MicroRNA-761 inhibits Angiotensin II-induced vascular smooth muscle cell proliferation and migration by targeting mammalian target of rapamycin

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Directed by Professor Namsik Chung

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ABSTRACT

MicroRNA-761 inhibits Angiotensin II-induced vascular smooth muscle cell proliferation and migration by targeting mammalian target of rapamycin

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Aberrant vascular smooth muscle cell (VSMC) proliferation and migration are a major pathological phenomenon in vascular disease characterized by intimal thickening. The important role of the mammalian target of rapamycin (mTOR) signaling in VSMC proliferation has been previously reported. Consequently, down-regulation of mTOR pathway may be an effective way of controlling excessive VSMC proliferation. Since microRNAs (miRNA) are newly emerging regulators of virtually all the biological processes including cellular proliferation, miRNAs targeting mTOR pathway may be able to suppress aberrant VSMC proliferation during pathologic vascular conditions. Thus, in the present study, we screened miRNAs targeting mTOR and identified miR-761 as a new mTOR targeting miRNA. Luciferase assay using luciferase vector containing 3'UTR of mTOR indicated that miR-761 directly targets mTOR mRNA leading to suppression of mTOR protein expression. Our data indicate that miR-761 expression decreases during angiotensin II (AngII)-induced proliferation of VSMCs, and exogenous miR-761 delivery effectively inhibit the AngII-induced VSMC proliferation. The results of migration assay demonstrated that down-regulation of mTOR using exogenous miR-761 suppressed AngII-induced migration of VSMCs as well. Taken together, the present study provided evidence that miR-761 can be a potent anti-proliferative agent for VSMCs in vascular diseases such as atherosclerosis and restenosis, and warrants further studies to validate the effectiveness of miR-761 in vivo.

Keywords: Ang II, miR-761, mTOR, VSMC, proliferation

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I. INTRODUCTION

Known as intimal hyperplasia, aberrant proliferation and migration of vascular smooth muscle cells (VSMCs) has been implicated in the development and progression of vascular diseases such as atherosclerosis and restenosis.[1,2] Under physiologic conditions, the phenotype of VSMCs is predominantly contractile and quiescent. However, in response to vascular injury, various growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and angiotensin II (AngII) are produced inducing VSMC proliferation.[3] These growth factors activate corresponding receptors leading to initiation of signaling pathways, and one of the important pathways for VSMC proliferation is phosphoinositide 3-kinase (PI3K) signaling.[4] The serine–threonine kinase Akt (also known as protein kinase B, PKB) is a key downstream component of PI3K pathway.[5] Upon PI3K activation, Akt phosphorylates and inhibits the activity of glycogen synthase kinase-3 beta (GSK3β),[6] which stimulates export and degradation of cyclin D,[7] hence activation of PI3K signaling pathway stabilizes cyclin D and enhances

proliferation.[8] Mammalian target of rapamycin (mTOR) is a critical regulator in cell proliferation that phosphorylated and activated by phosphatidylinositol 3-kinase (PI3K)/AKT.[9] Activated mTOR propagates signals through phosphorylation of downstream molecules p70 S6 kinase (P70S6K) and eukaryotic initiation factor 4E binding protein 1 (4EBP1).[10] Such mTOR pathway -mediated SMC proliferation has been demonstrated in previous study used AngII as a proliferation inducing agent.[11] Thus, considering the important role of mTOR pathway in SMC proliferation, disruption of mTOR pathway may be an effective way of preventing excessive SMC proliferation under pathologic conditions such as atherosclerosis or restenosis. MicroRNAs (miRNAs), small (~23 nucleotides) highly conserved non-coding RNAs, negatively regulate the expressions of target proteins by binding to 3' untranslated region (3'UTR) in the target genes, subsequently degrading or suppressing translation of the target mRNAs.[12] MicroRNAs regulate various biological functions including, apoptosis,[13] cell cycle,[14] differentiation,[15] and proliferation.[16] The aberrant changes of miRNAs in the vascular wall after angioplasty have been reported.[17] Thus, in the present study, we examined the feasibility of suppressing AngII-induced excessive VSMC proliferation using exogenous miRNA targeting PI3K/AKT/mTOR pathway. In the present study, we screened miRNAs for targeting mTOR based on miRNA target prediction program, and the effect of selected miRNA on AngII-induced VSMC proliferation was examined further.

II. MATERIALS AND METHODS

1. Isolation and culture of rat aortic VSMCs

All experimental procedures for animal studies were approved by the Committee for the Care and Use of Laboratory Animals, Yonsei University College of Medicine, and performed in accordance with the Committee's guidelines and regulations for animal care. The thoracic aortas from 6- to 8-week-old Sprague–Dawley rats were removed and transferred into serum-free DMEM (Dulbecco's modified Eagle's medium; Invitrogen, USA) containing 100 units/ml penicillin and 100 mg/ml streptomycin. The connective tissues were removed and the aorta was transferred to a petri dish containing 5 ml of an enzyme dissociation mixture containing DMEM with 1 mg/ml collagenase type I (Sigma, USA) and 0.5 mg/ml elastase (USB Bioscience, USA), and incubated for 30 min at 37°C. The adventitia was stripped with forceps under a microscope. The aorta was transferred into a plastic tube containing 5 ml enzyme dissociation mixture and incubated for 2 hr at 37°C. The suspension was centrifuged (1500 rpm for 10 min), and the pellet was re-suspended with 10% fetal bovine serum (FBS) containing DMEM with. Rat aortic SMCs were cultured in DMEM supplement with 10% FBS, 100 IU/ml penicillin and 100 mg/ml streptomycin in 75 cm2 flasks at 37°C in a humidified atmosphere of 95% air and 5% CO2 (Forma Scientific, USA).

2. Selection of miRNA targeting mTOR

First, miRNAs predicted to target mTOR were retrieved using a publicly available database (TargetScan, www.targetscan.org). The efficiency of miRNAs in down-regulating mTOR expression was empirically determined by

Western blot using mTOR specific antibodies.

3. MicroRNA transfection

Transfections of miRNA mimics were performed using siLentFect™ Lipid reagent (Life Science Research). Mature specific miRNA and miR-control (Genolution Pharmaceuticals, Inc., Korea) were used at a final concentration of 100 nM. After 4 hr incubation in a CO2 incubator at 37°C, the medium was changed to 10% FBS containing DMEM.

4. Cell proliferation assay

Rat aortic VSMCs were plated in 96-well plates at 5x103 per well. Cells were transfected with miRNA mimics 24 hr prior to AngII (Sigma) exposure for 48 hr. After treatment, 10ul of the WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetra zolium, monosodium salt] solution (Dojindo, Japan) was added to each well and incubated at 37°C for 2 hr to allow formation of WST-8 formazan. The absorbance of a water soluble formazan dye was measured at 450 nm using a microplate reader (Molecular Devices, USA). Experiments were performed in triplicate.

5. Real-time polymerase chain reaction (PCR)

Total RNA was isolated with TRIzol® Reagent (Life Technologies, USA). In brief, 100 ng purified total RNA was used for reverse transcription (Taqman® MicroRNA Reverse Transcriptase Kit, Applied Biosystems, USA) in combination with Taqman® MicroRNA Assays for quantification of specific miRNAs and U6 control transcripts, according to the manufacturer's conditions.

The threshold cycle (Ct) of each target gene was automatically defined, located in the linear amplification phase of the PCR, and normalized to control U6 (ΔCt value).

6. Reverse transcription polymerase chain reaction (RT-PCR)

Cellular total RNA was isolated with TRIzol® Reagent (Life Technologies, USA). Single-stranded cDNA was synthesized from total RNA using a reverse transcription system (Promega, USA) according to the manufacturer's protocol. The primer sequences were as follows: GAPDH, sense: 5' – GGT GAT GCT GAG TA - 3' and antisense: 5' –GGA TGC AGG GAT GAT GTT CT– 3' (369bp); mTOR, sense: 5' – TTG AGG TTG CTA TGA CCA GAG AGA A– 3' and antisense: 5' –TTA CCA GAA AGG ACA CCA GCC AAT G– 3' (540bp).

7. Western Blot Analysis

VSMCs cultured in 60-mm dishes were treated with or without miRNA mimics that were predicted to target mTOR and stimulated with AngII. Proteins were separated in a 10% SDS-polyacrylamide gel and transferred to PVDF membrane (Millipore, USA). After blocking the membrane with TBS-T (TBS-tween 20, 0.1% tween 20) containing 5% (w/v) non-fat dried skimmed milk powder for 1 hr at room temperature, membranes were washed twice with TBS-T and incubated with primary antibodies for 1 hr at room temperature or overnight at 4°C. The membrane was washed three times with TBS-T for 5 min and incubated with HRP (horseradish peroxidase)-conjugated secondary antibody for 1 hr at room temperature. After extensive washing, the bands were detected using ECL® (enhanced chemiluminescence) reagent (AbClon, Republic of Korea). The band intensities were detected using a Photo-Image

System (Molecular Dynamics, Canada). The primary antibodies were from Cell Signaling (mTOR:2972, p-mTOR:2971, P70S6K:9202, p-P70S6K:9206, 4EBP1:9452, p-4EBP1:9459, cyclin D1:9262), except the β-actin (Abcam, ab8227).

8. Luciferase reporter assay

We synthesized the 3′-UTR of rat mTOR containing the predicted binding sites for corresponding miRNA. The 3'-UTR fragments were then cloned into the pmirGLO vector (pmTOR-3UTR). HeLa cell was plated at 1×105 in 12 well plates. After 24 hr, the cells were co-transfected with pmTOR-3UTR vector, negative control miRNA mimic (NC), and miR-761 using siLentFect™ Lipid reagent. The Renilla luciferase was used for normalization. Luciferase activity was measured by the Dual Luciferase assay (Promega, USA) according to the manufacturer's instructions after 24 hr. Each assay was repeated three times.

9. Migration assay

VSMCs (8 × 103 cells) were seeded onto the upper chamber of a Transwell filter with 8 μm pores (Costar Corning, USA) coated with 10 μg/ml fibronectin. The cells were deprived of serum for 24 hr, and AngII-containing stimulating medium (50nM) was added to the lower chamber. Transwell chambers were incubated at 37°C for 24 hr. After incubation, the cells migrated through the pores of the filter were stained with 0.25% crystal violet. Non-migrating cells on the upper side of the filter were removed with cotton swabs.

10. Wound healing assay

VSMCs were plated at a density of 8 × 104 cells/well in six-well plates. After

the cells had reached 90% confluence, cells were deprived of serum for 24 hr. After incubation, wounds were produced by scratching with 200 μL pipette tips. The leading edge of the wounds was marked as a baseline. The medium was replaced with or without serum-deprived medium-containing AngII (50 nM), and the cells were incubated for 0 and 48 hr. Images were captured using an Axiovert 40C inverted microscope (Carl Zeiss, Germany) equipped with a Powershot A640 digital camera (Canon, Japan).

11. Ring assay

The rat aorta was isolated and cleaned of perivascular adipose tissue. The isolated rat aortas were cut into segments of 1 mm long aortic rings that were placed in Matrigel (BD Biosciences, USA). The aorta rings were transfected with miRNA mimics prior to treatment with media containing AngII. Over the next 7 days, the aorta rings were monitored for the outgrowth of VSMCs using microscope once per day.

12. Statistical analysis

Quantitative data were expressed as the means ± SEM. For statistical analysis, one-way ANOVA with Bonferroni correction was performed using the OriginPro 8 SR4 software (ver. 8.0951, OriginLab Corporation, Northampton, MA, USA) if there were more than 3 groups. For two group comparison, student's t-test was used. A p value of less than 0.05 was considered to be statistically significant.

III. RESULTS

1. AngII-induced rat aortic VSMC proliferation is mediated by mTOR

AngII significantly increased VSMC proliferation at the concentrations of 50 nM or higher (Fig. 1A). However, when the cells were pretreated with rapamycin, a potent inhibitor of mTOR,[18] AngII-induced VSMC proliferation was suppressed indicating that mTOR was involved in the AngII-induced proliferation of VSMCs (Fig. 1B). Since 50nM (or higher) AngII induced VSMC proliferation, 50nM of AngII was used to stimulate VSMC proliferation for further experiments.

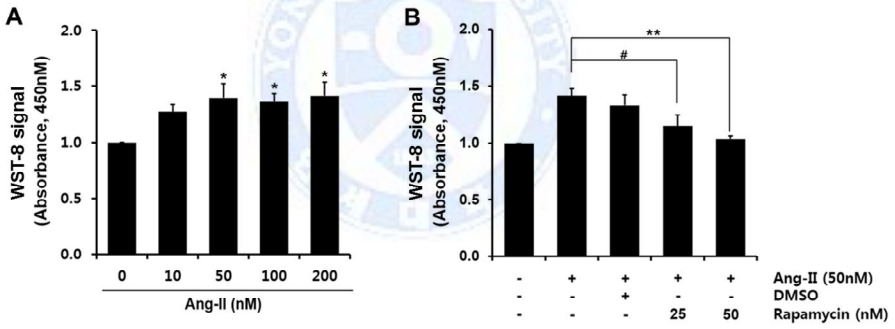

Fig. 1. AngII-induced VSMC proliferation is mediated by mTOR. (A) Relative VSMC proliferation with increasing concentrations of AngII (10~200nM) was measured. *P <0.05 (B) The effect of rapamycin (mTOR inhibitor) on AngII-induced VSMC proliferation was measured., **P<0.01, #p<0.05 vs. control.

2. miR-761 directly targets mTOR and is down-regulated during AngII-induced VSMC proliferation

We have hypothesized that AngII-induced proliferation of VSMCs may involve down-regulation of mTOR-targeting miRNAs, leading to increased mTOR activity. To test the hypothesis, first, we have selected 16 miRNAs based on miRNA database (www.TargetScan.org) (Table. 1).

Table. 1. miRNAs targeting mTOR from miRNA database (www.TargetScan.org)

miRNA	Position (of mTOR 3'UTR)	Sequence (miRNA)	Aggregate (Pct)
miR-96	809 - 815	3' - UCGUUUUUACACGAUCACGGUUU - 5'	0.79
let-7a	420 - 426	3' - UUGAUAUGUUGGAUGAUGGAGU - 5'	0.73
let-7b	420 - 426	3' - UUGGUGUGUUGGAUGAUGGAGU - 5'	0.73
let-7c	420 – 426	3' - UUGGUAUGUUGGAUGAUGGAGU - 5'	0.73
let-7d	420 - 426	3' - UUGAUACGUUGGAUGAUGGAGA - 5'	0.73
miR-98	420 - 426	3' - UUGUUAUGUUGAAUGAUGGAGU - 5'	0.73
miR-144	127 - 133	3' - UCAUGUAGUAGAUAUGACAU - 5'	0.31
miR-101b	127 - 133	3' - AAGUCGAUAGUGUCAUGACAU - 5'	0.31
miR-7	488 - 495	3' - UGUUGUUUUAGUGAUCAGAAGGU - 5'	0.14
miR-150	259 - 266	3' - GUGACCAUGUUCCCAACCCUCU - 5'	<0.1
miR-223	280 - 286	3' - CCCCAUAAACUGUUUGACUGU - 5'	<0.1
miR-15b	617 - 623	3' - ACAUUUGGUACUACACGACGAU - 5'	<0.1
miR-761	615 – 621	3' - ACACAGUCAAAGUGGGACGACG - 5'	<0.1
miR-214	615 – 621	3' - GACGGACAGACACGGACGACA - 5'	<0.1
miR-143	21 - 27	3' - ACUCGAUGUCACGAAGUAGAGU - 5'	<0.1
miR-99a	294 - 300	3' - GUGUUCUAGCCUAGAUGCCCAA - 5'	<0.1

Next, we transfected rat VSMCs with selected miRNAs, and then evaluated mTOR expression using Western blot (Fig. 2A). Four miRNAs (miR-143/150/214/761) significantly repressed mTOR expression. However, three of them (miR-143/150/214) induced significant cell death (data not shown), thus consequently excluded from further experiments. The expression of miR-761 was significantly decreased as the VSMCs proliferated in response to AngII treatment (Fig. 2B).

For luciferase assay, the 3'-UTR of mTOR was cloned into a pmirGLO vector using xhoI and xbaI endonuclease site to produce a pmTOR-3UTR vector. HeLa cells were transiently transfected with the pmTOR-3UTR vector (or pmirGLO control vector), in combination with miR-761 mimics or negative control mimics (scrambled miRNA; N.C.). Transfection with miR-761 significantly decreased luciferase activity in pmTOR-3UTR group, while it had no significant effect on pmirGLO control vector transfected group (Fig. 2C). Although mRNA expression of mTOR was not changed by miR-761 transfection (Fig. 2D), mTOR protein expression was decreased by miR-761 transfection (Fig. 2E), suggesting the inhibitory mechanism of miR-761 on mTOR expression was to disrupt translation of mTOR from mRNA rather than degradation of mRNA itself.

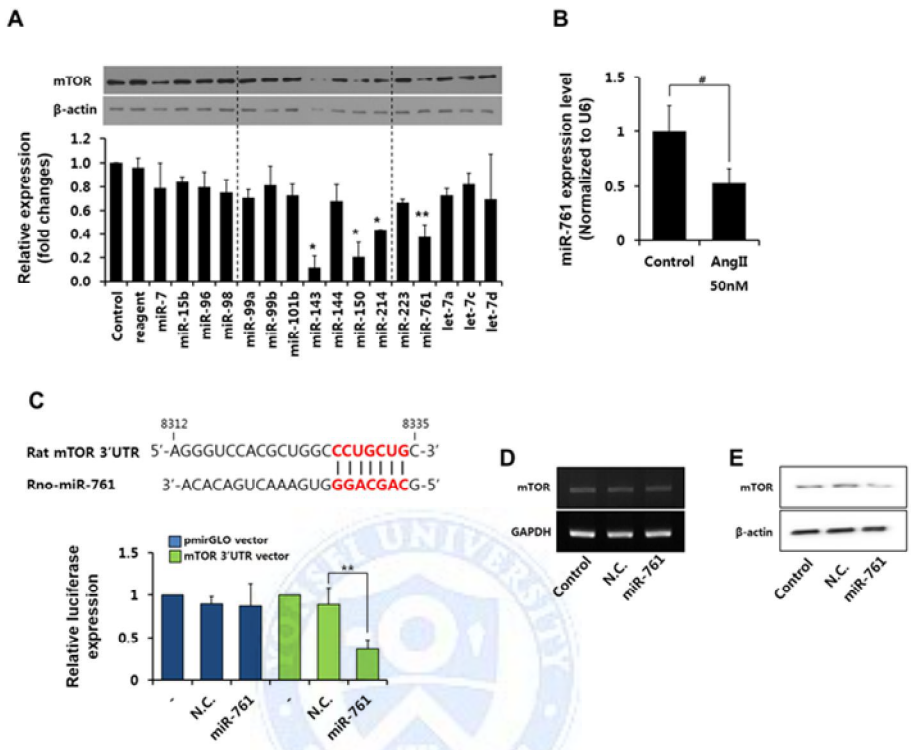

Fig. 2. miR-761 targets mTOR and AngII decreased miR-761 expression. (A) VSMCs transfected with candidate mTOR-targeting miRNAs and the expressions of mTOR protein was determined by Western blot. β-actin served as a loading control. *P<0.001 and **P<0.01 (B) Expression of miR-761 was detected by real time PCR in AngII stimulated VSMCs. #p<0.05. (C) miR-761 targeting 3'UTR of mTOR was verified by using luciferase reporter assay. Luciferase vector containing 3'UTR of mTOR was constructed and the resultant vector was co-transfected into HeLa cells with negative control (scrambled miRNA) or miR-761. **P<0.01. (D) The mRNA level of mTOR was analyzed by RT-PCR. GAPDH was used as a control. (E) VSMCs transfected with N.C. or miR-761 mimic were stimulated with Ang-II (50 nM) and analyzed for the mTOR protein level by western blot. β-actin was used as a control.

3. miR-761 suppresses AngII-induced cell cycle progression and subsequent proliferation of VSMCs by inhibiting mTOR signaling pathway

Although the percentage of cells in S phage of rapamycin-treated cells was lower than that of miR-761-treated cells, the percentage of cells in S phase of miR-761-treated cells was still lower than that of AngII-stimulated cells (Fig. 3A). The results of cell viability assay also indicated that transfection of miR-761 suppressed AngII-induced proliferation in a dose-dependent manner (Fig. 3B). Such anti-proliferative effect of miR-761 was further demonstrated by immunocyto-chemical staining of Ki-67, a proliferating cell marker.[19] The expression of Ki-67 was significantly decreased in miR-761-transfected VSMCs compared to AngII-stimulated cells (Fig. 3C). Additionally, we examined the phosphorylation status of p70S6k and 4EBP1 that is known to be regulated by mTOR.[20] The results of Western blot indicated that the phosphorylation of p70S6k and 4EBP1 were decreased in the miR-761 transfected group. These data suggested that exogenous miR-761 delivery decreased biological activity of mTOR, subsequently suppressing activation of mTOR/p70S6K/4EBP1 signal pathway (Fig. 3D).

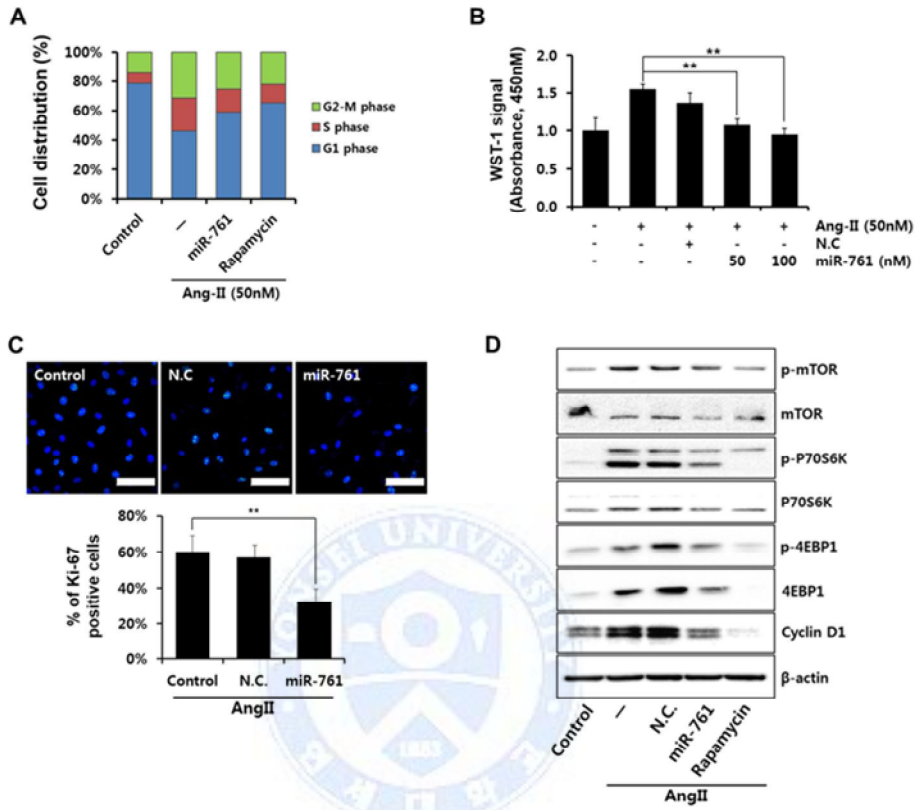

Fig. 3. miR-761 induces cell cycle arrest suppressing AngII-induced VSMC proliferation. (A) VSMCs were stimulated with 50nM of AngII with or without miR-761 or mTOR inhibitor (rapamycin) pretreatment. Cell cycle analysis was performed by using FACS. (B) The effect of miR-761 on 50nM AngII-treated VSMC proliferation. Relative proliferation was measured. **P<0.01. (C) Representative images of Ki-67 (Green) and DAPI (blue) staining. Scale bar (white bar) represents 100 μm. **P<0.01. (D) Phosphorylation status of downstream effectors of mTOR signaling.

4. miR-761 inhibits migration in Ang II-stimulated VSMCs

Migration of VSMC under pathologic condition has been reported to contribute to development of vascular diseases, including atherosclerosis and post-angioplasty restenosis.[21] AngII treatment significantly increased the number of migrated cells, and this increase was significantly suppressed by both miR-761 and rapamycin transfected VSMCs than Ang II-stimulated cells. MiR-761 also inhibited migration of Ang II-stimulated VSMCs (Figure. 4A). In wound healing assay, the distance between wound edges were shortest in the AngII-stimulated group, while the miR-761-treated group and the rapamycin-treated group showed longer distance compared to the AngII-treated group (Fig. 4B). The number of VSMC sprouting from the aortic rings of miR-761-treated group was lower compared to the AngII-treated group (Fig. 4C), indicating that miR-761 suppressed not only VSMC proliferation, but also cell migration.

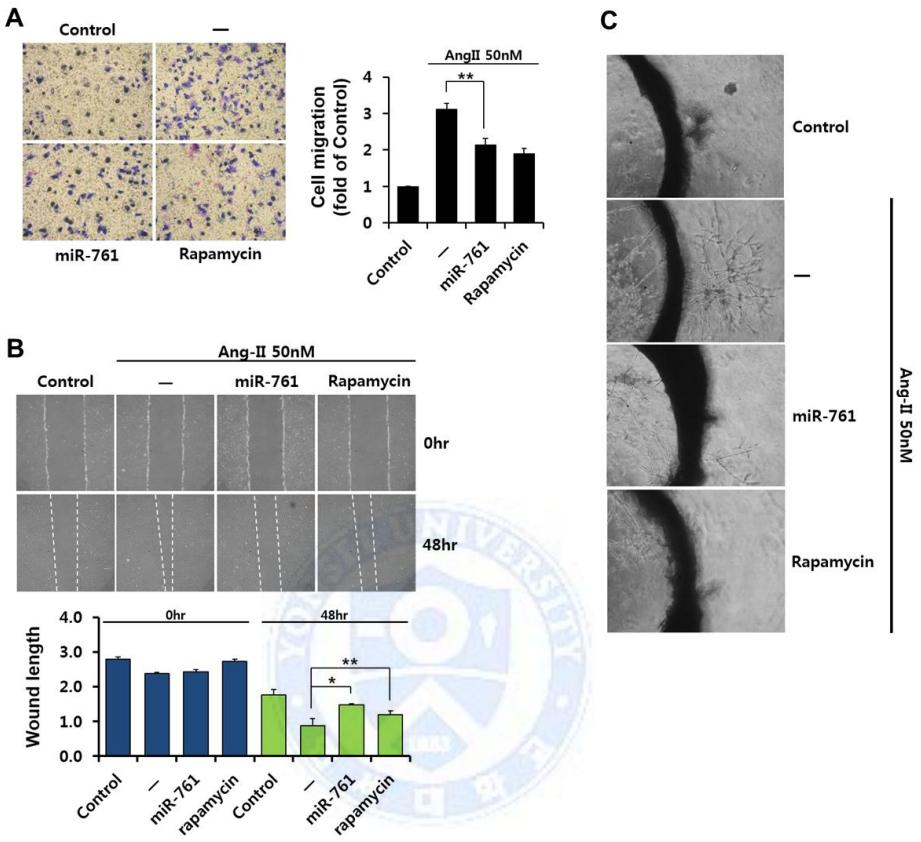

Fig. 4. Effects of miR-761 on AngII-stimulated VSMC migration. (A) Effects of miR-761 on VSMCs migration. Transwell migration assay was used to estimate the migration of VSMCs. To induce cell migration, the cells were incubated with 50 nM of AngII for 18 hr. **P<0.01. (B) Representative images of wound closing. VSMC migration was induced by 50nM of AngII migration. The distance between wound edges was measured. *P <0.001, **P<0.01. (C) Rat aortic rings (1 mm) were embedded and cultured in Matrigel in the presence of 50 nM of AngII with or without miR-761 transfection or rapamycin treatment. The images were taken on 7th day of stimulation.

IV. DISCUSSION

Accumulating evidence suggests that miRNAs play important roles in regulating VSMC proliferation. For example, miRNA-21 has been reported to increase VSMC proliferation after vascular injury by targeting phosphatase and tensin homolog (PTEN).[17] Furthermore, miR-221 and -222 also increased VSMC proliferation by targeting p27kip1 and p57kip2, respectively.[22] On the other hand, miR-26a, -143, and -145 have been demonstrated to decrease VSMC proliferation.[23,24] Additionally, involvement of miRNAs in phenotypic switch of VSMCs and their proliferation during neointima formation has been demonstrated.[25] These studies strongly suggest that miRNA-dependent regulation of VSMC proliferation and migration is an important component of vascular biology, as well as of development and progression of vascular diseases.

The correlation between AngII and vascular diseases such as atherosclerosis has long been recognized.[26] Furthermore, a previous study reported that AngII induced proliferation and migration of VSMCs by activating mTOR signal pathway,[11] indicating inhibition of mTOR pathway may be an effective way of suppressing excessive VSMC proliferation and migration under pathologic conditions such as atherosclerosis. In the present study, we identified a new miRNA, namely miR-761, which inhibits mTOR expression by binding to its 3' UTR. Our data showed that exogenous miR-761 delivery can effectively suppress the AngII-induced VSMC proliferation and migration. It has been reported that G1 cell cycle progression is regulated by PI3K/Akt/mTOR/p70S6k signaling.[27] Thus, disruption of this signaling pathway can lead to cell cycle arrest, and exogenous miR-761 produced such result in the present study.

To date, only few papers have been published regarding the role of miR-761 in biological systems. In fact, as of 2014 Dec, PubMed search using miR-761 as

a key word resulted only 2 research articles. One study implicated miR-761 in controlling nervous system development,[28] and the other study indicated that miR-761 acts as an anti-apoptotic molecule targeting mitochondrial fission factor (MFF).[29] Thus, this study is the first in vitro study proved the concept that delivery of exogenous miR-761 can be an effective anti-proliferative therapy by down-regulating mTOR signaling pathway. However, this anti-proliferative effect of miR-761 has to be further validated using an in vivo model, and there are few points need to be considered prior to any in vivo study.

One of the obvious limitations of the present study is that the anti-proliferative effect of miR-761 was examined in only one type of cells, VSMCs. In other words, the effect of miR-761 may not be VSMC specific when it is delivered in vivo. For example, if miR-761 is used to suppress neointima formation after balloon injury, miR-761 can be delivered via intravenous injection. However, this systemic delivery of miR-761 can be problematic because indiscriminate delivery of miR-761 can result in unwanted suppression of vascular endothelial cell (EC) growth as well. Since rapid regrowth of EC, called re-endothelialization, has been considered important in preventing intimal thickening as well as vascular thrombosis,[30,31] preventing proliferation of EC should not be occur. Considering such possibility, local delivery rather than systemic delivery seems to be logical approach because local delivery immediately after the balloon injury (denudation of existing endothelium) can directly target the VSMCs exposed rather than targeting the neighboring intact ECs. Additionally, a proven, effective mean of miRNA delivery should be ready prior to conducting an in vivo study. Consequently, further studies that can address these issues are warranted.

V. CONCLUSION

The present study, for the first time, provided evidence that miR-761 suppresses VSMC proliferation and migration through repression of mTOR expression and subsequent down-regulation of p70S6K/4EBP1. With further in vivo validation and optimization of delivery system, exogenous miR-761 can be a potent therapeutic agent for the treatment of restenosis and atherosclerosis.

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ABSTRACT(IN KOREAN)

mTOR 경로에 대한 miR-761의 안지오텐신-II 매개성 혈관평활근세포의 증식과 이동의 억제효과

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조 정 래

비정상적인 혈관 평활근세포의 증식과 이동은 내막비후가 특징적인 혈관질환의 주된 병태 현상이다. 혈관평활근세포의 증식에서 mTOR 신호전달체계의 중요성은 잘 알려져 있다. 따라서, mTOR 경로의 하향조절 (down-regulation)은 과도한 혈관평활근세포의 증식을 조절하는 효과적인 방법이 될 수 있다. MicroRNA (miRNA) 가 세포증식을 포함하는 다양한 생물학적 과정을 제어한다고 알려져 있고, 따라서 mTOR 경로를 겨냥하는 miRNA 는 병적인 혈관상태에서 비정상적인 혈관평활근세포의 증식을 억제할 수 있겠다. 본 연구에서는 mTOR 를 겨냥하는 miRNA 들을 선별하여 이중 miR-761를 연구에 이용하였다. 백서의 대동맥 평활근세포를 분리하고, 여기에 miR-761 를 투여하여 실험을 진행하였다. 본 연구에서는 miR-761이 혈관평활근세포 증식과 이동에서 중요한 역할을 하고 있음을 증명하였다. 첫째, mTOR 의 3'UTR을 함유하는 luciferase vector 를 사용한 luciferase assay 결과에서, miR-761 이 mTOR mRNA 를 직접 겨냥하여 mTOR 단백의 발현을 억제시켰다. 둘째, 안지오텐신-II 에 의해 유발된 혈관평활근세포의 증식시 miR-761의 발현이 감소되고, exogenous miR-761 이 이러한 세포증식을 효과적으로 억제함을 보였다. 셋째, migration assay 에서도 miR-761 의 투여로 인한 mTOR 의 하향조절이 안지오텐신-II 에 의해 유발된 혈관평활근세포의 증식을 억제하였다. 위 결과들은 토대로 miR-761이 동맥경화 또는 혈관재협착과 같은 혈관질환에서 혈관평활근세포의 강력한

항증식제로써 가능성을 확인하였으며, 생체내 (in vivo)에서의 miR-761의 유용성을 검증하는 후속 연구가 기대될 수 있겠다.

핵심되는 말: 안지오텐신-II, miR-761, mTOR, 혈관평활근세포, 증식