MicroRNA-182 prevents vascular smooth muscle cell dedifferentiation via FGF9/PDGFRβ signaling

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Abstract. The abnormal phenotypic transformation of vascular smooth muscle cells (SMCs) causes various proliferative vascular diseases. MicroRNAs (miRNAs or miRs) have been established to play important roles in SMC biology and phenotypic modulation. This study revealed that the expression of miR-182 was markedly altered during rat vascular SMC phenotypic transformation in vitro. We aimed to investigate the role of miR-182 in the vascular SMC phenotypic switch and to determine the potential molecular mechanisms involved. The expression of miR-182 gene was significantly downregulated in cultured SMCs during dedifferentiation from a contractile to a synthetic phenotype. Conversely, the upregulation of miR-182 increased the expression of SMC-specific contractile genes, such as α-smooth muscle actin, smooth muscle 22α and calponin. Additionally, miR-182 overexpression potently inhibited SMC proliferation and migration under both basal conditions and under platelet-derived growth factor-BB stimulation. Furthermore, we identified fibroblast growth factor 9 (FGF9) as the target gene of miR-182 for the phenotypic modulation of SMCs mediated through platelet-derived growth factor receptor β (PDGFRβ) signaling. These data suggest that miR-182 may be a novel SMC phenotypic marker and a modulator that may be used to prevent SMC dedifferentiation via FGF9/PDGFRβ signaling.

Introduction

Vascular smooth muscle cells (SMCs) retain remarkable plasticity to alternate from a differentiated to a dedifferentiated phenotype at different developmental stages or local environmental cues (1). The cellular switching process of SMCs from a quiescent contractile differentiated phenotype which is associated with the high expression of smooth muscle-specific marker genes, such as α-smooth muscle actin (SMA), smooth muscle 22α (SM22α) and calponin, to a synthetic dedifferentiated phenotype which is associated with decreased levels of the marker genes plays a critical role in many proliferative vascular diseases (1-3). This phenotypic switch is believed to be essential for vascular repair (3). However, for various cardiovascular diseases, the inhibition of abnormal switching and the control of SMC proliferation are critical therapeutic strategies.

MicroRNAs (miRNAs or miRs) are non-coding RNAs measuring ~22 nucleotides in length. They act as post-transcriptional negative repressors of protein expression by binding to the 3'-untranslated region (3'-UTR) of their target messenger RNAs (mRNAs) (4-6). miRNAs are known to play a critical role in cancer and cardiovascular disorders (7). Several miRNAs have emerged as important modulators of vascular SMC function and phenotype (8-10). The expression of miR-182 has been previously shown to be altered in various types of cancer, and it inhibits the proliferation and migration of rat Schwann cells (11-13).

Fibroblast growth factor (FGF) 9, a member of the FGF family, is a potent mitogen secreted from bone marrow cells (14,15). FGF9 has been reported to be one of the direct targets of miR-182, which is associated with a variety of vessel biological processes (13,16). Frontini et al revealed that FGF9 stimulates smooth muscle cells (SMCs) wrapping microvessels in implants required sonic hedgehog (SHH) and platelet-derived growth factor (PDGF)Rβ (16). PDGFs and their receptors (PDGFRs) are implicated in blood vessel pathophysiology (17). PDGFRβ, expressed in perivascular mesenchyme, particularly in vascular mural cell (vascular SMCs and pericytes), has been established to play important roles in SMC differentiation and dedifferentiation (17,18).

In this study, we found that miR-182 expression in cultured rat vascular SMCs was decreased with prolonged incubation (number of days) or with platelet-derived growth factor (PDGF)-BB treatment. Despite great advances in vascular SMC biology, the molecular mechanisms responsible for SMC phenotypic modulation remained unclear. Thus, the purpose of the study was to investigate the role of miR-182 in the vascular SMC phenotypic switch and to determine the underlying molecular mechanisms.

Key words: vascular smooth muscle cells, microRNA-182, fibroblast growth factor 9, platelet-derived growth factor receptor β
Materials and methods

Rat vascular smooth muscle cell isolation. The animal protocols used were approved by the Scientific Affairs Committee of Animal Research and Ethics of the 2nd Hospital of Harbin Medical University. Vascular SMCs were isolated using the enzymatic dissociation method as previously described (19). Abdominal and thoracic aortic segments were isolated from Sprague-Dawley (SD) rats (weighting about 180 g) under general anesthesia. All rats in this study were obtained from the Model Animal Center of Harbin Medical University. After removing the connective tissue, adventitia and the endothelial layer, aortas were sliced into 1-2-mm-thick fragments and incubated with Dulbecco's modified Eagle's medium (DMEM)/F12 with the addition of type 2 collagenase (1.4 mg/ml) (Sigma, St. Louis, MO, USA) for 4-6 h in a 37°C incubator with 95% air and 5% CO2. The fragments were agitated to release SMCs and the cells were centrifuged at 300 x g for 5 min. SMCs were then cultured in DMEM/F12 medium added with 10% fetal bovine serum in an incubator as described above at least for 5 days prior to the first time trypsin digestion. The primary cultured SMCS were then passed every 3 days, and the 4-6 passages were used. In addition, SMCS released from the arterial fragments (0 days), cultured SMCS at 5 day, 10 and 15 days were also collected for examination.

Transfection of cultured SMCS with oligonucleotides. Oligonucleotide (oligo; Bioneer Co., Ltd., Daedeon, Korea) transfection was based on the instruction of Roche X-tremeGENE siRNA Transfection Reagent (Roche, Mannheim, Germany). For miR-182 overexpression or silencing, miR-182 mimics or inhibitors were added directly to the complexes to different final concentrations (miR-182 mimic: sense, GCG GGU CUA GCU GCC GGA and antisense, CCG CAG CUA GAC CCG CUU; miR-182 inhibitor: UUU GCC AAG GGU AGA ACU CAC ACC G). A small interfering RNA (siRNA) was used to degrade the target mRNAs for fibroblast growth factor 9 (FGF9) gene silencing as previously described (sense, CUG UCU CAC ACC G; antisense, AGG UGU UCG UAC AGG UUG GAA G) (20). Briefly, following trypsin digestion, SMCS were inoculated in a 6-well plate. Twenty-four hours later, the X-tremeGENE siRNA transfection reagent was used to treat the cells in a 1:4 ratio for 20 min. Further treatment included transfection with siRNA followed by incubation in medium comprising 2 ml of serum-free Opti-MEM (Invitrogen, Carlsbad, CA, USA) using transfection reagents (vehicle), negative control, inhibitor negative control (Bioneer Co., Ltd.), and siRNA control (Invitrogen). The transfection efficiency of miR-182 mimic and inhibitor was then examined by reverse transcription-quantitative PCR (RT-qPCR) and the efficiency of FGF9 siRNA was examined by western blot analysis, as described below. Forty-eight hours later, the engineered SMCS were analyzed further.

miRNA and mRNA analysis by RT-qPCR. Following oligo transfection and stimulation with or without PDGF-BB, total RNA was extracted from the SMCS using TRIZol reagent (15596-026; Invitrogen) according to the instructions of the manufacturer. After the abdominal and thoracic aortic segments were isolated from the anesthetized rats, organs including aorta, muscle, heart, lung, liver and kidney were also removed. Total RNA was then extracted from these organs based on the instructions of the manufacturer of TRIZol reagent. RNA was then reverse transcribed into cDNA using the First Stand miRcute miRNA cDNA Synthesis kit according to the instructions of the manufacturer (Tiangen Biotech, Beijing, China). The miR-182 level was analyzed by quantitative polymerase chain reaction (qPCR) using the Tiangen miRcute miRNA qPCR detection system (Poly A tail addition method; miR-182 primer, TTT GGC AAT GGT AGA ACT CAC ACC; U6 primer sequence, ACA CGC AAA TTC GTG AAG CGT TCC). The RT-qPCR for FGF9, platelet-derived growth factor receptor β (PDGFRβ), SMA, SM22α and calponin was performed using the Bioneer Accupower GreenStar qPCR PreMix system (SMA forward, GTC AGG TCA TCA CTA TCC GCA GAT and reverse, AGA GGT CTT TAG CAA GTG CA; SM22α forward, ATG GCC AAC AGG GGT CCA TCC and reverse, TCC ATC TGC TTG AAG ACC ATG; calponin forward, AGAGAA GGC AGG AAC AAT TTC TGG AGG GTT GGC TTT; PDGFRβ forward, AGG ACA ACC GTA CTT TGG GTG ACT and reverse, CAG TCC TGA CAC GTA CCT CCG GGT CTG). The expression of miR-182 relative to U6 and SMA, SM22α, calponin, FGF9, and PDGFRβ relative to β-actin was determined by the 2^-ΔΔCt method.

Western blot analysis. Cellular proteins were extracted for western blot analysis as previously described (20). The cell lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and run under standard conditions. Subsequently, the proteins were removed to polyvinylidene fluoride (PVDF) membranes. After blocking with 5% nonfat milk at room temperature for 1 h, the PVDF membranes were incubated with diluted primary antibodies SMA (1:1400 dilution; sc-53142), SM22α (1:300 dilution; sc-51442) (both from Santa Cruz Biotechnology, Santa Cruz, CA, USA), FGF9 (1:800 dilution; ab9743), PDGFRβ (1:1000 dilution; ab32570) (both from Abcam, Cambridge, MA, USA), and antibody against GAPDH (1:10,000 dilution; sc-77274; Santa Cruz Biotechnology) at 4°C overnight. The membranes were washed, further incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies (anti-mouse and anti-rabbit, ZB-2305 and ZB-2305; both from Zhongshan Goldenbridge Biotechnology, Beijing, China) at 37°C. Subsequently, the membranes were detected with BeyoECL Plus (Beyotime Institute of Biotechnology, Haimen, China), and further analysis of protein band densitometry was facilitated by Quantity One software (Bio-Rad, Hercules, CA, USA).

SMC proliferation assay. SMCs at the 4th to 6th passages were trypsinized and plated into 96-well culture plates (5x10^3 cells/well) in DMEM/F-12 complete medium. After 24 h, the SMCs were rendered quiescent in DMEM/F-12 supplemented with 0.5% FCS for 24 h. The proliferation of the SMCs was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Solarbio, Beijing, China) or by 5-ethyl-2′-deoxyuridine (EdU) proliferation assay (Ribo-Bio Co., Ltd., Guangzhou, China). MTT assays were conducted as previously described (8,9). In brief, the SMCs in each well were incubated with 20 μl MTT mixture (5 mg/μl). Four hours later, formazan product was dissolved with dimethyl sulfoxide (DMSO) and the absorbance read at 570 nm with the help of a microplate reader.
miR-182 is a novel marker for rat vascular smooth muscle cell (SMC) phenotypic modulation. The levels of the differentiation marker genes, including SMA, SM22α and calponin, declined eventually with the prolonged incubation of rat vascular SMCs in vitro (Fig. 1A). During the phenotypic switch, the biosynthesis of miR-182 was also gradually diminished. On the 5th day of culture, the level of miR-182 in the SMCs decreased to almost half that of the level at 0 days. To examine the implications of this regulation, the SMCs were treated with various concentrations of PDGF-BB, a powerful stimulant of SMC dedifferentiation. Treatment with PDGF-BB induced a decrease in miR-182 expression in a dose-dependent manner; treatment with 10 ng/ml PDGF-BB markedly reduced miR-182 expression; the expression levels of miR-182 did not differ significantly between the SMCs stimulated with PDGF-BB 20 and 40 ng/ml (Fig. 1B). Furthermore, the miR-182 distribution levels in the aorta, heart, muscle, lung, liver and kidney were examined by RT-qPCR. The expression level of miR-182 in the aorta was higher than that in other tissues (Fig. 1C). Taken together, these findings suggest that miR-182 may be a novel marker for SMC phenotypic modulation.

miR-182 is a novel phenotypic modulator of vascular SMCs. To explore the potential effect of miR-182 during SMC phenotypic modulation, gain-of-function and loss-of-function experiments were performed to transfect oligos into rat vascular SMCs. For miR-182 overexpression, we added miR-182 mimics at a final concentration of 20, 40, 60 or 80 nM to culture medium. miR-182 expression was increased depending on the oligo concentrations (Fig. 2A). Conversely, miR-182 expression was markedly inhibited by transfection with 80 nM miR-182 inhibitors. Additionally, the gene levels of SMC-specific markers, including SMA, SM22α and calponin, were distinctly decreased by transfection with miR-182 mimics, and the marker gene levels were significantly decreased by transfection with miR-182 inhibitors (Fig. 2B). Similarly, as shown in Fig. 2C, the results of western blot analysis revealed that the transfection of SMCs with miR-182 mimic or inhibitor altered the protein levels of SMA and SM22α.

Subsequently, the biological role of miR-182 in primary cultured rat vascular SMC morphology was investigated. The primary cultured SMCs at passages 4 to 6 transfected with miRNA negative control presented a flattened morphology as a synthetic phenotype (Fig. 2D). The overexpression of miR-182 altered SMC morphology from a flattened to a spindle-like contractile state. The cells flattened progressively when transfected with miR-182 inhibitors or treated with PDGF-BB (10 ng/
miR-182 overexpression helped the SMCs retain their contractile morphology and prevented the phenotypic switch. On the whole, these findings suggest that miR-182 is essential in modulating the rat vascular SMC phenotype.

miR-182 inhibits rat vascular SMC proliferation and migration. Typical images of EdU cell proliferation are shown in Fig. 3A. miR-182 overexpression markedly suppressed SMC proliferation even with PDGF-BB treatment in vitro. In addition, PDGF-BB treatment increased SMC proliferation, which was further enhanced by transfection with miR-182 inhibitors. MTT assay was also performed to determine the mitochondrial activity. Transfection with miR-182 mimics at 80 nM significantly inhibited cell proliferation to a greater extent than at 20 and 40 nM (Fig. 3B).

Furthermore, we examined the effect of miR-182 on SMC migration in vitro by scratch-wound healing assay. The migration of SMCs transfected with miR-182 mimics was markedly inhibited even with PDGF-BB stimulation (Fig. 3C and D). However, transfection with miR-182 inhibitors increased the PDGF-BB-induced migration of SMCs.

To the best of our knowledge, for the first time, in this study, we established that miR-182 effectively suppressed the proliferation and migration of rat vascular SMCs under both quiescent conditions and PDGF-BB stimulation.

miR-182 prevents SMC phenotypic modulation via FGF9/ PDGFRβ signaling. miRNAs exert their biological functions by inhibiting the transcription or translation of their target genes. We used the microRNA.org database to identify the target genes of miR-182. FGF9 is a target gene of miR-182 across multiple species, such as human, rat, and mouse (Fig. 4A). Using qPCR, we discovered that transfection with miR-182 mimic or inhibitor slightly inhibited or increased the FGF9 mRNA levels, respectively (Fig. 4B). However, using western blot analysis, the protein levels of FGF9 were significantly affected by miR-182 regulation. The FGF9 levels were decreased when the SMCs were transfected with miR-182 mimics at 80 nM more than at 40 nM (Fig. 4C). On the contrary, the protein level of FGF9 was increased by transfection with miR-182 inhibitors at 80 nM more than 40 nM (Fig. 4D). These data suggest that FGF9 is the direct target gene of miR-182 in rat vascular SMCs.

To determine the role of FGF9 in rat SMCs, we used FGF9 siRNA transfection to achieve FGF9 downregulation. The protein levels of the SMC-specific contractile markers, SMA and SM22α, were significantly upregulated by FGF9 siRNA transfection (Fig. 5A). The protein levels of PDGFRβ were decreased with the knockdown of FGF9. Additionally, as shown in Fig. 5B and C, the knockdown of FGF9 significantly inhibited SMC proliferation with or without PDGF-BB stimulation, as determined by MTT and EdU assays. Moreover, when the SMCs were transfected with FGF9 siRNA, the migration induced by PDGF-BB was also significantly depressed (Fig. 5D and E). Therefore, the silencing of FGF9 suppressed the differentiation, proliferation and migration of rat SMCs.

Subsequently, miR-182 inhibitors and FGF9 siRNA were both transfected into the SMCs. The downregulation of miR-182 increased SMC proliferation and migration, and decreased the expression of dedifferentiated marker genes (Fig. 6A-C). However, FGF9 knockdown interfered with the function of miR-182 inhibitors on SMC proliferation and migration, but induced an increase in the expression of SMA, SM22α, and calponin. Overall, our data indicate that FGF9 is a direct target gene of miR-182 in rat vascular SMCs, and is critical in the process of miR-182-mediated rat vascular SMC phenotypic modulation.

We hypothesized that the inhibition of SMC phenotypic modulation via the upregulation of miR-182 or the downregulation of FGF9 was related to PDGFRβ signaling. PDGF-BB
induced an increase in PDGFRβ gene expression, while transfection with miR-182 mimics significantly inhibited PDGFRβ expression with or without PDGF-BB stimulation (Fig. 6D). Conversely, the gene levels of PDGFRβ were upregulated following transfection with miR-182 inhibitors. PDGF-BB-induced a significant increase in the PDGFRβ protein level, while miR-182 overexpression markedly suppressed PDGFRβ expression (Fig. 6E and F). In addition, transfection with FGF9 siRNA decreased PDGFRβ expression (Fig. 5A). Thus, these data indicate that FGF9/PDGFRβ signaling is critical for the miR-182-mediated prevention of the SMC phenotypic switch.
Discussion

In the present study, to the very best of our knowledge, we demonstrate for the first time miR-182 is a novel marker which has the capacity to modulate the rat vascular SMC phenotype. The critical target gene of miR-182 is FGF9, and miR-182 mediates the differentiation, proliferation and migration of rat vascular SMCs via FGF9/PDGFRβ signaling.

Despite great advances in vascular SMC biology, the molecular mechanisms responsible for the SMC phenotypic modulation remain unclear. miR-182 has been shown to predict survival in patients with various types of cancer (21). Increasing evidence indicates the important roles of miR-182 in cell biology. miR-182 has been shown to predict survival in patients with various types of cancer (21). Kouri et al. found that miR-182 targeted Bcl-2-like 12, c-Met and hypoxia-inducible factor 2α to regulate the apoptosis, growth and differentiation programs of glioma-initiating cells (31). miR-182 has also been shown to inhibit Schwann cell proliferation and invasion following nerve injury and repair (13).

In the present study, the overexpression of miR-182 inhibited the dedifferentiation of cultured vascular SMCs, as well as SMC proliferation and migration. Additionally, miR-182 overexpression prevented the SMCs from switching from a contractile morphology to a synthetic phenotype. Thus, miR-182 is a novel phenotypic modulator of SMC differentiation, proliferation and migration.

miRNAs exert their biological functions by inhibiting the transcription or translation of target genes (4,6). By using the findings of previous studies that dispersed SMCs cultured in vitro exhibited rapidly downregulated SMC-specific gene expression (29,30). During the period that the SMC phenotype switches from a contractile to a synthetic type, the expression of miR-182 was also demonstrated to gradually decrease. Additionally, the miR-182 levels significantly decreased with PDGF-BB stimulation in a dose-dependent manner. For the first time, to the best of our knowledge, we established that miR-182 is a novel phenotypic marker for SMCs.
microRNA.org database, we identified that FGF9 is one of the target genes of miR-182 across species. FGF9 combined with its cognate receptors is able to induce cell dedifferentiation and maturation (32). In addition, Yu et al proved that miR-182 inhibited FGF9 expression by binding to the 3'-UTR in rat Schwann cells using Luciferase reporter assay (13). These data suggest that FGF9 may be a direct target of miR-182 in rat vascular SMCs.

This study revealed a dose-dependent decrease in FGF9 protein expression in miR-182 mimic-transfected SMCs. Accordingly, FGF9 is the target gene of miR-182 in rat vascular SMCs.

The downregulation of FGF9 or upregulation of miR-182 induced a similarly protective effect on the SMC phenotype and an inhibitory effect on proliferation and migration. miR-182 downregulation induced SMC differentiation, proliferation and migration; however, these processes were prevented by FGF9 knockdown. Moreover, the inhibition of FGF9 itself was able to suppress the dedifferentiation, proliferation and migration of rat SMCs. Although FGF9 was only one of the target genes, the results suggest that FGF9 is critical in the process of miR-182-mediated rat vascular SMC phenotypic modulation.

The activation of PDGFRβ is essential for the proliferation and migration of a variety of cells, and FGF9 has been implicated in upregulating of PDGFRβ expression (16,18). Frontini et al reported that the FGF9-induced migration of cultured SMCs and neovascularization required both PDGFRβ and SHH, but not ERK1/2 (16). PDGF-AB or -BB binding with the PDGFRβ or the receptor overexpression results in the activation of PI3K/Akt, PLCγ1 and MAPK pathways (33-36). PDGFRβ is a critical downstream target of FGF9 and an important signaling for FGF9-induced mural cell recruitment and vessel maturation (16). Thus, we hypothesized that the inhibition of SMC phenotypic modulation via the upregulation of miR-182 or the downregulation of FGF9 was related to PDGFRβ signaling. In this study, the expression of PDGFRβ was demonstrated to be markedly altered by regulating the expression of miR-182 and FGF9. Indeed, the downregulation of FGF9 decreased PDGFRβ expression, whereas the upregulation of FGF9 increased the PDGFRβ levels in rat vascular SMCs. In addition, PDGF-BB induced an increase in the expression of PDGFRβ, which was significantly inhibited by miR-182 overexpression, but was upregulated following miR-182 knockdown. Thus, FGF9/PDGFRβ signaling is critical in the miR-182-mediated SMC phenotypic switch.

In conclusion, the present study demonstrated that the miR-182 level was altered markedly during the phenotypic transformation of rat vascular SMCs in vitro. The upregulation of miR-182 inhibited SMC dedifferentiation, proliferation and migration under both basal conditions and PDGF-BB stimulation. FGF9 was the critical target gene of miR-182 in the process of miR-182-mediated rat vascular SMC pheno-
typical mutation. The downregulation of FGF9 increased SMC-specific contractile marker expression, and inhibited the proliferation and migration of SMCs via PDGFRβ signaling. This study suggests that miR-182 is a novel phenotypic marker, which modulates the differentiation, proliferation and migration of rat vascular SMCs via FGF9/PDGFRβ signaling. miR-182 may thus play a potential role in the diagnosis and treatment of vascular proliferative diseases.

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References

1. Duband JL, Gimona M, Scatena M, Sartore S and Small JV: Calponin and SM 22 as differentiation markers of smooth muscle: Spatiotemporal distribution during avian embryonic development. Differentiation 55: 1-11, 1993.
2. Owens GK, Kumar MS and Warnhoff BR: Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 84: 767-801, 2004.
3. Davis-Dusenberg BN, Wu C, Hata A and Sessa WC: Micromanaging vascular smooth muscle cell differentiation and phenotypic modulation. Arterioscler Thromb Vasc Biol 31: 2747-2757, 2011.
4. Kim VN: MicroRNA biogenesis: Coordinated cropping and dicing. Nat Rev Mol Cell Biol 6: 376-385, 2005.
5. Niwa R and Slack FJ: The evolution of animal microRNA function.Curr Opin Genet Dev 17: 145-150, 2007.
6. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116: 281-297, 2004.
7. Hammond SM: RNAi, microRNAs, and human disease. Cancer Chemother Pharmacol 58 (Suppl 1): s63-s68, 2006.
8. Davis BN, Hilyard AC, Nguyen PH, Lagna G and Hata A: Induction of microRNA-221 by platelet-derived growth factor signaling is critical for modulation of vascular smooth muscle phenotype. J Biol Chem 284: 3728-3738, 2009.
9. Wang YS, Wang HY, Liao YC, Tsai PC, Chen KC, Cheng HY, Lin RT and Juo SH: MicroRNA-182 regulates vascular smooth muscle cell phenotype and prevents neointimal formation. Cardiovasc Res 95: 517-526, 2012.
10. Li P, Zhu N, Yi B, Wang N, Chen M, You X, Zhao X, Solomides CC, Qin Y and Sun J: MicroRNA-663 regulates human vascular smooth muscle cell phenotypic switch and vascular neointimal formation. Circ Res 113: 1117-1127, 2013.
11. Stenvold H, Donnem T, Andersen S, Al-Saad S, Busund LT and Bremnes RM: Stage and tissue-specific prognostic impact of miR-182 in NSCLC. BMC Cancer 14: 138, 2014.
12. Mitroukoua EA, Arvieux C, Vaishnav A, Johnson DN, Giangreco AA, Martens-Balla E, Bagasra O, Kajdacsy-Balla A and Nonn L: miR-183-96-182 cluster is overexpressed in prostate cancer cell lines and in primary tumors. Cancer Lett 361: 118-125, 2015.
13. Yu B, Qian T, Wang Y, Zhou S, Ding G, Ding F and Gu X: miR-182 inhibits Schwann cell proliferation and migration by targeting FGF9 and NTM, respectively at an early stage following sciatic nerve injury. Nucleic Acids Res 40: 10356-10365, 2012.
14. Nairouk K, Seko C, Kurokawa K, Matsutani E, Sasada R, Kondo T and Kurokawa T: Novel secretory heparin-binding factors from human glioma cells (glia-activating factors) involved in glial cell growth. Purification and biological properties. J Biol Chem 268: 2857-2864, 1993.
15. Miyawaka K, Hatsuzawa K, Kurokawa T, Asada M, Kuroiwa T and Imamura T: A hydrophobic region locating at the center of fibroblast growth factor-9 is crucial for its secretion. J Biol Chem 274: 29352-29357, 1999.
16. Frontini MJ, Nong Z, Gros R, Drangova M, O’Neill C, Rahman MN, Akawi O, Yin H, Ellis CG and Pickering JG: Fibroblast growth factor 9 delivery during angiogenesis produces durable, vasoresponsive networks developed by smooth muscle cells. Nat Biotechnol 29: 421-427, 2011.
17. Andrae J, Gallini R, and Betsholtz C: Role of platelet-derived growth factors in physiology and medicine. Genes Dev 22: 1312-1321, 2008.
18. Hellström M, Kalén M, Lindahl P, Abramsson A and Betsholtz C: Role of PDGF-B and PDGF-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development 126: 3047-3055, 1999.
19. Metz RP, Patterson JL and Wilson E: Vascular smooth muscle cells: Isolation, culture, and characterization. Methods Mol Biol 843: 169-176, 2012.
20. Liu J, Wang Y, Du W, Liu W, Liu F, Zhang L, Zhang M, Hou M, Liu B, Zhang S and Wang B: Wnt1 inhibits hydroxyurea-induced apoptosis in mouse cardiac stem cells. PLoS One 8: e58883, 2013.
21. Wang F, Zhong S, Zhang H, Zhang W, Zhang H, Wu X and Chen B: Prognostic value of MicroRNA-182 in cancers: A meta-analysis. Dis Markers 2015: 482146, 2015.
22. Zhang W, Qian P, Zhang X, Zhang M, Wang H, Wu M, Kong X, Tan S, Ding K, Perry JK, et al: Autocrine/paracrine human growth hormone-stimulated MicroRNA-19a-96-182 cluster promotes epithelial-mesenchymal transition and invasion in breast cancer. J Biol Chem 290: 13812-13829, 2015.
23. Li Y, Zhang D, Wang X, Yao X, Ye C, Zhang S, Wang H, Chang C, Xia H, Wang YC, et al: Hsa-miR-182 enhances HIF1α signaling via targeting PDH2 and FH1 in prostate cancer. Sci Rep 5: 12495, 2015.
24. Du C, Weng X, Hu W, Lv Z, Huan C, Gyaobaba A, Xie H, Zhou L, Wu J and Zheng S: Hypoxia-inducible miR-182 promotes angiogenesis by targeting KASA1 in hepatocellular carcinoma. J Exp Clin Cancer Res 34: 67, 2015.
25. Chen L, Chu F, Cao Y, Shao J and Wang F: Serum miR-182 and miR-331-3p as diagnostic and prognostic markers in patients with hepatocellular carcinoma. Tumour Biol 36: 7439-7447, 2015.
26. Tang L, Chen F, Pang EJ, Zhang ZQ, Jin BW and Dong WF: MicroRNA-182 inhibits proliferation through targeting onecogenic ANUBL1 in gastric cancer. Oncol Rep 33: 1707-1716, 2015.
27. Sato F, Fang R, Li C and Chen X: miR-182 suppresses lung tumorigenesis through downregulation of RGS17 expression in vitro. Biochem Biophys Res Commun 396: 501-507, 2010.
28. Shu L, Suh J, Mufua FM, Pan Y, Panchakrishi R, Gottipati P, Muschel RJ, Beech J, Kulsrethra R, Abdelmohsen K, Weinstock DM, et al: miR-182-mediated downregulation of BRCA1 impacts DNA repair and sensitivity to PARP inhibitors. Mol Cell 41: 210-220, 2010.
29. Chamley-Campbell J, Connell RB and Ross R: The smooth muscle cell in culture. Physiol Rev 59: 1-61, 1979.
30. Shanahan CM and Weissberg PL: Smooth muscle cell heterogeneity: Patterns of gene expression in vascular smooth muscle cells in vitro and in vivo. Arterioscler Thromb Vasc Biol 18: 333-338, 1998.
31. Kouri FM, Hurley LA, Daniel WL, Day ES, Hua Y, Hao L, Peng CY, Merkel TJ, Queisser MA, Ritner C, Kouri FM, Hurley LA, Daniel WL, Day ES, Hua Y, Hao L, Peng CY, Merkel TJ, Queisser MA, Ritner C, et al: MiR-182 integrates apoptosis, growth, and differentiation programs in glioblastoma. Genes Dev 29: 732-745, 2015.
32. Cinaroglu A, Ozmen Y, Ozdemir A, Ozcan F, Ergorul C, Cayirlooglu P, Hicks D and Bugra K: Expression and possible function of fibroblast growth factor 9 (FGF9) and its cognate receptors FGFR2 and FGFR3 in postnatal and adult retina. J Neurosci Res 79: 329-339, 2005.
33. Heldin CH, Ostman A and Rönnstrand L: Signal transduction via platelet-derived growth factor receptors. Biochim Biophys Acta 1278: F79-F113, 1995.
34. Cosedal R, Abedi H and Zachary I: Platelet-derived growth factor-BB (PDGF-BB) regulation of migration and focal adhesion kinase phosphorylation in rabbit aortic vascular smooth muscle cells: Roles of phosphorylinsitol 3-kinase and mitogen-activated protein kinases. Cardiovasc Res 41: 708-721, 1999.
35. Song MC, Kim EC, Kim WJ and Kim TJ: Meso-dihydroguaiaretic acid inhibits rat aortic vascular smooth muscle cell proliferation by depressing phosphorylation of platelet-derived growth factor receptor beta. Eur J Pharmacol 744: 36-41, 2014.
36. Caglayan E, Vantler M, Leppänen O, Gerhard F, Mustafø L, Ten Freyhausen H, Kappert K, Odenthal M, Zimmermann WH, Tallquist MD and Rosenkranz S: Disruption of platelet-derived growth factor-dependent hypoxia-inducible factor 1a promotes carcinogenesis in a mouse model of phospholipase Cβ1 activity abolishes vascular smooth muscle cell proliferation and migration and attenuates neointima formation in vivo. J Am Coll Cardiol 57: 2527-2538, 2011.