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# MicroRNA-139-5p Regulates Fibrotic Potentials via Modulation of Collagen Type 1 and Phosphorylated p38 MAPK in Uterine Leiomyoma

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**Purpose:** This study aimed to elucidate whether microRNA-139-5p is involved in the pathogenesis of uterine leiomyoma.
**Materials and Methods:** Human leiomyoma and matched human smooth muscle samples were obtained from 10 women who underwent hysterectomy for uterine leiomyoma. MicroRNA (miRNA) expression was analyzed by quantitative real-time polymerase chain reaction. To assess the effects of miR-139-5p on cultured leiomyoma cells, cell migration, collagen gel contraction, wound healing, and the expression levels of hallmark proteins were evaluated in cells transfected with a miR-139-5p mimic.
**Results:** The expression of miR-139-5p was significantly lower in leiomyoma tissues than in matched smooth muscle tissues. Restored miR-139-5p expression in miR-139-5p mimic-transfected human leiomyoma cells resulted in decreased contractility of the ECM and cell migration. In addition, upregulation of miR-139-5p decreased the protein expression of collagen type 1 and phosphorylated p38 MAPK.
**Conclusion:** Expression of miR-139-5p is downregulated in leiomyoma cells and modulation of miR-139-5p may be involved in the pathogenesis of leiomyomas through the regulation of collagen type 1 and phosphorylated p38 MAPK. Therefore, miR-139-5p is a potential therapeutic target for leiomyoma.

**Key Words:** MiR-139-5p, uterine leiomyoma, extracellular matrix, fibrosis

## INTRODUCTION

Uterine leiomyomas, or uterine fibroids, are the most common benign tumors of the reproductive tract.[1] They affect 30–50% of women of reproductive age and up to 80% of women in their lifetime.[2] Approximately 30% of patients with leiomyomas show clinically significant symptoms, including heavy menstrual bleeding, pelvic pain and pressure, dyspareunia, voiding and gastrointestinal problems, infertility, and pregnancy complications.[3] Symptomatic uterine leiomyomas can have devastating effects on women's quality of life and require proper management.[4] Therapeutic options include medical therapy, interventional radiology procedures, and surgical treatment. Improved understanding of the pathophysiology of uterine fibroids could contribute to the development of more effective, less invasive treatments.

MicroRNAs (miRNAs, miRs) are short (~22 nucleotides), non-coding single-stranded RNAs that play important roles in post-transcriptional gene regulation. miRNAs control target genes via translational inhibition and messenger RNA destabilization.[5] Dysregulated expression of specific miRNAs has been observed in a variety of human diseases.[6] Thus, several miRNAs have been proposed as potential diagnostic biomarkers and therapeutic targets for various human diseases. Studies have shown that aberrant miRNA expression is associated

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with numerous diseases of the female reproductive system, including gynecologic cancers,[7,8] pregnancy disorders such as preeclampsia,[9] and recurrent pregnancy loss,[10] endometriosis,[11] and uterine leiomyomas.[12] Since Wang's first transcriptome-miRNA microarray study of uterine tissue in 2007,[13] several miRNA species differentially expressed in leiomyoma and matched normal myometrium have been reported. Many of these miRNAs are associated with biological pathways related to uterine leiomyomas, including tumorigenesis (let-7 and miR-146), angiogenesis (miR-15 and miR-16), cell cycle regulation (miR-21, miR-150, miR-181, miR-182, and miR-197), inflammation (miR-93, miR-106, and miR-200), and extracellular matrix (ECM) accumulation (miR-21 and miR-29).[13-20]

Our microarray analysis showed that miR-139-5p was most significantly downregulated among aberrantly expressed 250 miRs.[16] However, its function and mechanism in uterine leiomyomas remain unknown. Therefore, this study aimed to determine the functional role of miR-139-5p in the pathophysiology of uterine leiomyomas. We hypothesized that downregulation of miR-139-5p expression is involved in the pathophysiology of uterine leiomyomas, particularly in ECM accumulation and fibrosis. We evaluated the expression of miR-139-5p in leiomyomas and matched myometrial tissues, and investigated whether modulation of miR-139-5p expression in cultured leiomyoma cells caused changes in cell characteristics and the expression of hallmark proteins associated with the leiomyoma pathogenesis.

## MATERIALS AND METHODS

### Study population and sample collection

After providing written informed consent, 10 women, aged 19–44 years who underwent hysterectomy for symptomatic uterine leiomyomas between June 2015 and July 2016, participated in the study. All of the uterine leiomyomas were intramural myomas. Patients with post-menopausal status, as well as those who had been on hormonal medications or GnRH agonists within 3 months of surgery, had an infectious disease, acute or chronic inflammatory disease, malignancy, or an autoimmune disease, were excluded from the study. Uterine leiomyoma and adjacent myometrium tissues within 2 cm of the excised leiomyoma were collected within 1 hour of removal. All samples were rinsed in cold phosphate-buffered saline three times, cut into 4 $mm^3$ pieces, and then stored at -80°C in vials containing RNAlater (Ambion, Austin, TX, USA) for nucleic acid preservation. The study was approved by the Institutional Review Board of Gangnam Severance Hospital (3-2015-0249).

### Culture of leiomyoma and myometrial smooth muscle cells

We utilized a previously published method to culture leiomyoma and myometrial smooth muscle cells.[16] Tissue samples were cut into small pieces (~2–5 $mm^3$) and incubated in Dulbecco's modified Eagle's medium without phenol red (Sigma-Aldrich, St. Louis, MO, USA), containing collagenase type I 2.0 mg/mL (Gibco, Waltham, MA, USA), 1% antibiotic-antimycotic mixture containing 100 IU/mL penicillin and 100 mg/mL streptomycin, and 10% heat-inactivated fetal bovine serum (FBS) for 45 min at 37°C in a shaker. The digested tissue was subsequently cultured using the explant method in a humidified incubator at 37°C and 5% $CO_2$ for 2–4 h. Cells were routinely passaged using Versene-EDTA (Gibco). Cultured human leiomyoma cells at 3–4 passages were used for analysis.

### MiR-139-5p isolation and expression analysis by quantitative real-time polymerase chain reaction

Total RNA was isolated from leiomyoma and matched myometrial tissues to estimate miR expression levels using the miRVana RNA Isolation Kit (Ambion) according to the manufacturer's instructions. The RNA was eluted in 30 μL of nuclease-free water. The concentration of the extracted RNA was assessed using a Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and 10 ng was used to generate cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems by Thermo Fisher Scientific, Baltics, Lithuania). Quantitative real-time polymerase chain reaction (qRT-PCR) for the targeted miRs was performed using TaqMan Universal Master Mix II, uracil-N-glycosylase (UNG) (Applied Biosystems), and sets of miR-139-5p and U6 small nuclear RNA (U6 snRNA) (Applied Biosystems). All RT-PCR reactions were performed on a 7300 RT-PCR system (Applied Biosystems) for 40 amplification cycles; each reaction was performed in triplicate. Threshold cycle (Ct) and melting curves were estimated using the 7300 software program (Applied Biosystems). Relative miR levels were calculated using the comparative Ct method and normalized to U6 levels.[21]

### Cell transfection

To modify miR-139-5p expression levels in leiomyoma cells, the cells were seeded into six-well plates, cultured to 70–80% confluence, and then transfected with hsa-miR-139-5p mimics (Thermo Fisher Scientific); hsa-miR-negative, as a control; or hsa-miR-139-5p inhibitor (Thermo Fisher Scientific) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) transfection reagent according to the manufacturer's instructions. Transfected cells were harvested 48 h after transfection.

### Migration assay

A migration assay was performed using 8-mm pore size polycarbonate membranes (Millipore, Billerica, MA, USA) and 24-well plates. Freshly trypsinized cells were washed and suspended in serum-free medium. These cells (200 μL, $5\times10^4$ cells/well) were placed in the top chamber of each insert; medium (600 μL) containing 10% FBS was added to the lower chamber. After

24 h incubation at 37°C in a 5% $CO_2$/95% air humidified incubator, the cells were fixed and stained with crystal violet. After removing the cells in the inner chamber with a cotton swab, the cells attached to the bottom side of the membrane were counted and imaged under an inverted microscope (Olympus, Shinjuku, Tokyo, Japan) at 200× magnification in 10 random fields per well.[21]

### Collagen gel contraction assay

Leiomyoma cells were suspended ($3.0\times10^5$ cells/mL) in a sterile solution of bovine type I collagen (Cell Biolabs, San Diego, CA, USA). This collagen/cell mixture was dispensed in 0.5 mL aliquots into 24-well culture plates (Corning, Corning, NY, USA), and the mixture was polymerized at 37°C for 1 h. Immediately after polymerization, 1 mL culture medium was added to each well. After 72 h incubation, the collagen gels were photographed, and the gel surface area was measured.[22,23]

### Wound healing assay

Leiomyoma cells, transfected with either a control miRNA or miR-139-5p mimic for 48 h, were seeded in 6-well culture plates ($5.0\times10^5$ cells per well) containing DMEM/F12 (1:1) with 10% FBS and antibiotics. The plates were maintained in a humidified atmosphere containing 5% $CO_2$/95% air at 37°C for 24 h. A linear wound (scratch) was generated using a sterile 200 μL pipette tip, and the debris was washed away washing with phosphate-buffered saline (PBS) twice. Culture medium was added to the cells, and they were incubated for 24 h at 37°C with 5% $CO_2$. Images of each well were acquired using an EVOS inverted microscope (Advanced Microscopy Group, Mill Creek, WA, USA) at 0 and 24 h. The wound area was calculated using ImageJ and the following formula: [(mean wound width×mean remaining width)/mean wound width×100 lrb%].

### Protein extraction and Western blot analysis

Proteins were extracted using the RIPA lysis buffer (Thermo Fisher Scientific) containing protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). The protein concentrations were measured using the bicinchoninic acid assay kit (Thermo Fisher Scientific). Equal protein (20 μg) was mixed with 5× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (Biosesang, Seongnam, Korea) and heated at 95°C for 5 min. The samples were separated using 10% SDS-PAGE, and electrotransferred to a polyvinylidene fluoride membrane (Millipore) using a Transblot apparatus (Bio-Rad). The membranes (Millipore) were blocked with 5% non-fat skim milk in Tris-buffered saline solution [10 mmol/L Tris-HCl (pH 7.4) and 0.5 mol/L NaCl] with Tween-20 (0.1% vol/vol). The blots were then probed with primary antibodies against the following proteins: matrix metalloproteinase (MMP)-2 (1:250; Santa Cruz Biotechnology, Dallas, TX, USA), MMP-9 (1:300; Santa Cruz Biotechnology), collagen type 1 (Col-1) (1:1,000, Santa Cruz Biotechnology), connective tissue growth factor (CTGF) (1:1,000, Santa Cruz Biotechnology), fibronectin (1:1,000, Santa Cruz Biotechnology), extracellular signal-regulated kinase 1/2 (ERK 1/2) (1:1,500 with 5% skim milk; Cell Signaling Technology, Danvers, MA, USA), phosphorylated ERK 1/2 (p-ERK 1/2) (1:1,000 with 5% skim milk; Cell Signaling Technology), p38 mitogen-activated protein kinases (p38 MAPK) (1:1,500 with 5% skim milk; Cell Signaling Technology), phosphorylated p38 MAPK (p-p38 MAPK; 1:1,000 with 5% skim milk; Cell Signaling Technology), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1,000, Abcam, Cambridge, UK). Proteins were detected using enhanced chemiluminescence (Santa Cruz Biotechnology). The ImageQuant LAS 4000 (GE Healthcare, Little Chalfont, UK) was used to measure the relative optical density of each specific band obtained after the Western blotting, and the images were further analyzed using ImageJ software (version 1.41o, Java 1.6.0_10, Wayen Rasband, US National Institutes of Health). These experiments were repeated three times for analysis.

### Statistical analysis

Results are reported as the mean±standard error of the mean or the median [interquartile range (Q1-Q3)]. Data were analyzed using the Kolmogorov-Smirnov test or Shapiro-Wilk test to assess normal distribution, and were compared using either the paired t-test or Wilcoxon signed rank test as appropriate. Statistical analyses were performed using SPSS v.25.0 (IBM Corp., Armonk, NY, USA), and statistical significance was set at $p<0.05$.

## RESULTS

### Clinical characteristics

Tissues were collected from 10 subjects, and their clinical characteristics are presented in Table 1. The median age of the participants was 44 years, and all patients underwent hysterectomy. The median maximal diameter was 7.3 cm, and the median number of leiomyomas was 1.5. Two patients were asymptomatic and underwent surgery due to an increased size of the

**Table 1.** Clinical Characteristics of Study Participants

| Variables | Patients (n=10) |
|---|---|
| Age (yr) | 44 (35–48) |
| Name of operation | Hysterectomy |
| Site of leiomyoma | Intramural |
| Maximal diameter of leiomyoma (cm) | 7.3 (4.7–10.7) |
| Number of leiomyoma | 1.5 (1–15) |
| Gravidity | 3 (1–7) |
| Parity | 1.5 (0–2) |
| Follicular phase | 4 |
| Proliferative phase | 6 |

Data are expressed as the median (minimum–maximum).


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leiomyoma. The other eight patients complained of one or more symptoms associated with leiomyomas (four with heavy menstrual bleeding, four with lower abdominal discomfort, three with urinary frequency, three with dysfunctional vaginal bleeding, and one with bearing sense).

### Expression of miR-139-5p in leiomyomas

The relative expression levels of miR-139-5p in leiomyomas and matched myometrium were assessed using qRT-PCR (Fig. 1). The expression level of miR-139-5p was significantly lower in the leiomyomas than in the control tissues [1 vs. 0.26 (0.086–0.296), $p$=0.005] (Fig. 1).

### Migration assay

The migration potential of miR-139-5p mimic-transfected leiomyoma cells was evaluated by performing a migration assay. Leiomyoma cells that migrated and attached to the bottom side of the membrane after 24 h of incubation were counted and imaged under an inverted microscope (Fig. 2A). The number of migrating miR-139-5p mimic-transfected leiomyoma cells was significantly lower than the number of migrating vehicle-treated leiomyoma cells (cell counts: miR-139-5p mimic vs. vehicle, 58.25±1.25 vs. 34.5±1.26, $p$=0.002, n=5) (Fig. 2B). In addition, the number of miR-139-5p mimic-transfected migrating leiomyoma cells was significantly lower than the number of miR-139-5p inhibitor-transfected migrating leiomyoma cells (cell counts: miR-139-5p mimic vs. miR-139-5p inhibitor, 34.5±1.26 vs. 56.5±2.68, $p$=0.004, n=5) (Fig. 2B).

### Wound healing assay

The wound healing assay revealed no significant differences in wound closure (expressed as a percentage of the area of the initial wound) between the vehicle treatment, miR-139-5p mimic-transfected, and miR-139-5p inhibitor-transfected groups (63.11±4.6 vs. 65.10±6.58 vs. 63.79±6.58, n=5) (Fig. 3).

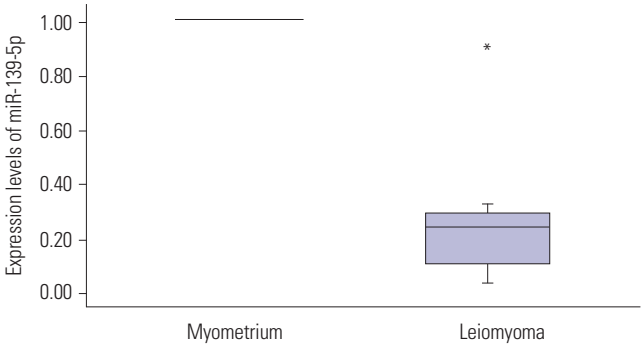


**Fig. 1.** Relative expression of miR-139-5p was significantly decreased in uterine leiomyomas (n=10) compared to the matched myometrium (n=10) according to quantitative real-time polymerase chain reaction analysis [1 vs. 0.26 (0.086–0.296), $p$=0.005]. Results are presented as the median [interquartile range (Q1–Q3)].

### Collagen gel contraction assay

A collagen gel contraction assay was conducted to determine the collagen gel contractility of miR-139-5p mimic-transfected leiomyoma cells. Collagen gel contraction significantly re-

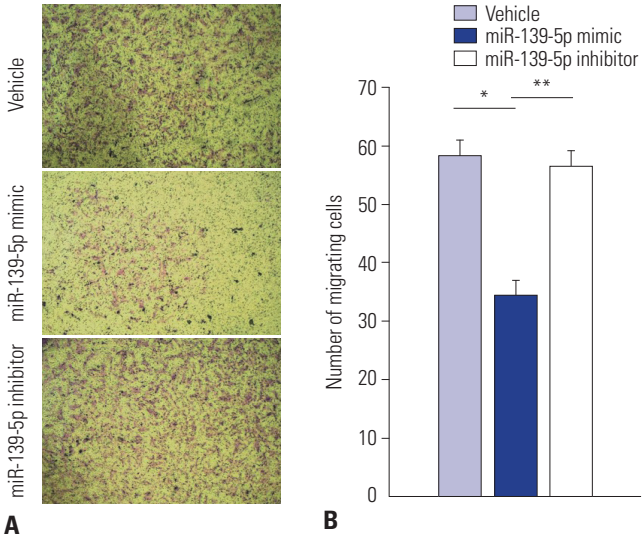


**Fig. 2.** (A) Migration assay of cultured leiomyoma cells was performed, and representative fields of migration cells on the membrane were obtained with magnification ×200. (B) After miR-139-5p mimic transfection, migration assays showed a significant decrease in cell numbers compared to the vehicle group (cell counts: miR-139-5p mimic vs. vehicle, 58.25±1.25 vs. 34.5±1.26, *$p$=0.002, n=5). Migration assays showed a significant difference in cell numbers between the miR-139-5p inhibitor transfected group and miR-139-5p mimic transfected group (cell counts: miR-139-5p mimic vs. miR-139-5p inhibitor, 34.5±1.26 vs. 56.5±2.68, **$p$=0.004, n=5). Results are presented as the mean±standard error of the mean values.

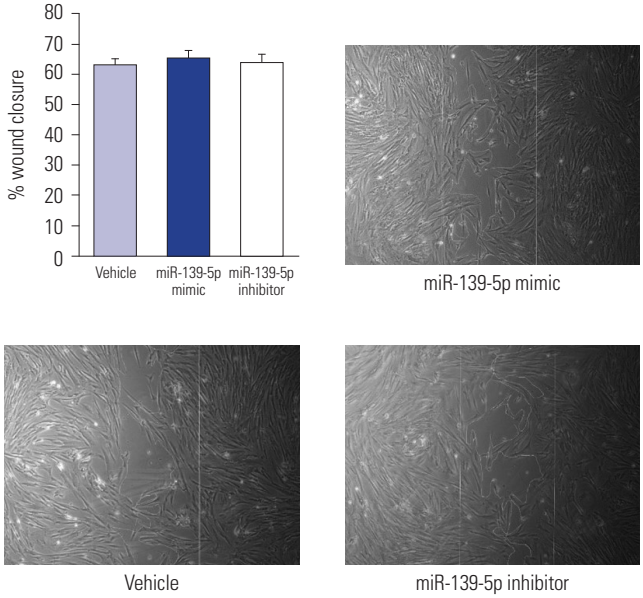


**Fig. 3.** Wound healing assay did not show significant differences between the vehicle treatment group, miR-139-5p mimic transfected group, and miR-139-5p inhibitor transfected group (63.11±4.6 vs. 65.10±6.58 vs. 63.79±6.58, n=5). The percentage migration was calculated by ImageJ. Results are presented as the mean±standard error of the mean values.

duced the following miR-139-5p mimic transfection compared to that of the control cells (relative contraction gel diameter: 0.57±0.032 vs. 0.39±0.026, $p$<0.001, n=5) (Fig. 4). There was no difference between miR-139-5p inhibitor-transfected cells and control cells (0.44±0.03 vs. 0.39±0.026, $p$=0.12, n=5) (Fig. 4).

### Effect of miR-139-5p mimic transfection and miR-139-5p inhibitor transfection on the expression of proteins related to the leiomyoma pathophysiology

Western blot analysis revealed significantly decreased expression of Col-1 (0.76-fold decrease, $p$=0.046) after miR-139-5p mimic transfection. However, the levels of MMP-2, MMP-9, and other markers of fibrosis, CTGF, and fibronectin were not significantly altered by the transfection of a miR-139-5p mimic or inhibitor. In addition, there were no significant differences in the levels of ERK 1/2 and phosphorylated ERK 1/2 between groups. However, significantly lower expression of phosphorylated p38 MAPK was observed after miR-139-5p mimic transfection (0.59-fold decrease, $p$=0.043). The expression of p38 MAPK protein decreased after miR-139-5p inhibitor transfection (0.61-fold decrease, $p$=0.031). When the relative ratio of phosphorylated p38 MAPK to p38 MAPK was compared between the control and miR-139-5p mimic-transfected groups, a significantly lower p-p38 MAPK/p38 MAPK ratio was observed in the miR-139-5p mimic transfection group (0.56-fold decrease, $p$=0.04). However, this ratio did not differ between the miR-139-5p inhibitor transfection and control groups (Fig. 5).

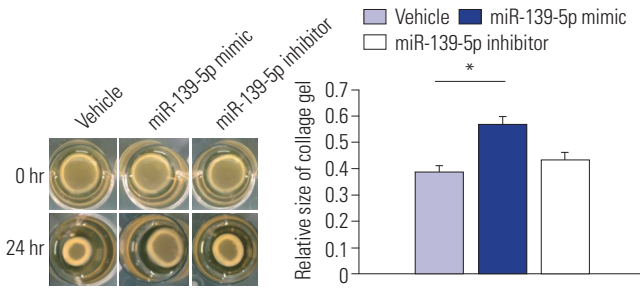


**Fig. 4.** Collagen gel contractility of leiomyoma cells was evaluated using the collagen gel contraction assay. Collagen gel contraction assay revealed significantly less collagen gel contraction compared to the control group (Relative contraction gel diameter: miR-139-5p mimic vs. vehicle, 0.57±0.032 vs. 0.39±0.026, *$p$<0.001, n=5). Results are presented as mean±standard error of the mean values.

## DISCUSSION

The present study showed that miRNA-139-5p is downregulated in leiomyomas when compared with the levels in matched myometrium. Using a transfected human leiomyoma cell culture model, we showed that miR-139-5p affects several cell-specific characteristics of uterine leiomyomas, including cell migration and fibrosis formation. In addition, decreased protein expression levels of Col-1 and phosphorylated p38 MAPK were observed following upregulation of miR-139-5p expression. To the best of our knowledge, this is the first study to highlight the functional role of miRNA-139-5p in uterine leiomyomas.

MiR-139-5p is one of the most commonly downregulated miRNAs in cancer, and is also related to other diseases such as

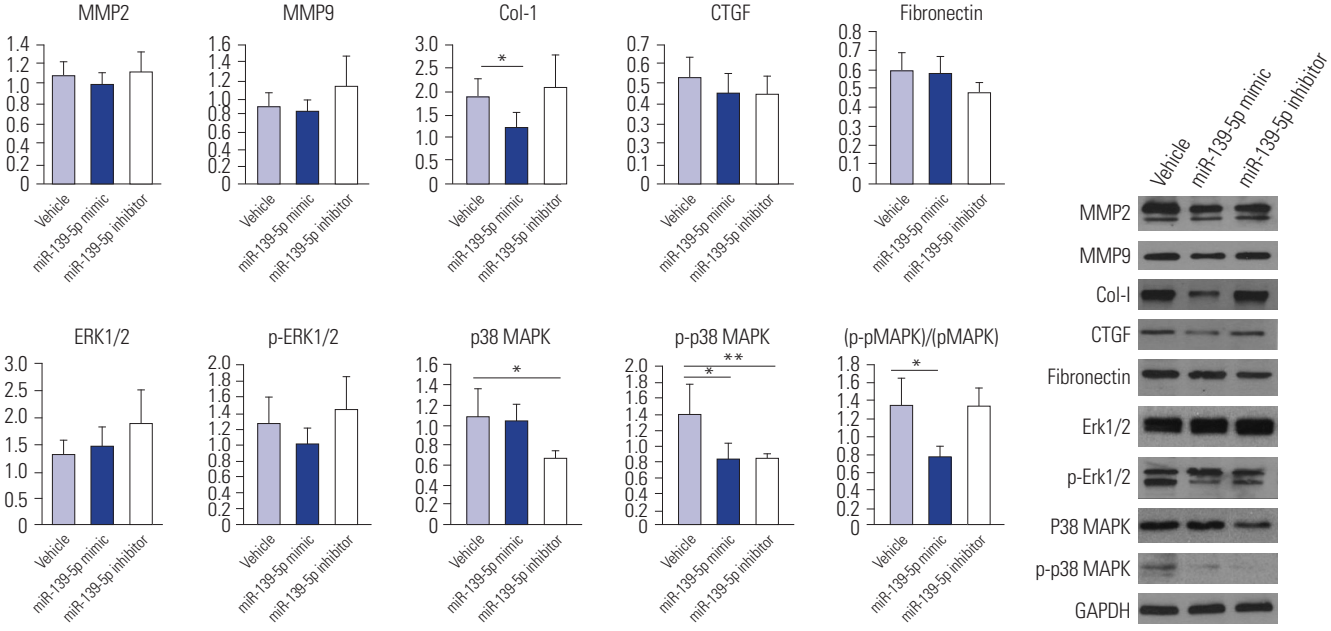


**Fig. 5.** Western blots analysis of proteins (MMP2, MMP9, Col-1, CTGF, fibronectin, ERK1/2, p-ERK1/2, p35 MAPK, p-p38 MAPK) in the vehicle treatment leiomyoma cells, miR-139-5p mimic transfected leiomyoma cells, and miR-139-5p inhibitor transfected leiomyoma cells (n=5). The expression of Col-1 and p-p38 MAPK were significantly decreased after miR-139-5p mimic transfection. The expression of p38 MAPK protein was decreased after miR-139-5p inhibitor transfection. The relative ratio of phosphorylated p38 MAPK to p-p38 MAPK showed a significant difference between the vehicle group and miR-139-5p mimic transfected group. Data are presented as the mean±standard error of the mean values. *$p$<0.05, **$p$=0.068. P, phosphorylated.


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preeclampsia,[9] myocardial ischemia,[24] neurodegenerative disease, and neuropsychiatric diseases.[25] Previous studies have demonstrated that miR-139-5p plays a tumor-suppressive role by regulating cell proliferation, apoptosis, migration, and invasion in various cancers, including endometrial cancer,[26] colorectal cancer,[27] and hepatocellular carcinoma.[28] Aberrant miR-139-5p expression is associated with fibroid formation in other organs. MiR-139-5p inhibits fibrosis in women with interstitial cystitis through the PI3K/Akt signaling pathway.[29] In addition, miR-139-5p is downregulated in patients with hypertrophic cardiomyopathy, and overexpression of miR-139-5p inhibited cardiac hypertrophy and fibrosis, possibly through regulation of c-Jun expression.[30] Consistent with these studies, the present study showed that miR-139-5p exerts a similar effect on the fibrotic process of uterine leiomyomas. However, its function and the detailed mechanism in uterine leiomyomas remain unknown.

Excessive ECM accumulation, which causes fibrosis, is a hallmark of uterine leiomyomas, and collagen is a crucial component of the ECM. Previous studies have shown overexpression of collagen type 1 and other collagen types in leiomyomas compared to their levels in the myometrium.[31,32] Collagen is also believed to affect smooth muscle cell proliferation, migration, and differentiation in uterine leiomyoma.[33] Several studies have investigated other miRNAs related to the changes associated with excessive ECM deposition observed in uterine fibroids.[34,35] Cardozo, et al.[35] reported that miR-21a-5p overexpression modulated the expression of ECM genes in both fibroid and myometrial cells and increased cell proliferation and migration in fibroid cells. However, there was no significant difference in the expression level of miR-21-5p between the fibroid and myometrial cells. Another study showed that overexpression of miRNA-29c decreased the protein expression of the major collagen subtypes COLA1, COL2A1, and COL3A1 in leiomyoma cells.[34] Our findings were consistent with the results of the previous study, and furthers our understanding by adding important findings about the changes in the expression of p-p38 MAPK, collagen gel contraction, and cell migration induced by miR-139-5p overexpression.

In the present study, miR-139-5p expression decreased the collagen contractility of leiomyoma cells. The collagen gel contraction assay has long been used as a tool to study cell-induced contraction of the ECM, which plays an important role in the pathophysiology of leiomyomas as well as imbalanced wound healing and scarring formation.[36] A recent study suggested that the rigid structure of leiomyoma, along with collagen accumulation, may be the cause of abnormal bleeding and pelvic pain.[36] Thus, inhibition of further deposition and remodeling of collagen could be viable options for the management of uterine leiomyomas. Our study showed that upregulation of miR-139-5p expression decreased leiomyoma cell migration. Previous studies revealed that the migration of fibroblast connective tissue cells contributes to the development of pulmonary fibrosis[37] and liver fibrosis.[38] Therefore, our results suggest that the promotion of fibroblast connective tissue cell migration induced by suppression of miR-139-5p expression may contribute to fibrosis in uterine leiomyomas. Similar to our results, miR-150-5p and miR-21a-5p are also known to induce changes in cell migration in uterine leiomyomas,[16,35] but further research is warranted to determine the pathways regulated by these miRNAs.

The p38 MAPK mediates a bidirectional signaling pathway that is activated by mechanical force exerted by the stiffness of the ECM, and leads to changes in cell proliferation, decreased apoptosis, and upregulated expression of genes that determine ECM composition in leiomyoma.[39,40] In the present study, phosphorylated p38 MAPK levels decreased after the restoration of miR-139-5p expression in leiomyoma cells compared to the levels in control leiomyoma cells. This finding may reflect the impact of miR-139-5p on the mechanotransduction of leiomyoma pathology through a pathway mediated by phosphorylated p38 MAPK.

This study had some limitations. First, we examined leiomyoma tissues and matched myometrium tissues of only 10 subjects. Although our results were highly consistent, additional studies with a larger sample size may be able to dilute the inter-subject variability. In addition, a single miRNA can be linked to multiple pathways with varying consequences, and these connections can be affected by numerous factors, such as hormones and other miRNAs from different cells. Therefore, further studies aimed at identifying the exact pathway, starting with the target genes regulated by miR-139-5p, and confirming its effect in vivo are warranted to enrich our current findings.

In conclusion, aberrant expression of miR-139-5p, which was significantly lower in uterine leiomyoma than in matched myometrium, was associated with overexpression of collagen type 1 protein and phosphorylated p38 MAPK protein, increased tissue contractility, and cell migration. These novel findings suggest that miR-139-5p plays a role in the pathophysiology of uterine leiomyoma, especially in the process of fibrosis. In addition, restoration of miR-139-5p led to decreased levels of collagen type 1 and phosphorylated p38 MAPK, collagen contractility, and cell migration in leiomyoma, indicating its potential as a non-invasive therapeutic option for leiomyoma.

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## AUTHOR CONTRIBUTIONS

Conceptualization: Jae Hoon Lee and SiHyun Cho. Data curation: So Hyun Ahn, Jae Hoon Lee, and SiHyun Cho. Formal analysis: So Hyun

Ahn and Jae Hoon Lee. Funding acquisition: Young Sik Choi and SiHyun Cho. Investigation: Inha Lee and Heeyon Kim. Methodology: Jae Hoon Lee and SiHyun Cho. Project administration: Young Sik Choi and SiHyun Cho. Resources: Young Sik Choi and SiHyun Cho. Software: Inha Lee and Heeyon Kim. Validation: Jae Hoon Lee, Inha Lee, and Heeyon Kim. Visualization: So Hyun Ahn and Jae Hoon Lee. Writing—Original draft: So Hyun Ahn. Writing—review & editing: So Hyun Ahn and Jae Hoon Lee. Approval of final manuscript: all authors.

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