lncRNA MIAT increases cell viability, migration, EMT and ECM production in age-related cataracts by regulating the miR-181a/CTGF/ERK signaling pathway

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Abstract. Age-related cataract (ARC) is a common cause of blindness in elderly individuals. Long non-coding RNA (lncRNA) myocardial infarction associated transcript (MIAT) has been reported to participate in various biological processes in a number of diseases; however, the biological mechanism underlying MIAT during ARC is not completely understood. The expression levels of MIAT, microRNA (miR)-181a and connective tissue growth factor (CTGF) were measured by reverse transcription-quantitative PCR. The protein expression levels of CTGF, α-smooth muscle actin, fibronectin, collagen type I, ERK, phosphorylated (p)-ERK, mitogen-activated protein kinase (MEK), and p-MEK were detected by western blotting. Cell viability and migration were assessed using MTT and Transwell assays, respectively. Moreover, a dual-luciferase reporter assay was performed to investigate the interaction between miR-181a and MIAT or CTGF. MIAT and CTGF were upregulated, while miR-181a was significantly downregulated in ARC tissues compared with normal tissues. MIAT or CTGF knockdown decreased cell viability, migration, epithelial-mesenchymal transition and extracellular matrix production in TGF-β2-treated SRA01/04 cells. It was hypothesized that miR-181a may be sponged by MIAT and may target CTGF. Furthermore, the miR-181a inhibitor reversed the inhibitory effect of MIAT knockdown on the progression of TGF-β2-treated SRA01/04 cells. Moreover, CTGF knockdown also reversed MIAT overexpression-mediated progression of TGF-β2-treated SRA01/04 cells. In addition, MIAT and CTGF regulated the activity of the ERK signaling pathway. The results suggested that MIAT may regulate the progression of ARC via the miR-181a/CTGF/ERK signaling pathway, which may serve as a novel therapeutic target for ARC.

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Introduction

Age-related cataract (ARC) causes ~50% of blindness worldwide (1). Posterior capsule opacification (PCO) is a common complication of cataract, which can result in secondary loss of vision (2). Increasing evidence has suggested that residual lens epithelial cells (LECs) can be transformed into myofibroblasts via epithelial-mesenchymal transition (EMT) and accumulation of extracellular matrix (ECM) components (3-5). Previous studies have also reported that transforming growth factor-β2 (TGF-β2), a TGF-β homology isomer, promoted EMT and ECM synthesis in LECs (6,7).

Long non-coding RNAs (lncRNAs) are a class of RNAs >200 nucleotides in length that lack translational capacity (8) and have been reported to participate in numerous different diseases, including PCO. For example, lncRNA HOX transcript antisense RNA upregulation mediated TGF-β2-induced EMT in SRA01/04 cell lines (9). lncRNA KCNQ1OT1, taurine upregulated 1 and FEX family zinc finger 1-antisense RNA have also been reported to facilitated LEC progression (10-12). Moreover, certain lncRNAs have been reported to be associated with oxidative stress during cataract (13-15). LncRNA myocardial infarction associated transcript (MIAT) aberrant expression has been identified in a number of diseases, including coronary artery disease (16,17), ischemic stroke (18) and ARC (19). However, the biological mechanism underlying MIAT during ARC is not completely understood.

MicroRNAs (miRNAs), a class of short non-coding RNAs ~22 nucleotides in length, affect gene expression by inhibiting mRNA translation or mediating mRNA degradation (20). miRNA dysregulation has been identified in a number of diseases, including ARC. For example, miR-221 accelerated LEC apoptosis by regulating sirtuin 1 and E2F transcription factor 3 expression (21). Furthermore, miR-181a has been reported to be involved in ARC development (22). Connective tissue growth factor (CTGF), a downstream effector of TGF-β2, has been reported to be associated with several cellular functions, Including proliferation, migration and adhesion in LECs (7,23,24). However, the regulatory mechanisms underlying miR-181a and CTGF during ARC have not been investigated.

The aim of the present study was to investigate the role and mechanism underlying MIAT during ARC development. The results of the present study may provide a theoretical basis for further investigation of ARC.

Materials and methods

Tissue samples. A total of 20 ARC posterior capsular tissue samples (cataract) were collected from 12 female patients and 8 male patients (age, 58-75 years; mean age, 65 years) recruited from the Department of Ophthalmology, Renmin Hospital between January 2017 and July 2018. A further 20 normal posterior capsule tissue samples were obtained from 10 female patients and 10 male patients from the Department of Ophthalmology; Renmin Hospital (age, 49-71 years; mean age, 57.7 years) who had been in an accident but did not exhibit eye damage between March 2017 and November 2018. All tissues were frozen at -80˚C until further analysis. All participants or their guardians provided written informed consent. The present study was approved by the Ethics Committee of the Department of Ophthalmology, Renmin Hospital, Hubei University of Medicine.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from ARC tissue samples and SRA01/04 cells using TRIzol® reagent (Thermo Fisher Scientific, Inc.). Subsequently, reverse transcription was performed using the miScript RT kit (Takara Biotechnology Co., Ltd.) at 37˚C for 60 min, according to the manufacturer's protocol. qPCR was performed using the ABI Prism 7700 Sequence Detection system (Thermo Fisher Scientific, Inc.) and TaqMan miRNA assay (for miRNA; Applied Biosystems; Thermo Fisher Scientific, Inc.) and SYBR Premix Ex Taq II (for lncRNA and mRNA; Takara) were used, according to their manufacturer's protocols. The thermocycling conditions for qPCR were initial denaturation at 95˚C for 10 min followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. The following primer pairs were synthesized by Sangon Biotech Co., Ltd. and used for qPCR: MIAT forward, 5'-GGACGT TCACAACCACACTG-3' and reverse, 5'-TCCCACTTTGGC ATTCTAGG-3'; miR-181a forward, 5'-GCGGTAACATTC AACGCTGTCG-3' and reverse, 5'-GTGCAGGGTCCGAGG T-3'; CTGF forward, 5'-GGAAATGCTGTGAGGAGTGGG TGT-3' and reverse, 5'-TGTCTTCCAGTCGGTAGGCAG CTA-3'; GAPDH forward, 5'-TGTTCGTCATGGGTGTGA AC-3' and reverse, 5'-ATGGCATGGACTGTGGTCAT-3'; and U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. mRNA and miRNA levels were quantified using the $2^{-\Delta\Delta Cq}$ method (25). mRNA levels of MIAT and CTGF were normalized to the internal reference gene GAPDH. miR-181a levels were normalized to the internal reference gene U6.

Western blot assay. Total protein was extracted from ARC tissues or TGF-β2-treated SRA01/04 cells by RIPA buffer (Thermo Fisher Scientific, Inc.). Protein concentration was detected using BCA Protein Assay Kit (Beyotime Institute of Biotechnology). Subsequently, total protein was boiled with loading buffer (Beyotime Institute of Biotechnology) for 10 min at 95˚C. Proteins (30 μg/lane) were separated by 10% SDS-PAGE and transferred onto PVDF membranes (EMD Millipore). Subsequently, the membranes were blocked with skim milk for 4 h at 37˚C and incubated overnight at 4˚C with primary antibodies targeted against: CTGF (1:1,000; ab6992), α-smooth muscle actin (α-SMA; 1:5,000; ab32575), fibronectin (FN; 1:5,000; ab2413), collagen I (COL-1; 1:1,000; ab34710), extracellular signal-regulated kinase (ERK; 1:1,000; ab17942), phosphorylated-ERK (p-ERK; 1:500; ab214362), mitogen-activated protein kinase kinase (MEK; 1:10,000; ab32091), p-MEK (1:1,000; ab96379) and GAPDH (1:5,000; ab9485). Following primary incubation, the membranes were incubated with a horseradish peroxidase-conjugated anti-rabbit lgG secondary antibody (1:10,000; ab205718) for 3 h at 37˚C. All antibodies were purchased from Abcam. Protein bands were visualized using the RapidStep ECL reagent (EMD Millipore) and analyzed by Imagequant software (version 5.1; Amersham-Pharmacia's Biotech). GAPDH was used as the loading control.

Cell culture and transfection. The human LEC line SRA01/04 was purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Cells were cultured in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% FBS (Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.). TGF-β2 (Sigma-Aldrich; Merck KGaA) was dissolved in PBS to make a 5 ng/ml stock solution for subsequent experiments.

Small interfering (si)RNAs targeting MIAT (si-MIAT, 5'-CCUUACCAUUCCUCCACUUTT-3') or CTGF (si-CTGF, 5'-GCUGACCUGGAAGAGAACATT-3'), a negative control (NC) siRNA (si-NC, 5'-UUCUCCGAACGUGUCA-3'), MIAT overexpression vector (MIAT), CTGF overexpression vector (CTGF), an NC vector (pcDNA), miR-181a mimic (miR-181a, 5'-AACAUUCAACGCUGUCGGUGAGU-3'), an NC mimic (miR-NC, 5'-UUCUCCGAACGUGUCACGUTT-3'), miR-181a inhibitor (anti-miR-181a, 5'-ACUCACCGACAGCGUUGA AUGUU-3') and a NC inhibitor (anti-miR-NC, 5'-CAGUAC UUUUGUGUAGUACAA-3') were obtained from Shanghai GenePharma Co., Ltd. SRA01/04 cells were seeded into 6-well plates, si-RNAs (50 nM), miRNA mimics (50 nM), miRNA inhibitors (100 nM) and plasmids (100 nM) were transfected into SRA01/04 cells when the cell density reached 70% using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. At 24 h post-transfection, cells were used for subsequent experiments.

Cell viability assay. An MTT assay (Sigma-Aldrich; Merck KGaA) was used to detect SRA01/04 cell viability, according to the manufacturer's protocol. SRA01/04 cells ($6x10^3$ cells/well) were seeded into 96-well plates. Following transfection for 24 h, cells were incubated with 5 ng/ml TGF-β2 for another 48 h. Subsequently, MTT was added to each well and incubated for 4 h at 37˚C. DMSO was used to dissolve the purple formazan crystals for 15 min in the dark at 37˚C. Cell viability was measured at a wavelength of 570 nm using an Evolution™ 350 UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc.).

Transwell assay. The Transwell assay was conducted using Transwell plates (Corning Life Sciences). Following a 24 h

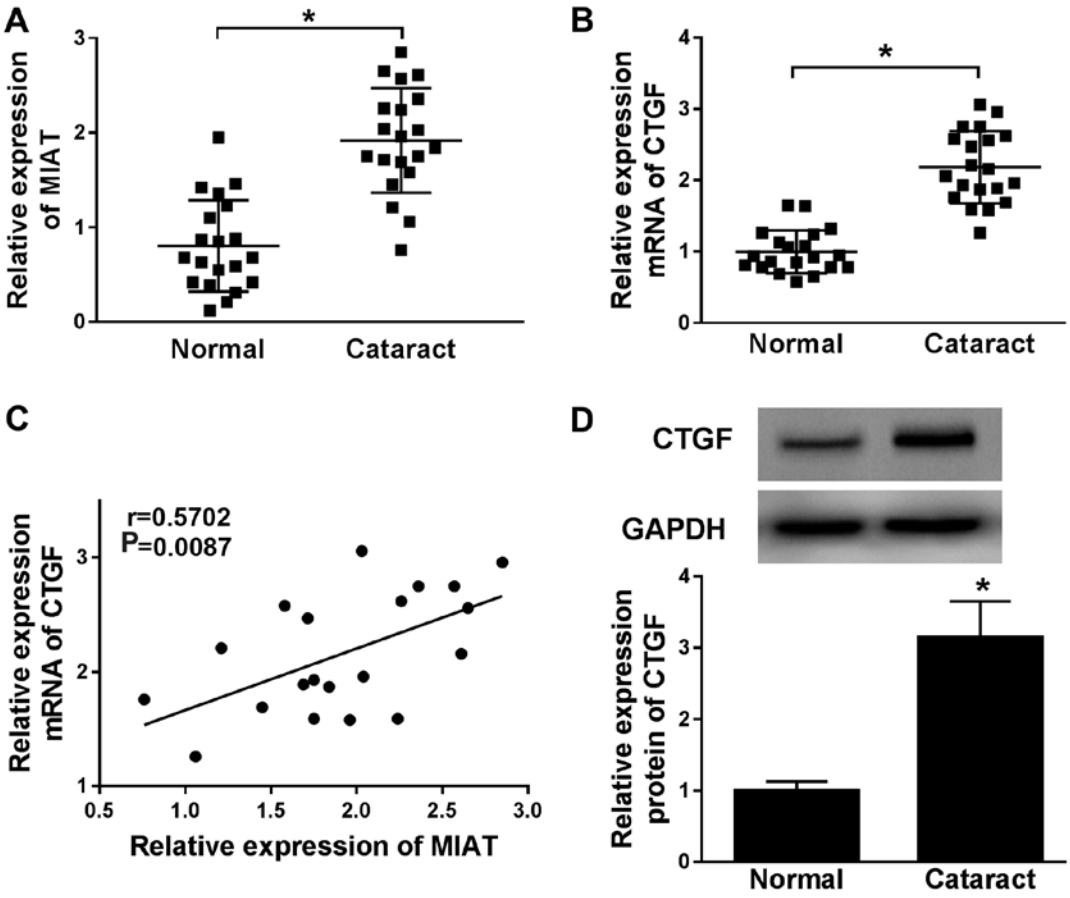

Figure 1. MIAT and CTGF are upregulated in ARC tissues. The expression levels of (A) MIAT and (B) CTGF in ARC tissue samples (cataract) and normal posterior capsule tissue samples (normal) were detected by reverse transcription-quantitative PCR. (C) The correlation between CTGF and MIAT expression was analyzed by Pearson's correlation coefficient. (D) The protein expression level of CTGF in ARC tissue samples was measured by western blotting. *P<0.05 vs. the normal group. MIAT, myocardial infarction associated transcript; CTGF, connective tissue growth factor; ARC, age-related cataract.

culture and treatment with TGF-β2 for another 48 h at 37˚C, SRA01/04cells ($5x10^5$ cells/ml) were seeded into the upper chambers of the Transwell plates with serum-free DMEM. DMEM containing 10% FBS was plated in the lower chambers of the Transwell plates. Following incubation for 24 h, cells on the lower surface of the Transwell membrane were fixed with 4% methanol at room temperature for 30 min and stained with 0.1% crystal violet at room temperature for 20 min. Migratory cells were counted in 10 random fields of view using a light microscope a magnification of x100 (Olympus Corporation).

Dual-luciferase reporter assay. The interaction between miR-181a and MIAT or CTGF was predicted using the StarBase online database (version 2; starbase.sysu.edu.cn). The wild-type (WT) and mutant (MUT) sequences of MIAT and 3'-untranslated regions (3'-UTRs) of CTGF were amplified and inserted into the pGL3 vector (Promega Corporation), WT-MIAT, MUT-MIAT, CTGF 3'UTR-WT and CTGF 3'UTR-MUT, respectively. The pGL3 vector and miR-181a mimic or miR-NC were co-transfected into SRA01/04 cells using Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Luciferase activity was detected, according to the manufacturer's protocol, using the Dual-Luciferase Reporter assay kit (Promega Corporation) 48 h post-transfection. Firefly luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. Statistical analyses were performed using GraphPad Prism software (version 7; GraphPad Software, Inc.). Data from three repeated independent experiments are presented as the mean ± standard deviation. Comparisons between two groups or among multiple groups were assessed using the Student's t-test or one-way ANOVA with Tukey's post hoc test, respectively. The correlation between CTGF or miR-181a and MIAT was analyzed by Pearson's correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

MIAT and CTGF are upregulated in ARC tissues. To investigate the roles of MIAT and CTGF during ARC, the levels of MIAT and CTGF in ARC tissues were detected. The levels of MIAT and CTGF were upregulated in ARC tissue samples compared with the normal posterior capsule tissue samples (Fig. 1A and B). The correlation analysis indicated that the level of CTGF mRNA expression was positively correlated with the level of MIAT mRNA expression in ARC tissues (Fig. 1C). In addition, the western blotting results also indicated

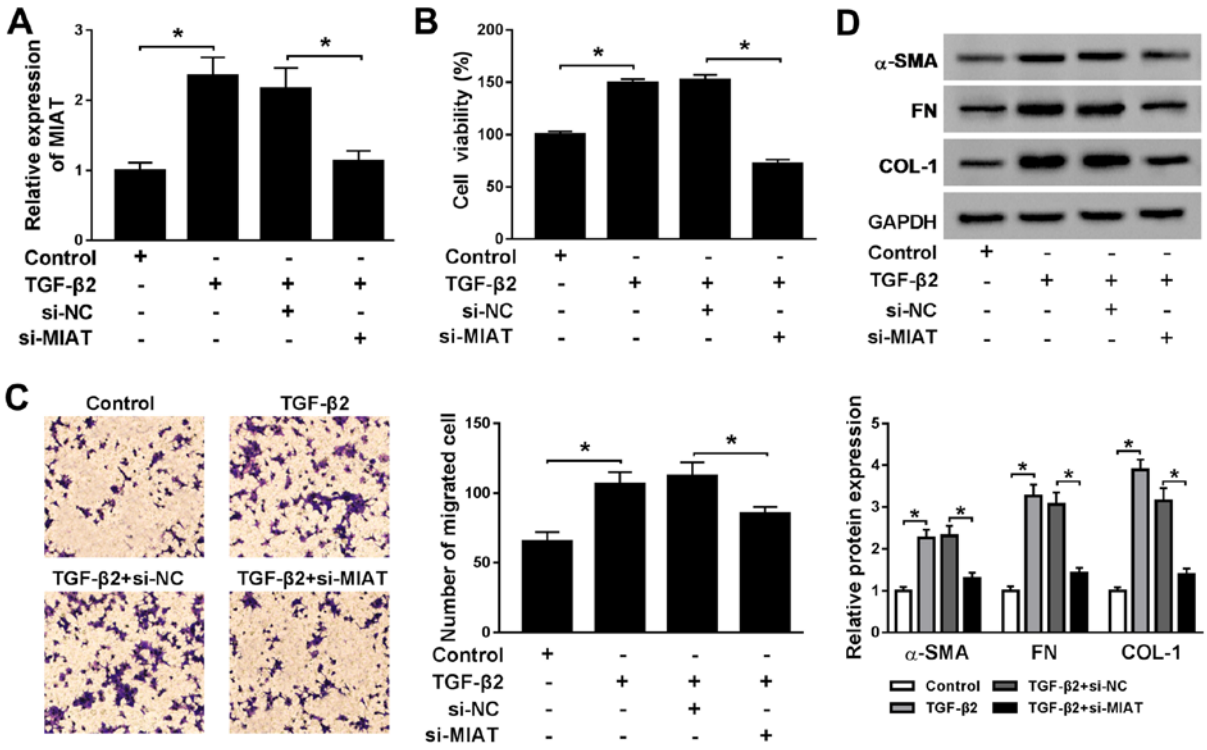

Figure 2. MIAT knockdown reverses TGF-β2-induced upregulation of SRA01/04 cell viability, migration, EMT and ECM production. SRA01/04 cells were treated with TGF-β2 and subsequently transfected with si-NC or si-MIAT. (A) The expression levels of MIAT in SRA01/04 cells were measured by reverse transcription-quantitative PCR. (B) SRA01/04 cell viability was assessed by the MTT assay. (C) The number of migratory SRA01/04 cells was examined using the Transwell assay. (D) The protein expression levels of α-SMA, FN and COL-1 in SRA01/04 cells were detected by western blotting. *P<0.05, as indicated. MIAT, myocardial infarction associated transcript; TGF-β2, transforming growth factor-β2; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix; si, small interfering RNA; NC, negative control; α-SMA, α-smooth muscle actin; FN, fibronectin; COL-1, collagen 1.

that the protein level of CTGF was upregulated in ARC tissues compared with the normal tissues (Fig. 1D). Therefore, the data suggested that MIAT and CTGF might play an important role during ARC, and a relationship between the two factors may be present.

MIAT knockdown reverses TGF-β2-induced effects on cell viability, migration, EMT and ECM productioninSRA01/04 cells. To further investigate the effects of MIAT in ARC, MIAT expression was knocked down in SRA01/04 cells using si-MIAT and the efficiency of MIAT knockdown was verified using RT-qPCR (Fig. S1A). The RT-qPCR results indicated that TGF-β2 elevated the expression levels of MIAT in SRA01/04 cells compared with the control group and this effect was reversed by MIAT knockdown (Fig. 2A). Furthermore, TGF-β2 increasedSRA01/04 cell viability and migration compared with the control group, whereas si-MIAT reversed the TGF-β2-induced effects (Fig. 2B and C). α-SMA is a marker of EMT (26), and FN and COL-1 are ECM markers (27). Therefore, the effect of MIAT knockdown on the protein expression levels of α-SMA, FN and COL-1 was investigated. TGF-β2 significantly upregulated the protein expression levels of α-SMA, FN and COL-1 in SRA01/04 cells compared with the control group, while si-MIAT decreased TGF-β2-induced protein expression (Fig. 2D). Additionally, MIAT overexpression increased SRA01/04 cell viability, migration, EMT and ECM production compared with the control group (Fig. S1B and S2A-C). The results suggested that MIAT expression was critical for the development of ARC.

MIAT promotes cell viability, migration, EMT and ECM production in TGF-β2-treated SRA01/04 cells by sponging miR-181a. To further explore the biological mechanism underlying MIAT during ARC, the putative target of MIAT was predicted using the StarBase online database. miR-181a displayed a complementary base pairing with MIAT (Fig. 3A). By investigating miR-181a expression, it was indicated that miR-181a was downregulated in ARC tissue samples compared with normal tissue samples (Fig. 3B). Furthermore, correlation analysis indicated that the level of miR-181a expression was negatively correlated with MIAT expression in ARC tissues (Fig. 3C). The dual-luciferase reporter assay results indicated that the luciferase activity of the WT-MIAT reporter was suppressed by miR-181a over-expression and enhanced by miR-181a inhibition compared with the control group; however, the luciferase activity of the MUT-MIAT reporter was not significantly altered by either transfection (Fig. 3D). The efficiency of miR-181a overexpression and miR-181a inhibition experiments are presented in Fig. S1C and D. Moreover, miR-181a expression was upregulated by MIAT knockdown and downregulated by MIAT overexpression, compared with the control group (Fig. 3E). To explore the role of miR-181a during ARC, si-MIAT1 and anti-miR-181a were co-transfected into TGF-β2-treated SRA01/04 cells. The results of the MTT, Transwell and western blot assays suggested that miR-181a inhibitor reversed the inhibitory effects of MIAT1 knock-down on TGF-β2-treated SRA01/04 cell viability, migration, and the protein expression levels of α-SMA, FN and COL-1

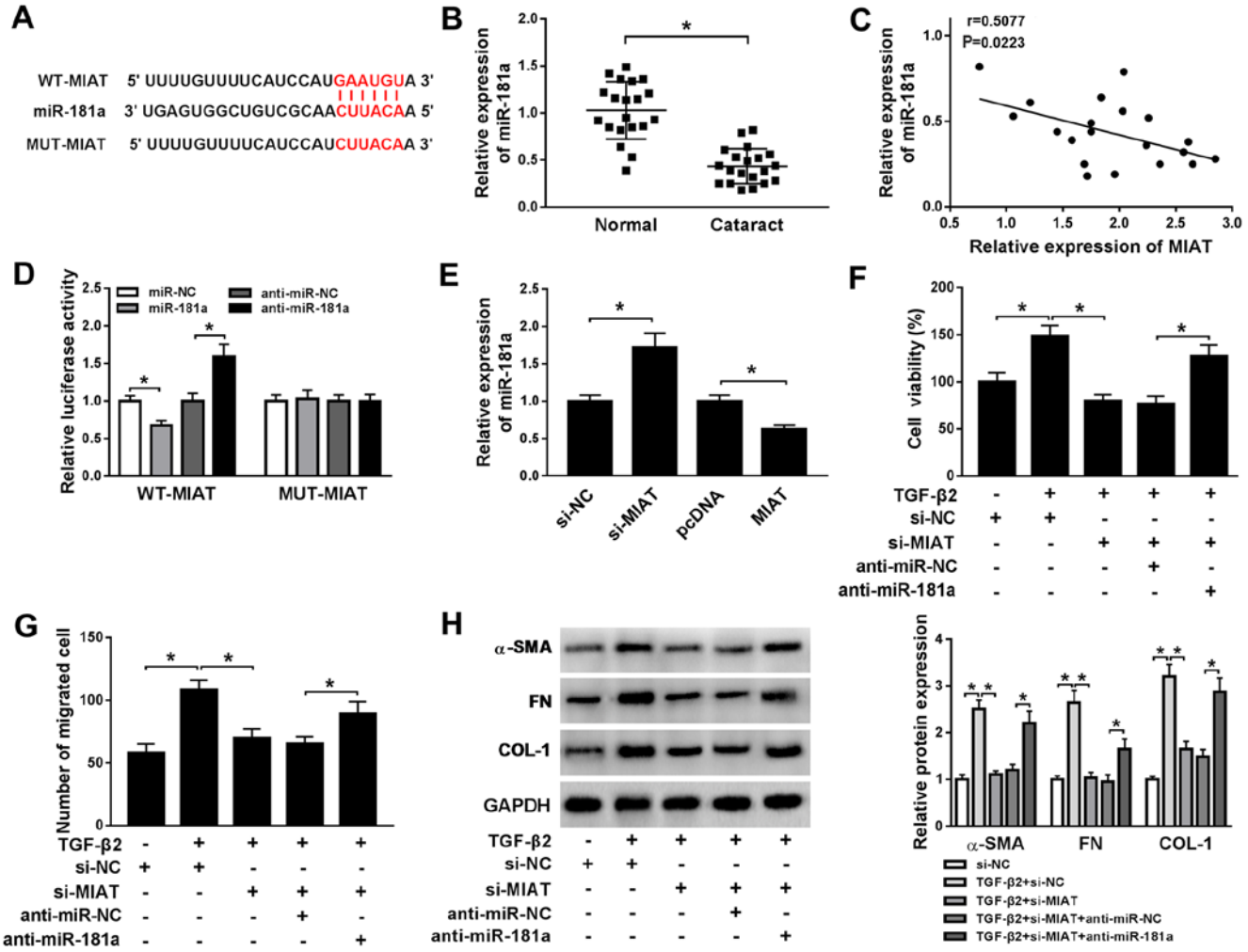

Figure 3. MIAT promotes cell viability, migration, EMT and ECM production in TGF-β2-treated SRA01/04 cells by sponging miR-181a. (A) The putative complementary binding sequences or mutant sequences between MIAT and miR-181a. (B) The level of miR-181a expression in ARC tissue samples (cataract) and normal posterior capsule tissue samples (normal) was measured by RT-qPCR. (C) The correlation between miR-181a and MIAT expression was assessed by Pearson's correlation coefficient. (D) The luciferase activities of the WT-MIAT and MUT-MIAT reporters were detected using the dual-luciferase reporter assay. (E) The level of miR-181a expression in TGF-β2-treated SRA01/04 cells transfected with si-MIAT, MIAT overexpression plasmid or the corresponding matched controls was measured by RT-qPCR. SRA01/04 cells were transfected with si-NC, si-MIAT, si-MIAT + anti-miR-NC or si-MIAT + anti-miR-181a and incubated with TGF-β2 for 48 h. (F) SRA01/04 cell viability was assessed by the MTT assay. (G) The number of migratory SRA01/04 cells was analyzed using the Transwell assay. (H) The expression levels of α-SMA, FN and COL-1 in SRA01/04 cells were detected by western blotting. *P<0.05, as indicated. MIAT, myocardial infarction associated transcript; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix; miR, microRNA; ARC, age-related cataract; RT-qPCR, reverse transcription-quantitative PCR; WT, wild-type; MUT, mutant; TGF-β2, transforming growth factor-β2; si, small interfering RNA; NC, negative control; α-SMA, α-smooth muscle actin; FN, fibronectin; COL-1, collagen 1.

(Fig. 3F-H). Therefore, the results suggested that miR-181a was sponged by MIAT and participated in the regulation of MIAT during ARC progression.

CTGF knockdown diminishes TGF-β2-induced cell viability, migration, EMT and ECM production in SRA01/04 cells. The expression of CTGF in TGF-β2-treated SRA01/04 cells was also investigated. CTGF expression was significantly increased in TGF-β2-treated SRA01/04 cells compared with the control group, which further indicated that the cell model of ARC was successfully established (Fig. 4A and B). The RT-qPCR and western blotting results demonstrated that si-CTGF successfully knocked down CTGF expression in SRA01/04 cells compared with the control group (Fig. 4C and D). To assess the effects of CTGF on ARC development, si-CTGF was transfected into TGF-β2-treated SRA01/04 cells. CTGF knockdown reversed TGF-β2-induced promotion of SRA01/04 cell viability, migration, EMT and ECM production (Fig. 4E-G). The results indicated that CTGF may have an active role during ARC development.

CTGF is targeted by miR-181a. The StarBase online database indicated that the 3'-UTR of CTGF had complementary binding sites with miR-181a (Fig. 5A). Subsequently, the dual-luciferase reporter assay demonstrated that the luciferase activity of the CTGF 3'-UTR-WT reporter was significantly decreased by miR-181a mimic compared with the control group, while the luciferase activity of the CTGF 3'UTR-MUT reporter was not altered by miR-181a overexpression (Fig. 5B). Furthermore, anti-miR-181a resulted in the upregulation of luciferase activity of the CTGF 3'UTR-WT reporter compared with the control group, but the luciferase activity of the CTGF 3'UTR-MUT

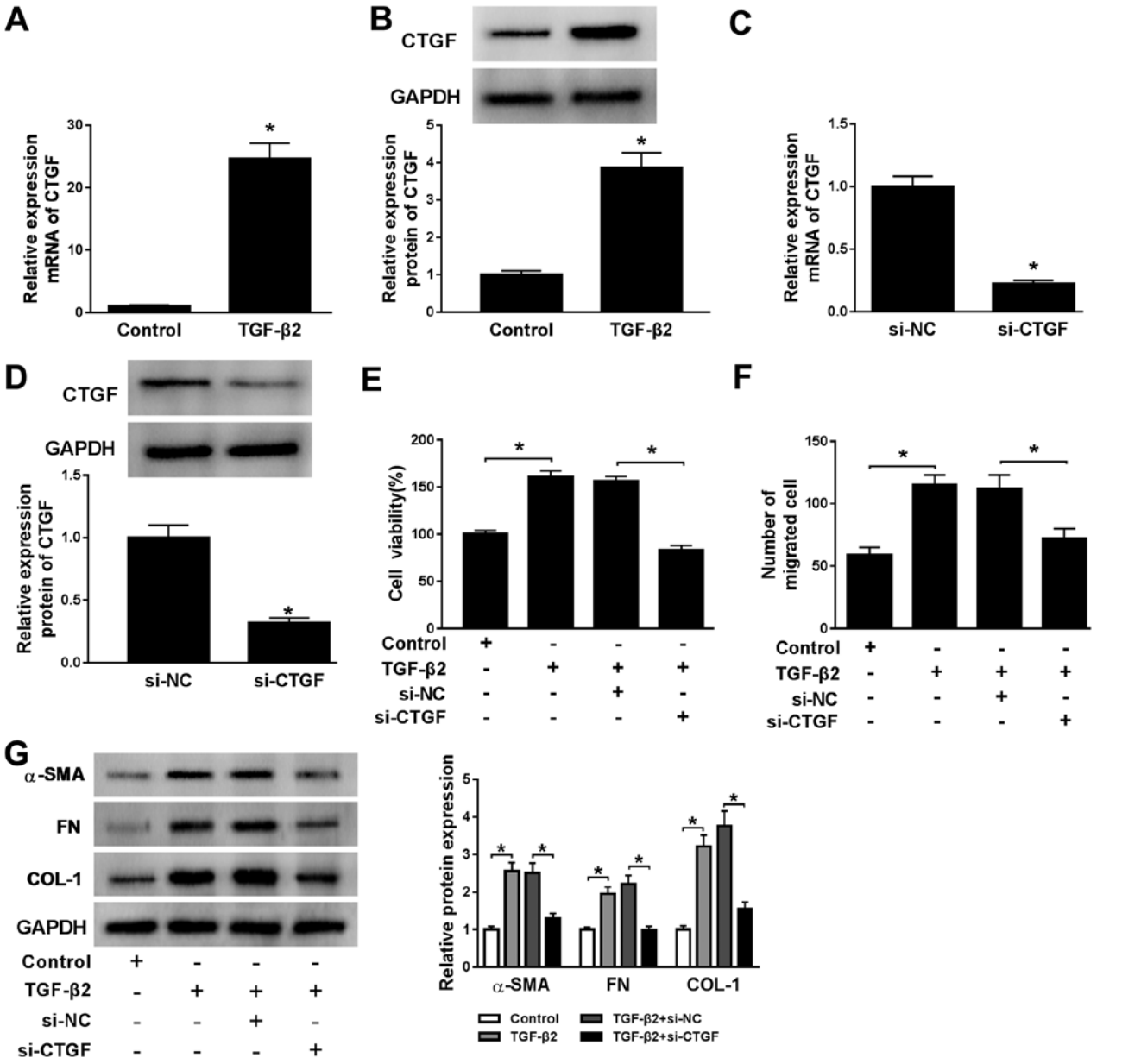

Figure 4. CTGF knockdown reverses TGF-β2-induced cell viability, migration, epithelial-mesenchymal transition and extracellular matrix in SRA01/04 cells. mRNA and protein expression levels of CTGF in TGF-β2-treated SRA01/04 cells were measured by (A) RT-qPCR and (B) western blotting, respectively. mRNA and protein expression levels of CTGF in SRA01/04 cells transfected with si-CTGF or si-NC were measured by (C) RT-qPCR and (D) western blotting, respectively. SRA01/04 cells were incubated with TGF-β2 and transfected with si-NC or si-CTGF. (E) SRA01/04 cell viability was examined using the MTT assay. (F) The number of migratory SRA01/04 cells was assessed by the Transwell assay. (G) The protein expression levels of α-SMA, FN and COL-1 in SRA01/04 cells were detected by western blotting. *P<0.05 vs. the control group. CTGF, connective tissue growth factor; TGF-β2, transforming growth factor-β2; RT-qPCR, reverse transcription-quantitative PCR; si, small interfering RNA; NC, negative control; α-SMA, α-smooth muscle actin; FN, fibronectin; COL-1, collagen 1.

reporter also displayed no alteration following transfection with anti-miR-181a (Fig. 5C). To further explore the effect of miR-181a expression on CTGF expression, miR-181a mimic or miR-181a inhibitor were transfected into TGF-β2-treated SRA01/04 cells. CTGF expression was decreased by miR-181a overexpression and increased by miR-181a inhibition, compared with the control group (Fig. 5D and E). The results indicated that miR-181a may target CTGF in ARC.

MIAT increases CTGF expression to promote cell viability, migration, EMT and ECM production in TGF-β2-treated SRA01/04 cells via miR-181a. To explore the biological mechanism linking MIAT, miR-181a and CTGF, TGF-β2-treated SRA01/04 cells were co-transfected with miR-181a mimic and MIAT overexpression plasmid to evaluate the effect of MIAT expression on CTGF expression. The mRNA and protein expression levels of CTGF were increased by MIAT overexpression in TGF-β2-treated SRA01/04 cells compared with the control group. miR-181a overexpression reversed the enhancing effects of MIAT overexpression, indicating that CTGF expression was regulated by MIAT and miR-181a. To further verify the hypothesis, MIAT overexpression plasmid and si-CTGF were co-transfected into TGF-β2-treated SRA01/04 cells to detect cell viability, migration, EMT and ECM production. CTGF

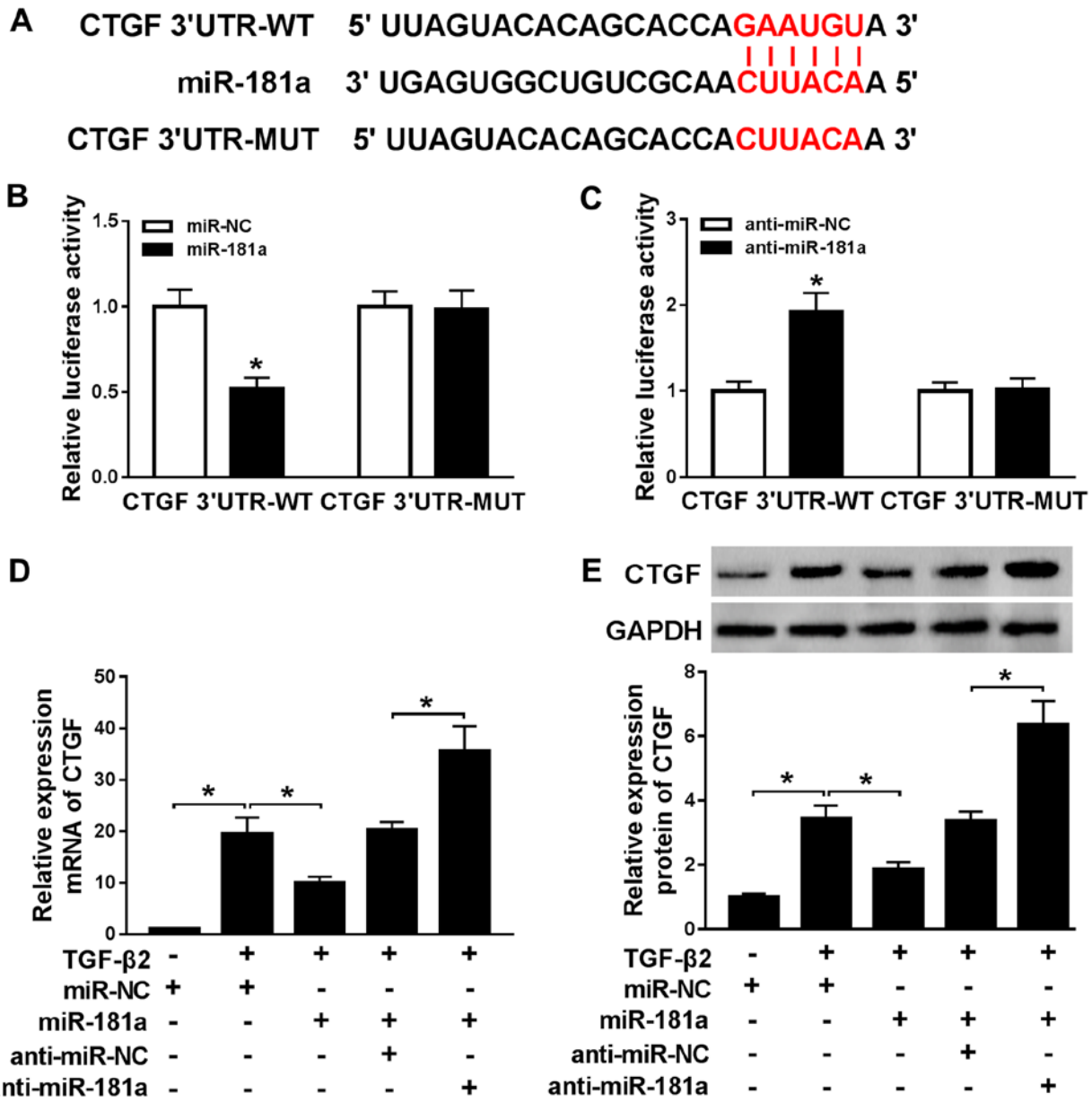

Figure 5. CTGF is targeted by miR-181a. (A) The putative complementary or mutant sequences between miR-181a and CTGF were identified. The luciferase activities of the CTGF 3'UTR-WT and CTGF 3'UTR-MUT reporters following transfection with (B) miR-NC or miR-181a and (C) anti-miR-NC or anti-miR-181a were estimated using the dual-luciferase reporter assay. mRNA and protein expression levels of CTGF in TGF-β2-treated SRA01/04 cells transfected with miR-181a, anti-miR-181a, miR-NC or anti-miR-NC were measured by (D) reverse transcription-quantitative PCR and (E) western blotting, respectively. *P<0.05, vs. the control group. CTGF, connective tissue growth factor; miR, microRNA; UTR, untranslated region; WT, wild-type; MUT, mutant; NC, negative control.

knockdown reversed the effects of MIAT overexpression on TGF-β2-treated SRA01/04 cell viability, migration and protein expression levels of α-SMA, FN and COL-1 (Fig. 6C-E). The results indicated that MIAT modulated CTGF to regulate the progression of ARC by sponging miR-181a.

MIAT and CTGF regulate the activity of the ERK signaling pathway. ERK signaling is related to the development of a number of diseases (24); therefore, si-MIAT and CTGF overexpression plasmid were co-transfected into TGF-β2-treated SRA01/04 cells to detect the expression levels of ERK signaling pathway-related proteins. The efficiency of CTGF overexpression transfection is presented in Fig. S1C and D. si-MIAT successfully decreased the expression of CTGF and CTGF overexpression plasmid successfully reversed si-MIAT-mediated downregulation of CTGF expression compared with the control group (Fig. 7A and B). TGF-β2 increased the protein expression levels of p-MEK and p-ERK compared with the control group, while MIAT knockdown reversed TGF-β2-induced effects on protein expression. Additionally, CTGF overexpression reversed the effects of MIAT knockdown on the protein expression levels of p-MEK and p-ERK (Fig. 7A, C and D). The results indicated that the ERK signaling pathway was involved in MIAT-mediated regulation of ARC.

Discussion

ARC can lead to blindness in elderly individuals (28). Increasing evidence suggests that lncRNAs affect a number of biological

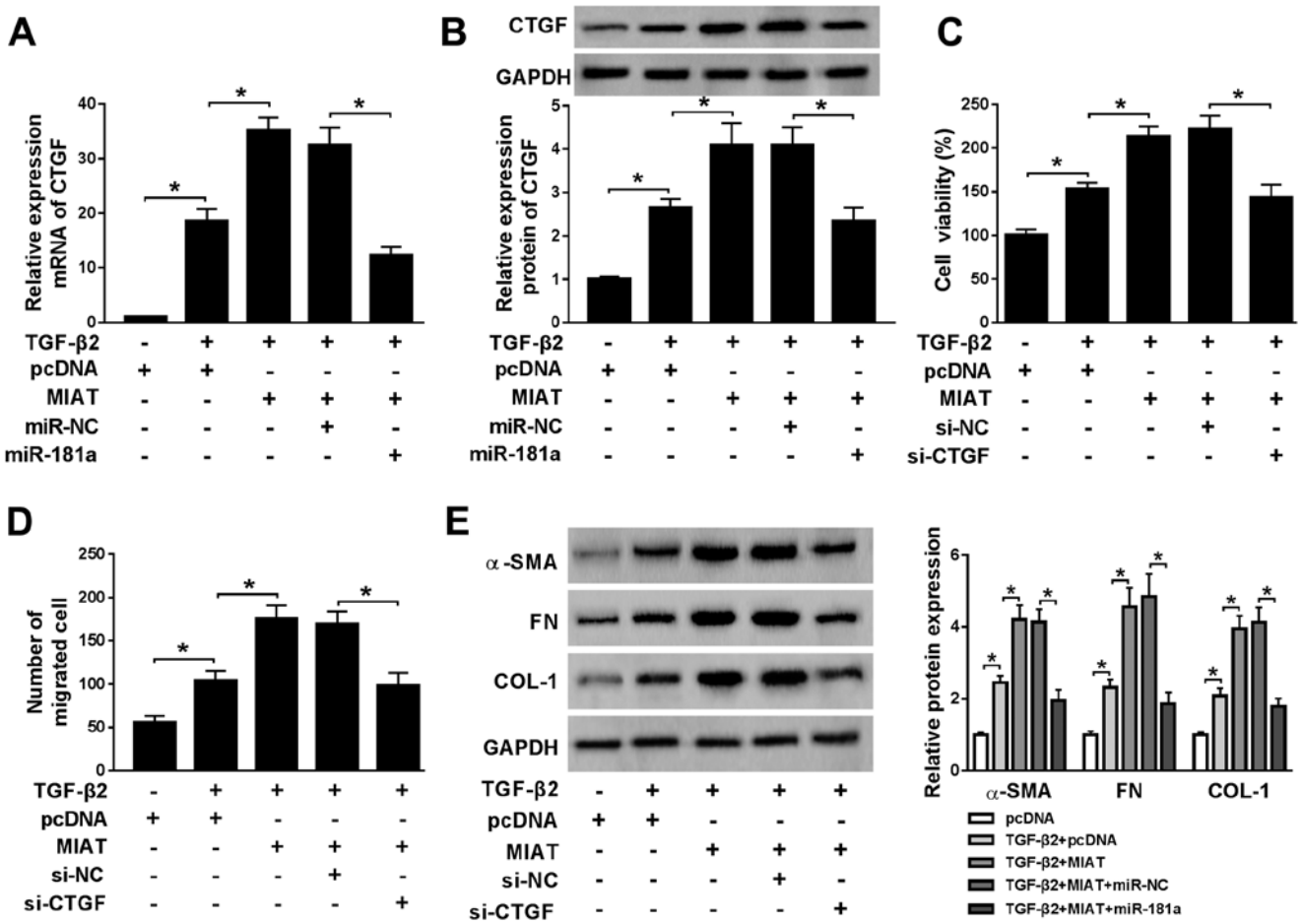

Figure 6. MIAT increases CTGF expression to promote cell viability, migration, epithelial-mesenchymal transition and extracellular matrix in TGF-β2-treated SRA01/04 cells via miR-181a. SRA01/04 cells were treated with TGF-β2 and subsequently transfected with pcDNA, MIAT, MIAT + miR-NCor MIAT + miR-181a. mRNA and protein expression levels of CTGF in SRA01/04 cells were measured by (A) reverse transcription-quantitative PCR and (B) western blotting, respectively. SRA01/04 cells were treated with TGF-β2 and subsequently transfected with pcDNA, MIAT, MIAT + si-NC or MIAT + si-CTGF. (C) SRA01/04 cell viability was assessed using the MTT assay. (D) The number of migratory SRA01/04 cells was examined by the Transwell assay. (E) The protein expression levels of α-SMA, FN and COL-1 in SRA01/04 cells were assessed by western blotting. *P<0.05, as indicated. MIAT, myocardial infarction associated transcript; CTGF, connective tissue growth factor; TGF-β2, transforming growth factor-β2; miR, microRNA; si, small interfering RNA; NC, negative control; α-SMA, α-smooth muscle actin; FN, fibronectin; COL-1, collagen 1.

processes in numerous different diseases, including ARC (9-12). The present study aimed to explore the biological mechanism underlying MIAT during ARC development. Collectively, the results indicated that MIAT regulated CTGF expression to facilitate cell viability, migration, EMT and ECM production during ARC via the ERK signaling pathway by sponging miR-181a.

MIAT dysregulation has been identified in a number of different diseases. For example, previous studies investigating coronary artery disease indicated that MIAT expression was significantly elevated in the serum of patients with coronary artery disease (16,17). Furthermore, MIAT upregulation has been reported in ischemic stroke (18). In the present study, MIAT was upregulated in ARC tissues and TGF-β2-treated SRA01/04 cells compared with the corresponding control groups. MIAT knockdown reversed TGF-β2-induced cell viability, migration, EMT and ECM production in SRA01/04 cells. The aforementioned results were consistent with a previous report (19), indicating that MIAT may play an active role during the development of ARC.

Accumulating evidence has suggested that miRNAs are associated with the development of a number of diseases (20,29). For example, MIAT knockdown inhibited the viability, migration and invasion of pancreatic carcinoma cells by targeting miR-133 (30). Zhang *et al* (31) reported that MIAT promoted cell viability and migration, and inhibited cell apoptosis in osteosarcoma via sponging miR-128-3p *in vitro*. The present study further indicated that miR-181a was downregulated in ARC tissues compared with normal tissues. Similar results were also reported in a previous study (22). Emerging evidence indicated that MIAT acted as a molecular sponge to regulate miRNA expression, resulting in altered target gene mRNA expression (32). In the present study, the results indicated that MIAT sponged miR-181a. Furthermore, miR-181a inhibitor reversed the inhibitory effects of MIAT knockdown on cell viability, migration, EMT and ECM in TGF-β2-treated SRA01/04 cells. Overall, the results suggested that MIAT facilitated ARC progression by sponging miR-181a.

Previous studies revealed that CTGF is involved in a number of biological processes, including ECM production, cell proliferation, adhesion, migration, fibrosis and differentiation (33-35). Wang *et al* (36) reported that CTGF was increased *in vivo* and *in vitro* during hypertension, and its overexpres-

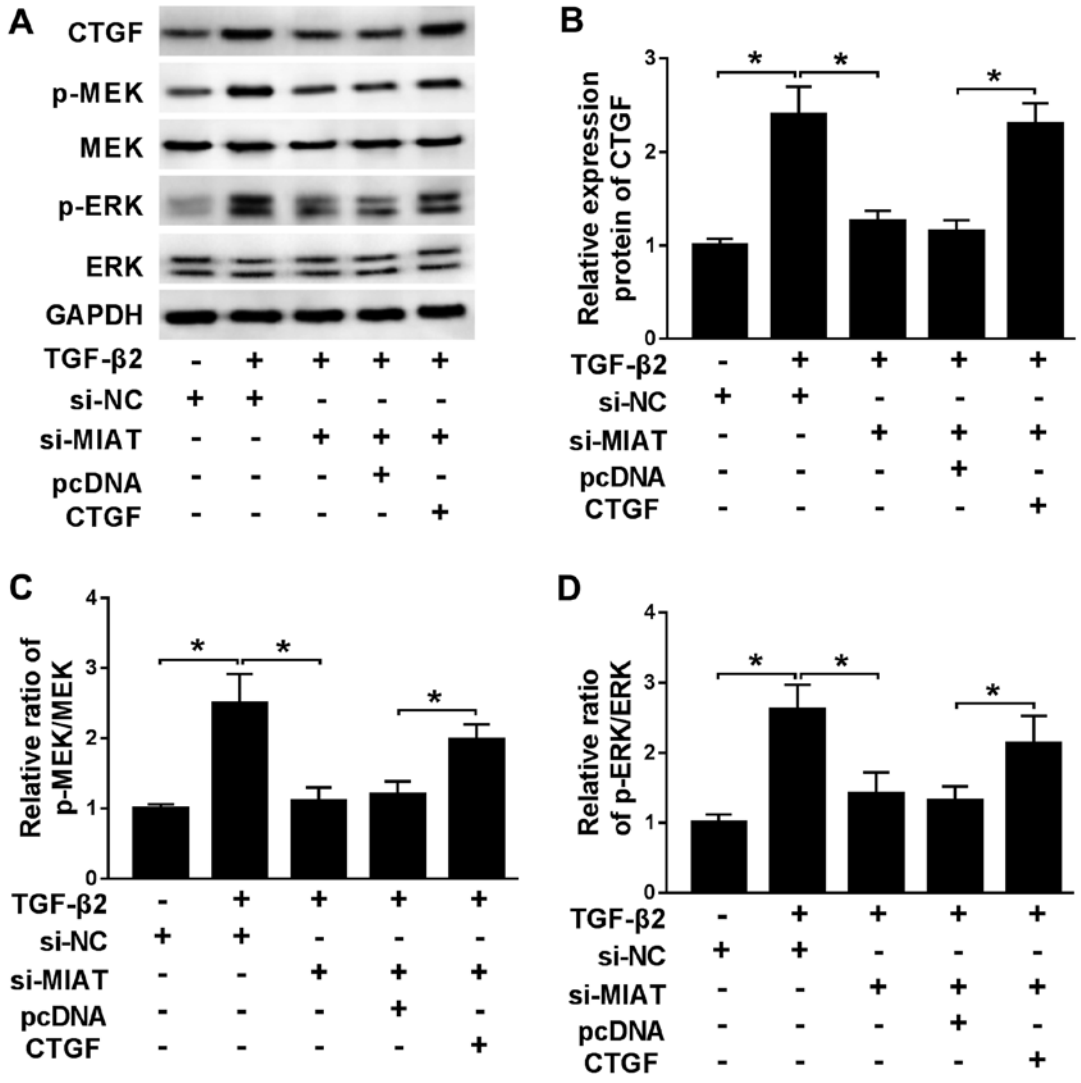

Figure 7. MIAT and CTGF regulate the activity of the ERK signaling pathway. TGF-β2-treated SRA01/04 cells were transfected with si-NC, si-MIAT, si-MIAT + pcDNA or si-MIAT + CTGF. Protein expression levels were (A) determined by western blotting and quantified for (B) CTGF, (C) p-MEK/MEK and (D) p-ERK(p-ERK1/2)/ERK (ERK1/2). Fig. 7A presents two bands for p-ERK as anti-erk1/2 and anti-p-ERK1/2 were used. *P<0.05, as indicated. MIAT, myocardial infarction associated transcript; CTGF, connective tissue growth factor; TGF-β2, transforming growth factor-β2; si, small interfering RNA; NC, negative control; p, phosphorylated; MEK, mitogen-activated protein kinase.

sion promoted vascular smooth muscle cell proliferation. In the present study, CTGF expression was enhanced in ARC samples and TGF-β2-treated SRA01/04 cells compared with the control groups. CTGF knockdown reduced cell viability, migration, EMT and ECM production in TGF-β2-treated SRA01/04 cells. Furthermore, CTGF was a direct candidate target of miR-181a. MIAT promoted cell viability, migration, EMT and ECM production in TGF-β2-treated SRA01/04 cells by modulating CTGF expression. The results suggested that CTGF expression was crucial for MIAT-mediated regulation of ARC progression.

Accumulating evidence has suggested that the ERK signaling pathway is associated with the development of multiple diseases (26). For example, one study reported that ERK signaling was activated during the development of Kashin-Beck disease (37). Another study reported that Notch1 expression relieved cigarette smoke extract-induced apoptosis by inhibiting the ERK signaling pathway during chronic obstructive pulmonary disease (38). In the present study, MIAT knockdown inhibited the activity of the ERK signaling pathway and CTGF overexpression reversed the inhibitory effect of MIAT. The results suggested that MIAT was involved in the development of ARC by activating the ERK signaling pathway.

The present study had a number of limitations. A previous study has reported that MIAT affects the progression of PCO by altering the proliferation, metastasis and EMT of LECs (19); however, the role of MIAT in cataract also remains largely unclear. Therefore, the effect of MIAT on the progression of other types of cataract requires further investigation. In addition, the inhibitory effect of miR-181a inhibitor on MIAT-induced effects was partial; therefore, other miRNAs may be involved in MIAT-mediated regulation of ARC progression, which requires further investigation.

To conclude, MIAT and CTGF expression were increased, while miR-181a was decreased in ARC tissues and TGF-β2-treated SRA01/04 cells. Mechanical and functional experiments indicated that MIAT increased cell viability, migration, EMT and ECM production during ARC by acti-

vating the ERK signaling pathway via the miR-181a/CTGF axis. Therefore, the novel regulatory network identified in the present study may serve as a therapeutic target for ARC and provide theoretical basis for the mechanism underlying ARC development.

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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

JL and KT conceptualized the current study. JL, KT, LLu and FY acquired data. All authors contributed to writing and reviewing the manuscript. JL and LLian supervised the current study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All participants or their guardians provided written informed consent. The present study was approved by the Ethics Committee of the Department of Ophthalmology, Renmin Hospital, Hubei University of Medicine.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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