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MicroRNA-370-3p inhibits human vascular smooth muscle cell proliferation via targeting KDR/AKT signaling pathway in cerebral aneurysm

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Abstract. – OBJECTIVE: Cerebral aneurysm is a common vascular disease with high morbidity and mortality. Vascular smooth muscle deletion or dysplasia is an important reason for the development of cerebral aneurysm. MiRNAs participate in a variety of biological functions through inhibiting target gene translation. The aim of the present study was to evaluate the role of miRNAs in the regulation of vascular smooth muscle cell proliferation.

MATERIALS AND METHODS: MiRNA and mRNA expressions were tested by Real-time PCR. Cell cycle was detected by flow cytometry. Cell viability was evaluated by MTT assay. HUASMC cell proliferation was determined by BrdU assay. Protein expressions were determined using Western blot. MiRNA target gene was confirmed by luciferase assay.

RESULTS: MiR-370-3p expression was increased in cerebral aneurysm tissues. Ectopic expression of miR-370-3p suppressed proliferation of vascular smooth muscle cells and blocked cell cycle. Numerous cell proliferation and apoptosis-related factors were down-regulated by miR-370-3p. Results of target prediction database and dual-luciferase assay revealed that KDR is a direct target of miR-370-3p. Importantly, FOXO1 activity and AKT and FOXO1 phosphorylation were inhibited by miR-370-3p. We suggest that miR-370-3p directly targets KDR, resulting in the activation of AKT signaling pathway.

CONCLUSIONS: MiR-370-3p was involved in the development of cerebral aneurysm by targeting KDR and blocking AKT/FOXO1 signaling pathway. The results provide theoretical basis for further investigation of potential clinical prevention and treatment of cerebral aneurysm.

Key Words:
miR-370-3p, KDR, Smooth muscle cell, Cerebral aneurysm, AKT signaling pathway.

Introduction

Cerebral aneurysm is a kind of common vascular disease with extremely high morbidity and mortality, which leads to fatal vascular rupture[1,2]. The molecular mechanism underlying cerebral aneurysm formation and rupture remains poorly understood. Considering the pathogenesis of cerebral aneurysm, current studies[3-5] mainly focus on hemodynamic factors, acquired degenerative changes in arterial walls, and genetic factors. However, it's also of great significance to investigate an effective way to intervene the growth of cerebral aneurysm through studying the molecular biology pathogenesis. Cell proliferation and apoptosis of vascular smooth muscle cells are involved in maintaining the balance and stability of blood vessels. However, the occurrence of cerebral aneurysm leads to the apoptosis of vascular smooth muscle cells, as a result of which attenuate walls of blood vessels in the brain fail to bear the impact of blood flow[6]. Starke et al[7] showed that deletion and hypoplasia of vascular smooth muscle are important reasons for the formation of cerebral aneurysm. MicroRNA (miRNA) is a short and single-stranded non-coding RNA (consisting of 22 nucleotides) that widely participates in many post-transcriptional regulation processes and plays an important role in regulating various cellular processes[8,9]. miRNA has been found to be involved in tumorigenesis and progression of various cancers, including cerebral aneurysm[10-12]. Previous reports[13,14] have demonstrated that some miRNAs are implicated in vascular smooth muscle proliferation and development. It has been reported that miR-146a triggered vascular smooth

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muscle cell apoptosis through NF-κB signaling pathway[15]. In a human tissue research, a group of lupus nephritis patients was selected to evaluate the mechanism of renal interstitial vascular damage. The result showed that renal interstitial vascular damage exhibited an association of miR-145 expression in renal vascular smooth muscle[16]. Our work aims to investigate the potential role of miRNA in the prevention and treatment of cerebral aneurysm.

Materials and Methods

Clinical Samples

Cerebral aneurysm tissues were obtained from patients who underwent micro-neurosurgery for cerebral aneurysms resection in Jinling Hospital from 2014 to 2016. The intracranial aneurysm was diagnosed according to digital subtraction angiography (DSA). The normal cerebral arteries used in the present study were obtained from intracranial tumor resection or intracranial hematoma removal. The tissue samples were comprised of 8 cerebral aneurysm tissues and 3 normal tissues. Informed consent was signed by all the patients. The tumor tissue samples were stored for the RNA studies.

The institutional ethics committee of JinLing Hospital approved this study according to the principles presented in the Declaration of Helsinki.

Cell culture

The human umbilical artery smooth muscle cells (HUASMC) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplement by 10% fetal bovine serum (FBS), 50 U/ml penicillin G, and 250 μg/ml streptomycin at 37˚C and 5% CO_2.

Cell Transfection

MiR-370-3p ectopic expression clones and KDR siRNA were generated in HUASMCs by transfection using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. The miR-370-3p expression was detected in HUASMCs transfected with miR-370-3p mimics and inhibitor. For luciferase assays, 3'UTR of KDR was amplified by using gene specific primers. The fragment spanning putative binding site for miR-370-3p was cloned into pMIR-REPORT miRNA Expression Reporter Vector (Life Technologies, Grand Island, NY, USA). The specificity of the 3'UTR KDR clone was confirmed by DNA sequencing.

Methyl Thiazolyl Tetrazolium Bromide (MTT) Assay

Cell viability was assessed by the MTT assay. Cells (2×10^3 cells/well) were cultured in 96-well plate and given fresh media every other day. Cells were then transfected with miR-370-3p mimic or miR-370-3p inhibitor as described above for 24 h. Cells were treated with MTT solution, 50 mg per well (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 4 h. Dimethyl sulfoxide was used to dissolve the formazan and the absorbance was measured at 450 nm using an ELISA plate reader (BioTek Instrument Inc., Winooski, VT, USA).

Cell Cycle

HUASMC cells were collected and washed with PBS for twice. A total of 1 ml of 70% precooled ethanol was added to cells and incubated for 8 h at 4°C. Then, the cells were washed by phosphate buffered saline (PBS) and treated with 100 mg/l RNase at 37°C for 30 min. After stained by 50 mg/l PI at 4°C in the dark for 30 min, the cells were detected by flow cytometry at the wavelength of 488 nm. The primary result was analyzed by cell cycle matching software to record hypodiploid peak, namely sub-G1 phase, G0/G1 phase, S phase, and G2/M phase. All of the experiments were repeated for three times.

BrdU Incorporation Assay

HUASMCs were seeded on coverslips in 6-well plates to 60-70% confluences before they were made quiescent in Dulbecco's Modified Eagle Medium (DMEM) containing 0.5% fetal bovine serum (FBS) for 24 h. BrdU incorporated into cellular DNA was detected by immunofluorescence assay using a BrdU Labeling and Detection Kit I (Hoffmann-La Roche, Basel, Switzerland). Total cellular nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). The results were reported as the percentage of BrdU-labelled cells.

Luciferase Reporter Gene Assay

The FoxO1 promoter was amplified by polymerase chain reaction (PCR) from human genomic DNA containing a FRE (AGTAAACAAA), and then was cloned into the pGL3-Basic plasmid (Promega, Madison, WI, USA) at the KpnI and BgIII (TaKaRa Biotechnology, Dalian, China) sites. The constructed plasmid was named as pGL3-FoxO1. pGL3-FoxO1 was obtained by introducing four mutations into FRE using a Mut Express II Fast Mutagenesis Kit (Vazyme, Nanjing, Jiangsu, China). HUASMCs were seeded

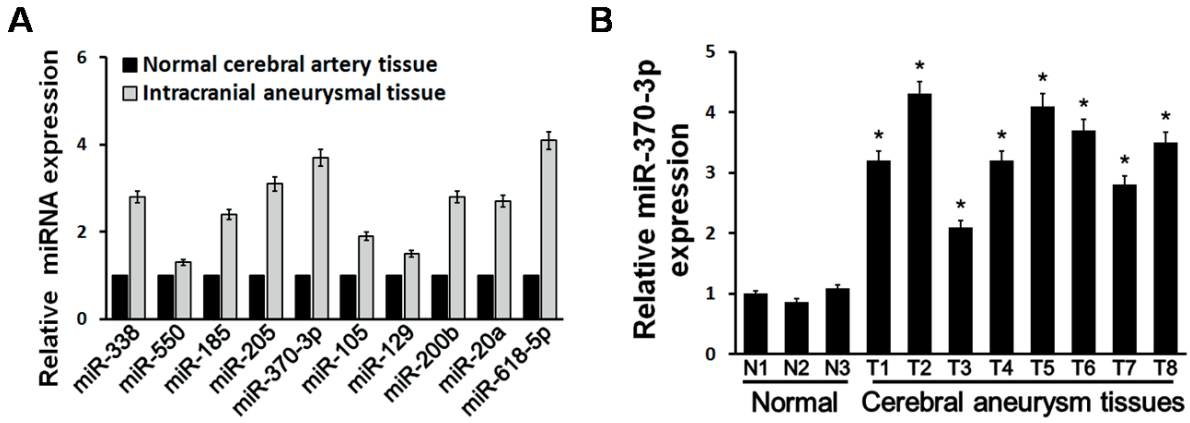

Figure 1. MiR-370-3p overexpressed in cerebral aneurysm tissues. ***(A)*** MiRNAs expression in cerebral aneurysm tissues based on TCGA database. ***(B)*** MiR-370-3p expression in cerebral aneurysm tissues. *$p < 0.05$, compared with control.

in a 12-well plate and grown to 70% density before transfection with Lipofectamine 2000 (Life Technologies, Grand Island, NY, USA). After 36 h, cells were processed for reporter assay on Modulus Microplate Luminometer (TurnerBio Systems, Sunnyvale, CA, USA) according to the manufacturer's instructions. All of the inserts were verified by sequencing.

Real-time PCR

Total RNA was extracted by using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. RNA was eluted in 50 mL of RNase-free water (Promega, Madison, WI, USA) and stored at -70°C. To analyze gene expression, the qRT-PCR mixture system containing cDNA templates, primers, and SYBR Green qPCR Master Mix were subjected to Real-time qPCR according to standard methods. Fold changes in gene expression were calculated using $2^{-\Delta\Delta Ct}$ method. The 18S rRNA used as an internal control.

Western Blot

Cells were lysed with loading lysis buffer (containing 0.5 mol/l Tris-HCl 2.5 ml, dithiothreitol 0.39 g, sodiumdodecyl sulfate (SDS) 0.5 g, bromophenol blue 0.025 g, glycerin 2.5 ml). An equal amount of protein was transferred onto the polyvinylidene fluoride (PVDF) membrane. The membrane was then incubated with primary antibodies against p-AKT, AKT, p-FOXO1, FOXO1, KDR, and GAPDH (Cell Signaling Technology, Danvers, MA, USA) at 4°C for 1 h. Then the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at room temperature. The binding signals were visualized by enhanced chemiluminescence (Thermo-Scientific, Rockford, IL, USA).

Dual-Luciferase Assay

The predicted binding site of miR-370-3p on 3'UTR of KDR was amplified via RT-PCR from the cDNA library of HUASMC cells. A recombinant mutant 3'UTR of KDR was also constructed through a Site-Directed Mutagenesis Kit (SBS Genetech, Beijing, China). The amplified wild type and mutant KDR 3'UTRs were then inserted into a pmiR-REPORT luciferase reporter vector (Ambion, Austin, TX, USA). The vectors were named as Luc-KDR (wild-type) and Luc-mKDR (mutant). A negative control luciferase vector (Luc-C) was also generated. Then, the three vectors were co-transfected with β-galactosidase and miR-370-mimic (RiboBio, Guangzhou, Guangdong, China) into HEK293cells by Lipofectamine 2000 (Life-Technologies, Grand Island, NY, USA) according to the manufacturer's instruction. HEK293 cells were then cultured at 37°C with 5% CO_2 for another 24 h. The dual-luciferase activities were measured by a luciferase reporter assay system (Promega, Madison, WI, USA), and normalized to β-galactosidase activity of the cells transfected with Luc-C.

Statistical Analysis

All data were presented as the mean ± standard deviation. All experiments were repeated at least three times. Statistical analysis was applied

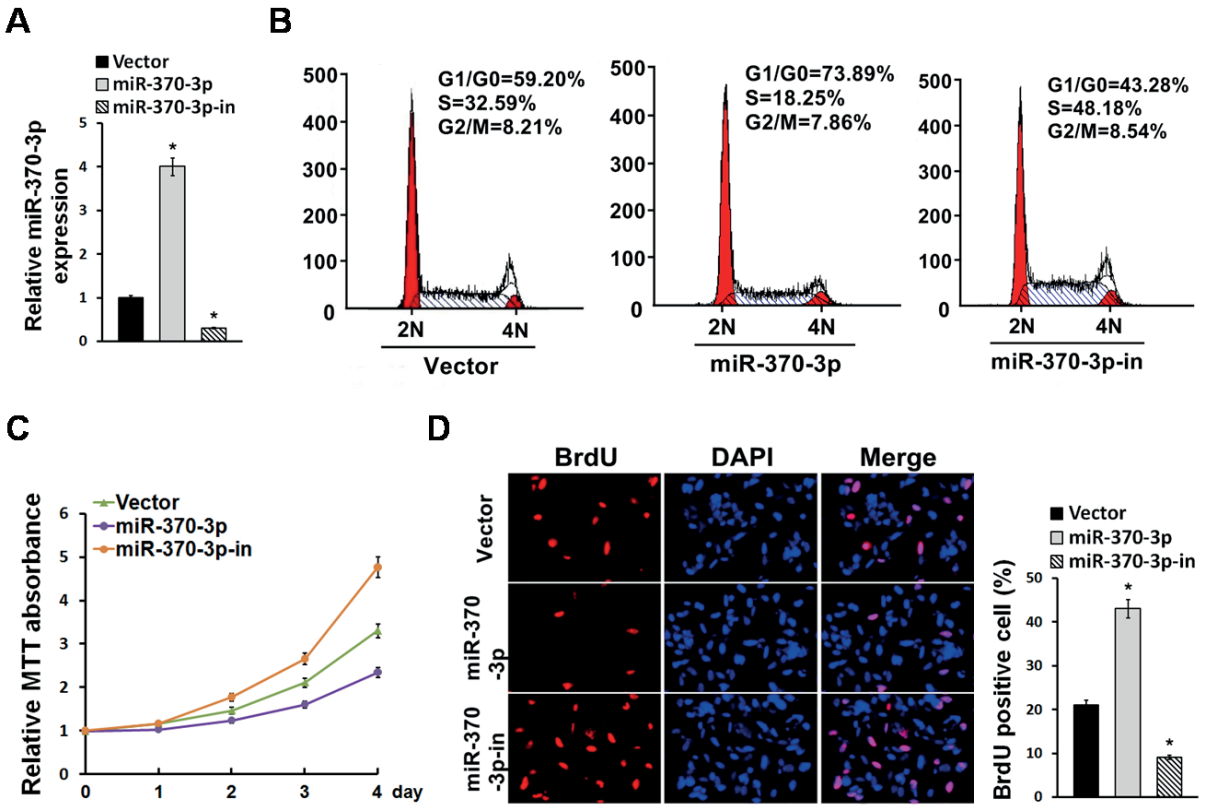

Figure 2. MiR-370-3p suppressed HUASMCs cell proliferation and cell cycle. ***(A)*** MiR-370-3p expression in HUASMCs after miR-370-3p or miR-370-3p-in transfection. ***(B)*** HUASMC cell cycle tested by flow cytometry after miR-370-3p or miR-370-3p-in transfection. ***(C)*** HUASMC cell viability detected by MTT assay after miR-370-3p or miR-370-3p-in transfection. ***(D)*** HUASMC cell proliferation determined by BrdU assay after miR-370-3p or miR-370-3p-in transfection. *$p < 0.05$, compared with control.

to SPSS16.0 software (SPSS Inc., Chicago, IL, USA). The differences between groups were determined using two-tail unpaired Student's *t*-test. A *p*-value of <0.05 was depicted significant difference.

Results

MiR-370-3p was Upregulated in Human Cerebral Aneurysm Tissues

To identify the expression of miRNAs in human cerebral aneurysm, we searched TCGA database; the expression levels of several miRNAs in human intracranial aneurysm tissues and normal brain tissues were compared. A set of miRNAs, such as miR-618-5p, miR-370-3p, and miR-205, exhibited significantly elevated levels in intracranial aneurysm tissues compared with normal control group ($p<0.05$, Figure 1A). Moreover, 8 cerebral aneurysm tissues and 3 normal tissues from our hospital were tested using Real-time PCR. It was demonstrated that miR-370-3p level in cerebral aneurysm tissues was obviously higher than that of control ($p<0.05$, Figure 1B).

MiR-370-3p Suppressed HUASMCs Proliferation and Blocked Cell Cycle

Since miR-370-3p was upregulate in cerebral aneurysm tissues, we suggested that miR-370-3p may play a crucial role in the vascular smooth muscle cells of cerebral aneurysm. To investigate its role in vascular smooth muscle, miR-370-3p mimics or inhibitor were transfected into HUASMCs to enhance or inhibit miR-370-3p expression. The results of Real-time PCR confirmed that the miR-370-3p level in HUASMCs was changed (Figure 2A). Furthermore, we examined the effect of miR-370-3p on cell cycle. Flow cytometry also revealed that miR-370-3p mimics promoted HUASMCs in S phase, while miR-370-3p inhibitor blocked cell cycle (Figure 2B).

MTT assay revealed that miR-370-3p elevated, whereas miR-370-3p inhibitor markedly reduced HUASMCs cell viability (Figure 2C). BrdU assay showed that miR-370-3p significantly suppressed cell proliferation, whereas miR-370-3p inhibitor facilitated cell growth compared with control group (Figure 2D). The results indicated that cell proliferation and cell cycle of HUASMCs were influenced by miR-370-3p.

MiR-370-3p Activated Akt/FOXO1 Signaling Pathway

Akt signaling pathway plays an important role in vascular smooth muscle cell proliferation. Therefore, the effect of miR-370-3p on Akt signaling pathway was explored. Real-time PCR exhibited that cell proliferation associated factors, such as Cyclin D2, XIAP, and MYC was upregulated by miR-370-3p in HUASMCs, while the miR-370-3p inhibitor showed the opposite effect of miR-370-3p (Figure 3A). Also, apoptosis-related genes, including Bim, FASL, and caspase-9, were found to be enhanced in HUASMCs after miR-370-3p transfection (Figure 3B). We also examined the impact of miR-370-3p on FOXO1 activity, which is a downstream transcription factor of Akt signaling pathway. Luciferase reporter gene assay showed that FOXO1 activity was elevated by miR-370-3p and restrained by miR-370-3p inhibitor (Figure 3C). Moreover, Western blot showed that Akt and FOXO1 phosphorylation were inhibited by miR-370-3p, whereas enhanced by miR-370-3p inhibitor (Figure 3D).

MiR-370-3p Targeted KDR to Trigger Akt Signaling Pathway

Previous evidence showed that miR-370-3p affects Akt signaling pathway activity. Thus, we searched the miRNA database and found that miR-370-3p was complimentary pairing with KDR mRNA (Figure 4A). Western blot demonstrated that KDR expression was upregulated in miR-370-3p inhibition group (Figure 4B). Luciferase assay showed that the level of KDR

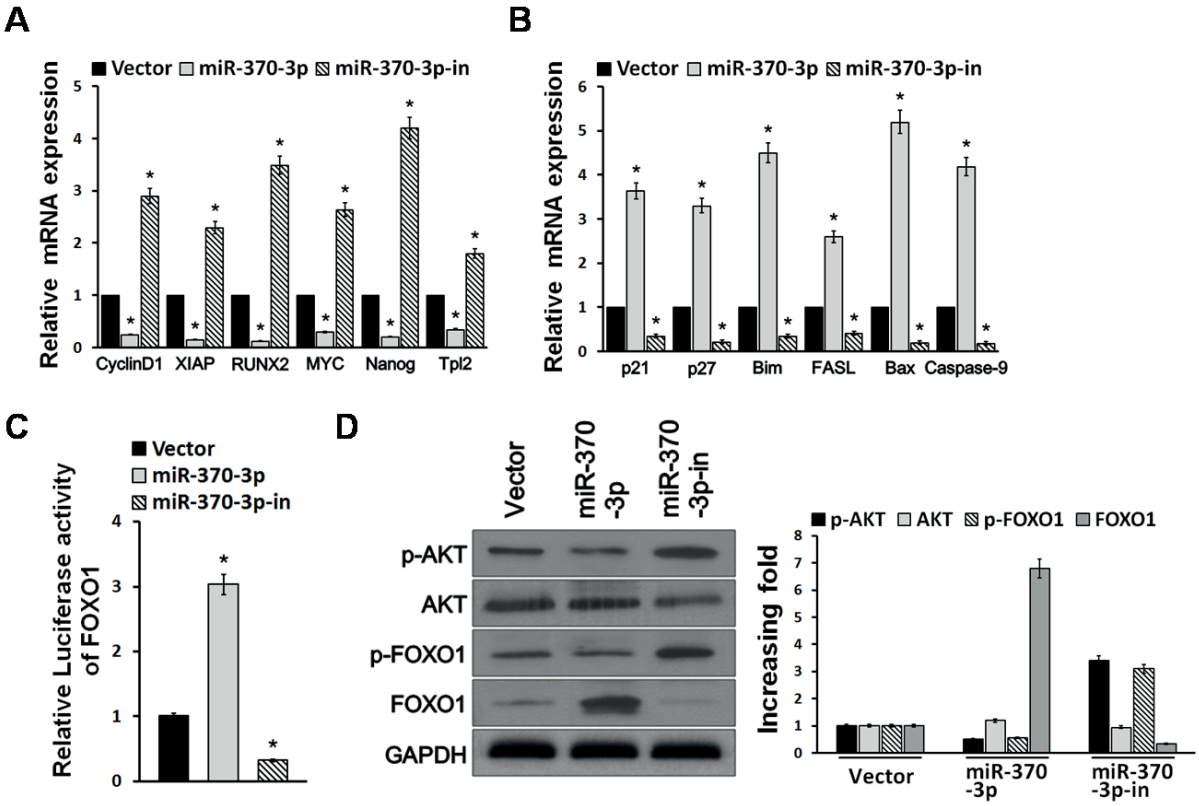

Figure 3. MiR-370-3p activated Akt/FOXO1 signaling pathway. ***(A)*** Cell proliferation related mRNAs expression detected by Real-time PCR in miR-370-3p or miR-370-3p-in transfected HUASMCs. ***(B)*** Cell apoptosis-related mRNAs expression tested by Real-time PCR in miR-370-3p or miR-370-3p-in transfected HUASMCs. ***(C)*** Luciferase assay detection of FOXO1 activity in miR-370-3p or miR-370-3p-in transfected HUASMCs. ***(D)*** Western blot detection of Akt and FOXO1 phosphorylation in miR-370-3p or miR-370-3p-in transfected HUASMCs. * $p < 0.05$, compared with control.

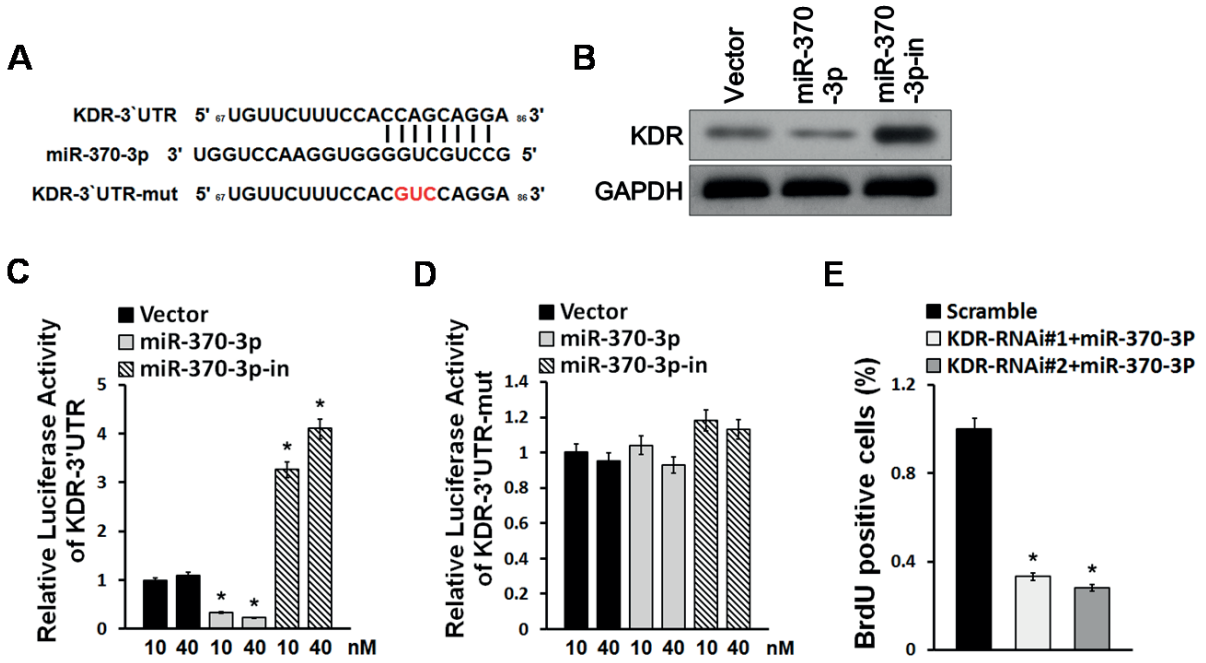

Figure 4. MiR-370-3p targeted KDR to activate Akt signaling pathway. ***(A)*** Predicted miR-370-3p target sequence in the 3' UTR of KDR. ***(B)*** Western blot detection of KDR protein expression in miR-370-3p or miR-370-3p-in transfected HUASMCs. ***(C)*** Dual-luciferase reporter assay of the HUASMCs transfected with the KDR-3' UTR reporter and miR-370-3p or miR-370-3p-in. ***(D)*** Dual-luciferase reporter assay of the HUASMCs transfected with the KDR-3' UTR-mut reporter and miR-370-3p or miR-370-3p-in. ***(E)*** BrdU assay determination of cell proliferation in miR-370-3p together with KDR siRNA transfected HUASMCs. * $p < 0.05$, compared with control.

3'UTR was declined in miR-370-3p transfected HUASMCs in a dose-dependent manner (Figure 4C). Meanwhile, there was no significant impact on the mutated KDR 3'UTR (Figure 4D). Moreover, we co-transfected miR-370-3p and KDR siRNA to judge their influences on Akt signaling pathway (Figure 4E).

Discussion

Intracranial aneurysm refers to the limitation of the cerebral artery lumen expansion of the arterial wall caused by a tumor-like prominent. Intracranial aneurysms, due to the local cerebral arterial wall of the congenital defects and increased pressure by cystic bulge, it is the main cause of subarachnoid hemorrhage.

Vascular smooth muscle deletion or hypoplasia is found in a variety of cerebral aneurysms[7,17]. As an important channel on cell surface, KDR contains a cerebral arterial current by electrophysiological experiments[18]. Moreover, since it is also known to be a vascular endothelial growth factor receptor, KDR plays multiple roles in the neointimal formation and adventitial angiogenesis[19,20]. At present, KDR is found to play a role in mediating vascular smooth muscle cell proliferation of venous-derived grafts under hypoxia[21]. Therefore, further elucidating the mechanism of vascular smooth muscle cell proliferation is of great significance in cerebral aneurysm. MiRNAs are small, single-stranded, and noncoding RNAs that are derived from the hairpin pre-miRNA precursors at 70-100 nucleotides in the cytoplasm, which was cleaved by RNaseIII Dicer to the mature form at 18-22 nucleotides. MiRNAs perfect or non-perfect complementarily bind with the 3'UTR of mRNAs with potentially numerous of genes, thus to degrade or inhibit the translation of target mRNAs[22]. Moreover, miRNAs are critical biological molecules in biological development[23]. The results of the present study revealed that miR-370-3p plays an important role in cell proliferation of vascular smooth muscle cells. Each miRNA has various target genes[24,25]. We found that miR-370-3p functions as negative regulator of vascular smooth muscle cell growth. It was also showed that miR-370-3p has a suppressor role in glioma via inducing cell cycle arrest and restoring

sensitivity to temozolomide[26,27]. We observed that miR-370-3p exerts its function in HUASMCs by inhibiting KDR expression. Therefore, it is possible that miR-370-3p acts a crucial role in cerebral aneurysm development. Since miR-370-3p may interact with several other mRNAs, the network of proteins, which are regulated by miR-370-3p, needs further investigation.

It has been reported that KDR was regulated by miRNA in many kinds of research. MiR-424 has been revealed to play a negative role in a KDR-dependent manner in regulating endothelial differentiation of dental pulp cells[28]. Additionally, miR-126 regulates innate response that operates through multiscale control of plasmacytoid dendritic cells via targeting KDR[29]. Although KDR is known to play a role in promoting angiogenesis, whether KDR is also affected by miRNAs in cerebral vascular smooth muscle proliferation is unclear. In our study, miR-370-3p is overexpressed in cerebral aneurysm tissues, which in turn suppressed KDR protein. These results proved a negative correlation between miR-370-3p and KDR in cerebral aneurysm. HUASMCs were obtained to test the regulation of miR-370-3p and KDR. The luciferase activity was applied to test whether miR-370-3p targets KDR. The result demonstrated that miR-370-3p directly targets to 3'UTR of KDR. It is also showed that FOXO1 activity is significantly enhanced in HUASMCs with miR-370-3p transfection. Meanwhile, Akt and FOXO1 phosphorylation were reduced by miR-370-3p mimics, and were elevated by the miR-370-3p inhibitor. Thus, it is reasonable to assume that miR-370-3p suppressed Akt/FOXO1 signaling pathway which plays an important role in cell proliferation. In summary, miRNAs have exhibited as therapeutic targets to study protein networks and gene regulation in cerebral aneurysm. Numerous miRNAs are overexpressed in cerebral aneurysm tissues, which could be the causative factor of intracranial aneurysm development. MiR-370-3p is implicated in the progression of various cancers, and appears to be involved in inhibiting cell proliferation related signal pathways. KDR contributes to the angiogenesis of vascular smooth muscle cells in cerebral aneurysm. KDR regulation could be a crucial step in preventing the development of intracranial aneurysm.

Conclusions

Our current report supplies experimental evidence that supports the effect of miR-370-3p in regulating Akt/FOXO1 signaling pathway. Further characterization of these signaling pathways and methods of suppressing miR-370-3p activity might prove valuable information for the treatment of cerebral aneurysm.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Molyneux A, Kerr R, Stratton I, Sandercock P, Clarke M, Shrimpton J, Holman R, International Subarachnoid Aneurysm Trial Collaborative G. International Subarachnoid Aneurysm Trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomised trial. Lancet 2002; 360: 1267-1274.
2) Ding W, Gao N, Li MX, Ding LJ, Li FF, Mou L. Clinical evaluation of the efficacy of the combination of aneurysm embolization and cerebrospinal fluid replacement in the treatment of aneurysmal subarachnoid hemorrhage. Eur Rev Med Pharmacol Sci 2015; 19: 402-405.
3) Mohan D, Munteanu V, Coman T, Ciurea AV. Genetic factors involves in intracranial aneurysms--actualities. J Med Life 2015; 8: 336-341.
4) Poelma C, Watton PN, Ventikos Y. Transitional flow in aneurysms and the computation of haemodynamic parameters. J R Soc Interface 2015; 12: pii: 20141394.
5) Matsukawa H, Shinoda M, Fujii M, Uemura A, Takahashi O, Niimi Y. Arterial stiffness as a risk factor for cerebral aneurysm. Acta Neurol Scand 2014; 130: 394-399.
6) Shimamura N, Ohkuma H. Phenotypic transformation of smooth muscle in vasospasm after aneurysmal subarachnoid hemorrhage. Transl Stroke Res 2014; 5: 357-364.
7) Starke RM, Chalouhi N, Ding D, Raper DM, McKisic MS, Owens GK, Hasan DM, Medel R, Dumont AS. Vascular smooth muscle cells in cerebral aneurysm pathogenesis. Transl Stroke Res 2014; 5: 338-346.
8) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
9) Sun G, Hou YB, Jia HY, Bi XH, Yu L, Chen DJ. MiR-370 promotes cell death of liver cancer cells by Akt/FoxO3a signalling pathway. Eur Rev Med Pharmacol Sci 2016; 20: 2011-2019.
10) Bekelis K, Kerley-Hamilton JS, Teegarden A, Tomlinson CR, Kuintzle R, Simmons N, Singer RJ, Roberts DW, Kellis M, Hendrix DA. MicroRNA and gene expression changes in unruptured human cerebral aneurysms. J Neurosurg 2016; 37: 1-10.
11) Su XW, Chan AH, Lu G, Lin M, Sze J, Zhou JY, Poon WS, Liu Q, Zheng VZ, Wong GK. Circulating microRNA 132-3p and 324-3p profiles in patients after acute aneurysmal subarachnoid hemorrhage. PloS One 2015; 10: e0144724.

12) Lee HJ, Yi JS, Lee HJ, Lee IW, Park KC, Yang JH. Dysregulated expression profiles of micrornas of experimentally induced cerebral aneurysms in rats. J Korean Neurosurg Soc 2013; 53: 72-76.

13) Chaturvedi P, Chen NX, O'Neill K, McClintick JN, Moe SM, Janga SC. Differential miRNA expression in cells and matrix vesicles in vascular smooth muscle cells from rats with kidney disease. PLoS One 2015; 10: e0131589.

14) Elliott KJ, Bourne AM, Takayanagi T, Takaguri A, Kobayashi T, Eguchi K, Eguchi S. ADAM17 silencing by adenovirus encoding miRNA-embedded siRNA revealed essential signal transduction by angiotensin II in vascular smooth muscle cells. J Mol Cell Cardiol 2013; 62: 1-7.

15) Wu ZW, Liu YF, Wang S, Li B. miRNA-146a induces vascular smooth muscle cell apoptosis in a rat model of coronary heart disease via NF-kappaB pathway. Genet Mol Res 2015; 14: 18703-18712.

16) Ding Y, Liao W, Yi Z, Xiang W, He X. Association of miRNA-145 expression in vascular smooth muscle cells with vascular damages in patients with lupus nephritis. Int J Clin Exp Patho 2015; 8: 12646-12656.

17) Ali MS, Starke RM, Jabbour PM, Tjoumakaris SI, Gonzalez LF, Rosenwasser RH, Owens GK, Koch WJ, Greig NH, Dumont AS. TNF-alpha induces phenotypic modulation in cerebral vascular smooth muscle cells: implications for cerebral aneurysm pathology. J Cerebr Blood F Met 2013; 33: 1564-1573.

18) Luykenaar KD, Welsh DG. Activators of the PKA and PKG pathways attenuate RhoA-mediated suppression of the KDR current in cerebral arteries. Am J Physiol Heart Circ Physiol 2007; 292: H2654-2663.

19) Bhardwaj S, Roy H, Babu M, Shibuya M, Yla-Herttuala S. Adventitial gene transfer of VEGFR-2 specific VEGF-E chimera induces MCP-1 expression in vascular smooth muscle cells and enhances neointimal formation. Atherosclerosis 2011; 219: 84-91.

20) Zaccone V, Flore R, Santoro L, De Matteis G, Giupponi B, Li Puma DD, Santoliquido A. Focus on biological identity of endothelial progenitors cells. Eur Rev Med Pharmacol Sci 2015; 19: 4047-4063.

21) Chanakira A, Dutta R, Charboneau R, Barke R, Santilli SM, Roy S. Hypoxia differentially regulates arterial and venous smooth muscle cell proliferation via PDGFR-beta and VEGFR-2 expression. Am J Physiol Heart C 2012; 302: 1173-1184.

22) Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, Liu CG, Bhatt D, Taccioli C, Croce CM. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA 2007; 297: 1901-1908.

23) Ruvkun G, Wightman B, Ha I. The 20 years it took to recognize the importance of tiny RNAs. Cell 2004; 116: 93-96.

24) Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15-20.

25) Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003; 115: 787-798.

26) Peng Z, Wu T, Li Y, Xu Z, Zhang S, Liu B, Chen Q, Tian D. MicroRNA-370-3p inhibits human glioma cell proliferation and induces cell cycle arrest by directly targeting beta-catenin. Brain Res 2016; 1644: 53-61.

27) Gao YT, Chen XB, Liu HL. Up-regulation of miR-370-3p restores glioblastoma multiforme sensitivity to temozolomide by influencing MGMT expression. Sci Rep 2016; 6: 32972.

28) Liu W, Gong Q, Ling J, Zhang W, Liu Z, Quan J. Role of miR-424 on angiogenic potential in human dental pulp cells. J Endodont 2014; 40: 76-82.

29) Agudo J, Ruzo A, Tung N, Salmon H, Leboeuf M, Hashimoto D, Becker C, Garrett-Sinha LA, Baccarini A, Merad M, Brown BD. The miR-126-VEGFR2 axis controls the innate response to pathogen-associated nucleic acids. Nat Immunol 2014; 15: 54-62.