, we suggested that miR-27a-3p might promote EMT by regulating the expression of its target genes. To identify the target genes of miR-27a-3p, we used miRanda, a commonly used prediction algorithm (http://www.microrna.org/microrna/home.do). Numerous target genes, including NOVA1, were identified. Decreased NOVA1 expression has

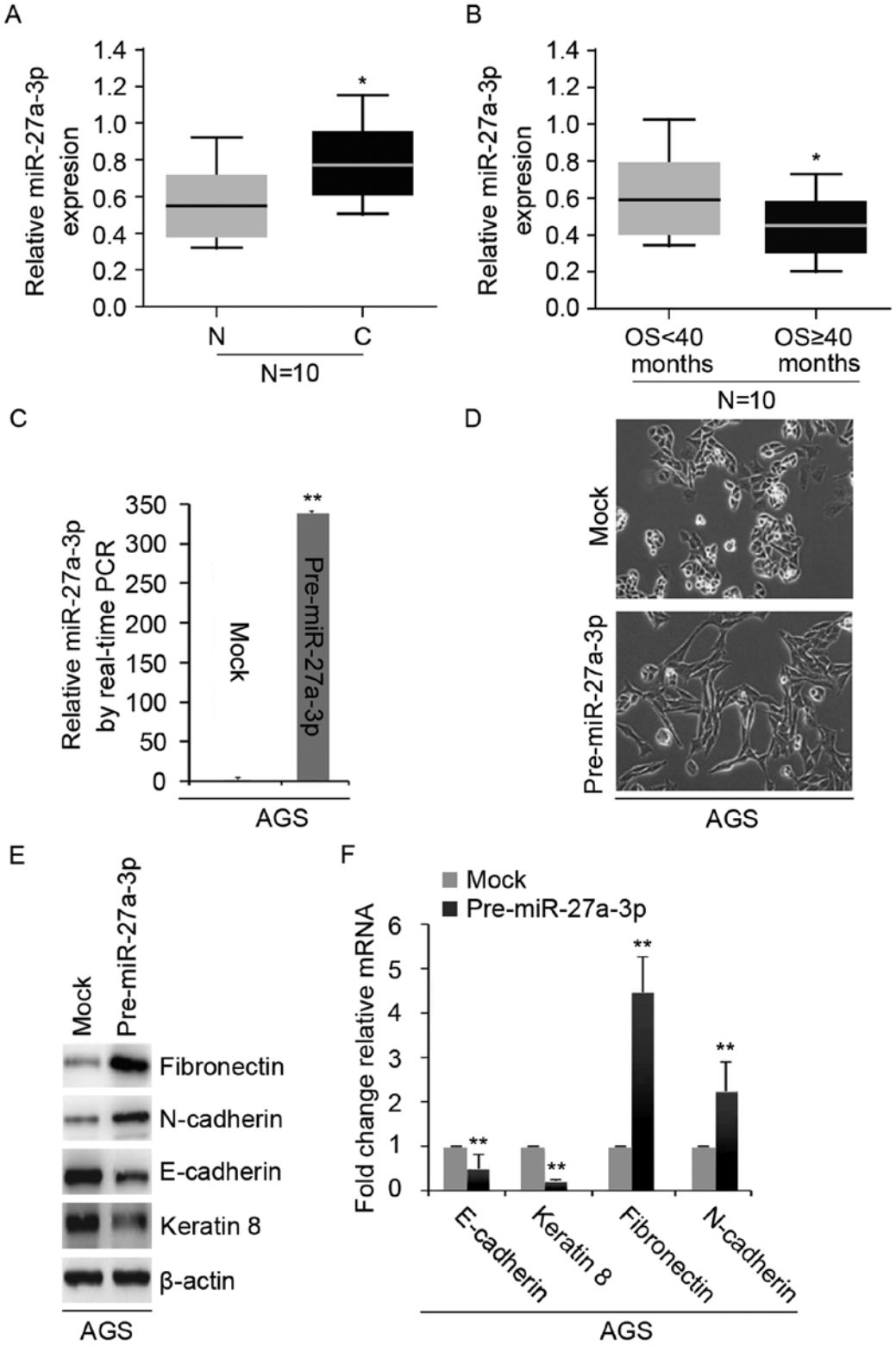

Figure 1. miR-27a-3p expression is inversely associated with overall survival and overexpression of miR-27a-3p promotes epithelial-mesenchymal transition in AGS cells. (A) RT-qPCR for the analysis of miR-27a-3p expression in 10 pairs of gastric cancer tissues and adjacent normal tissues. *P<0.05 vs. N. (B) RT-qPCR for the analysis of miR-27a-3p expression in gastric cancer tissues and adjacent normal tissues. *P<0.05 vs. OS<40 months. (C) RT-qPCR for the analysis of miR-27a-3p expression in AGS cells transfected as indicated. **P<0.01 vs. mock. (D) AGS cells were transfected as indicated and analyzed via microscopy (n=3), Scale bars, 50 μm. (E) Western blot analysis for fibronectin, N-cadherin, E-cadherin and keratin 8 expression in AGS cells transfected as indicated (n=3). (F) RT-qPCR for fibronectin, N-cadherin, E-cadherin and keratin 8 in AGS cells transfected as indicated. **P<0.01 vs. mock. C, cancer tissues; miR, microRNA; mock, control miR; N, adjacent normal tissues; OS, overall survival; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

been observed in gastric cancer, and correlated with the progression and poor prognosis of this disease (4). Thus, we reasoned that miR-27a-3p may promote EMT by regulating NOVA1 expression. The target sites on the 3'UTR of NOVA1

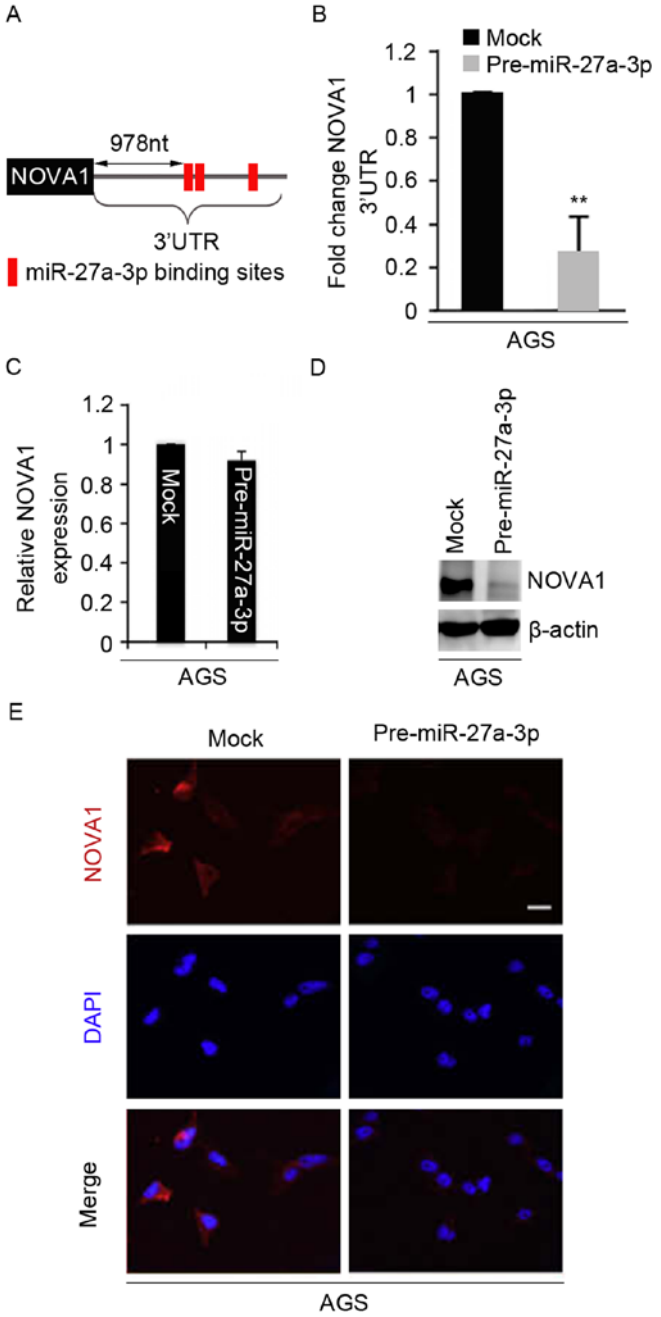

Figure 2. miR-27a-3p regulates NOVA1 expression in AGS cells. (A) Predicted binding sites of miR-27a-3p and NOVA1 by miRanda algorithm. (B) Reporter assay, with cotransfection of 500 ng reporter plasmids and 50 nM mock or pre-miR-27a-3p. **P<0.01 vs. mock. (C) Reverse transcription-quantitative polymerase chain reaction for NOVA1 mRNA in AGS cells transfected as indicated (n=3). (D) Western blotting for NOVA1 in AGS cells transfected as indicated (n=3). (E) Immunofluorescence analysis of NOVA1 protein in AGS cells transfected as indicated. Scale bar, 10 μm. n=3. miR, microRNA; mock, control miR; NOVA1, NOVA alternative splicing regulator 1; nt, nucleotide; 3'UTR, 3'-untranslated region.

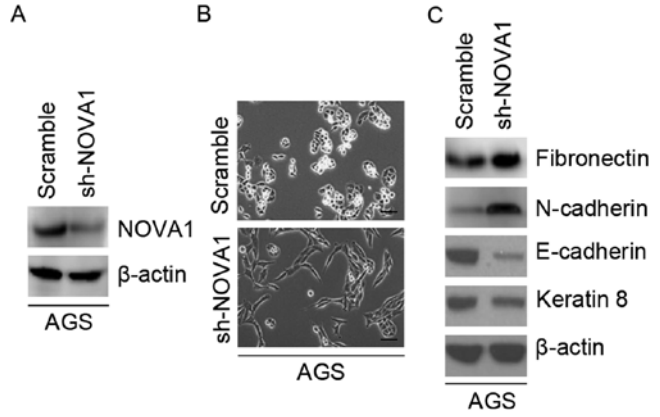

Figure 3. Silencing of NOVA1 promotes epithelial-mesenchymal transition in AGS cells. (A) Western blotting for the analysis of NOVA1 expression in AGS cells transfected as indicated (n=3). (B) AGS cells were transfected as indicated and then were analyzed via microscopy (n=3), Scale bars, 50 μm. (C) Western blotting for the analysis of fibronectin, N-cadherin, E-cadherin and keratin 8 expression in AGS cells transfected as indicated (n=3). shNOVA1, short hairpin RNA-NOVA alternative splicing regulator 1.

are presented in Fig. 2A. To examine whether miR-27a-3p regulates the 3'UTR of NOVA1, we performed a luciferase reporter assay. We observed that the luciferase activities of luciferase reporter plasmids were significantly inhibited by pre-miR-27a-3p compared with the control. To identify whether miR-27a-3p could regulate NOVA1 expression in AGS cells, we used RT-qPCR to determine NOVA1 mRNA expression. The results demonstrated that miR-27a-3p did not inhibit NOVA1 mRNA expression (Fig. 2C). Furthermore, we performed western blotting and an immunofluorescence assay to examine NOVA1 protein expression in AGS cells. We observed that NOVA1 protein expression was notably inhibited by pre-miR-27a-3p (Fig. 2D and E).

Silencing of NOVA1 promotes EMT in AGS cells. To study the role of NOVA1 in gastric cancer, we transfected AGS cells with shNOVA1- or scramble-expression plasmids. We observed that NOVA1 protein expression was inhibited by shNOVA1 (Fig. 3A). Silencing of NOVA1 promoted visible changes in the morphology of AGS cells (Fig. 3B). The phenotype was changed from a spindle-like to a cobblestone-like morphology. In order to confirm whether the morphological changes of AGS cells were promoted by EMT, we performed western blotting to examine the expression of epithelial and mesenchymal markers in AGS cells. We observed that E-cadherin and keratin 8 (epithelial markers) were downregulated, while that of N-cadherin and fibronectin (mesenchymal markers) were promoted by shNOVA1 (Fig. 3C).

Association between NOVA1 expression and the clinicopathological features of gastric cancer. We observed that the expression of NOVA1 was associated with the majority of the clinicopathological variables (age, sex, tumor size and histological classification); however, decreased NOVA1 expression was linked to lymph node metastasis and tumor stages (Table I).

Expression of NOVA1 protein and overall survival. Kaplan-Meier curves were generated to investigate overall survival; patients were stratified based on the expression of tumor NOVA1 protein. Using a log-rank test, we observed that the two overall survival curves were significantly different; survival among gastric cancer patients with low NOVA1 protein expression was significantly poorer than those with high NOVA1 protein expression (Fig. 4).

Table I. NOVA1 expression in relation to the clinicopathological characteristics of gastric cancer.

Clinicopathological features	n	NOVA1 expression		P-values[b]
		Positive	Negative (%)[a]	
Age (median)		65	64	>0.05
Sex				
M	75	21	54 (73)	>0.05
F	33	7	26 (80)	
Tumor size (cm)		7.1	7.6	>0.05
Histology				>0.05
Undifferentiated carcinoma	50	11	39 (77)	
Differentiated carcinoma	58	16	42 (73)	
Lymph node metastasis				<0.05
-	34	10	24 (72)	
+	74	18	56 (76)	
Stage				<0.05
1	21	6	15 (70)	
2	20	6	14 (69)	
3	29	8	21 (72)	
4	38	7	31 (81)	

The AJCC Cancer Staging system was used for tumor classification (18). [a]Values in parentheses represent percentages. [b]P-values were calculated using χ^2 tests for categorical variables and Student's t-test for continuous variables. NOVA1, NOVA alternative splicing regulator 1.

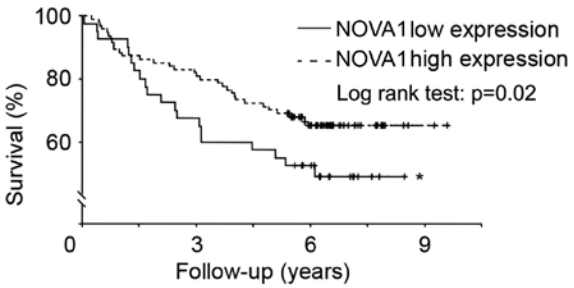

Figure 4. Association between the expression of NOVA1 and overall survival of patients with gastric cancer. The overall survival of gastric cancer patients was analyzed by the Kaplan-Meier method and compared with a log-rank test, according to NOVA1 expression. Each drop in the survival curve indicates the point of patient mortality death. Vertical lines indicated the time of censoring. NOVA1, NOVA alternative splicing regulator 1.

Discussion

Decreased NOVA1 expression has been observed in the tumor microenvironment, and correlates with progression and poor prognosis of gastric cancer (2,4). EMT has been proposed as one of main causes for cancer progression and poor prognosis (25,26). additionally, >4,000 genes are involved in EMT (27). In the present study, we showed that silencing of NOVA1 promoted EMT in gastric cancer cells. For example, silencing of NOVA1 promoted visible changes in the morphologies of gastric cancer cells; cellular morphologies were changed from a cobblestone-like phenotype to a spindle-like phenotype. Decreased E-cadherin and keratin 8 expression, or increased fibronectin and N-cadherin expression have been proposed as hallmarks of EMT (28,29). We observed that E-cadherin and keratin 8 expression were decreased, while that of fibronectin and N-cadherin were increased in response to sh-NOVA1. These results suggested that decreased NOVA1 expression in tumors may be associated with the progression and poor prognosis of patients with gastric cancer (2,4). Consistent with a recent report (4), we observed that NOVA1 expression was associated with lymph node metastasis and tumor stage in Chinese patients with gastric cancer.

Accumulating evidence indicates that miRNAs are involved in tumorigenesis and tumor progression of various cancers, via EMT and the formation of cancer-initiating cells (30). In esophageal squamous cell carcinoma, miR-27a-3p has been proposed as a tumor suppressive miRNA (31). However, an experimental model demonstrated that it could be an oncogene in osteosarcoma (32), laryngeal cancer (33), breast cancer (34), colorectal cancer (35) and gastric cancer (9,36,). Several genes (MAX-interacting protein 1, F-box and F-box WD-repeat containing protein 7) have been proposed as target genes of miR-27a-3p (37,38).

Bioinformatics analysis indicated that NOVA1 could be a potential target gene of miR-27a-3p, and three putative binding sites of miR-27a-3p were found in the 3'UTR of NOVA1 mRNA. We observed that overexpression of miR-27a-3p inhibited NOVA1 protein expression in AGS cells. The results suggested that NOVA1 was a target gene of miR-27a-3p. The levels of circulating miR-27a-3p are increased in patients with gastric cancer (11); miR-27a-3p expression was determined to be upregulated in gastric cancer tissues (9). Consistent with a recent report (9), we observed a statistically significant difference in the expression of miR-27a-3p between adjacent

normal tissues and gastric cancer tissues. In additionally, miR-27a-3p expression is correlated with distant metastasis, lymph node metastasis and advanced clinical stage (9). This is in line with our results, in which upregulated miR-27a-3p levels were associated with shorter survival. EMT participates in the tumorigenesis and is involved in the progression of cancer (39). Our results suggested that miR-27a-3p promoted EMT in gastric cancer cells. These findings indicate a possible molecular mechanism underlying the clinical observations of gastric cancer, in which miR-27a-3p may participate in the progression of gastric cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KL and XZ conceived the study, collected the experimental data and drafted the manuscript. XC and XW contributed to the experimental work and data analysis. All authors edited and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Renmin Hospital of Wuhan University and Cixi People's Hospital. All subjects gave explicit written informed consent at the time of enrollment.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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