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Original Article

MiR-126 enhances VEGF expression in induced pluripotent stem cell-derived retinal neural stem cells by targeting spred-1

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Abstract: Pathological retinal neovascularization (RNV) is a leading cause of vision loss in several ocular diseases; however, the underlying molecular mechanisms involved in the development of RNV remain unclear. It has been shown that microRNAs contribute to the process of angiogenesis, which has received greater attention by investigators who study the progression of RNV. In the present study, we investigated the function of miR-126 expression in retinal neural stem cells derived from induced pluripotent stem cell (IPSs) obtained from patients with RNV. During the induction process, the levels of both miR126 and vascular endothelial growth factor C (VEGF-C) gradually decreased, while the levels of spred-1 significantly increased. The existence of conserved miR-126-binding sites in spred-1 mRNA was predicted by computational algorithms, and verified by the luciferase reporter assay. The use of miR-126 mimics revealed dramatically reduced levels of spred-1, and increased levels of VEGF. When using shRNA to target spred-1, the resultant decreased levels of spred-1 were associated with significantly enhanced levels of VEGF expression. Our results demonstrate that miR-126 promotes VEGF expression in IPS cells by suppressing spred-1 expression, which contributes to angiogenesis during the progression of RNV. These findings suggest that miR-126 and spred-1 might serve as novel molecular targets for treating RNV-related ocular diseases.

Keywords: Pathological retinal neovascularization, induced pluripotent stem cell, micro-126, VEGF

Introduction

Pathological retinal neovascularization (RNV) is a leading cause of vision loss in several ocular diseases, including diabetic retinopathy, *retinal vein occlusion,* retinopathy of prematurity, and age-related macular degeneration. As RNV progresses, new capillary sprouts from the retinal veins grow out of the vitreous surface of the retina, causing vitreous hemorrhage and retinal detachment that eventually lead to vision loss and even blindness [1-4]. As the population ages, the incidence rates of ocular diseases caused by RNV have increased [5]. Several different mediators, including ADAM8, ROCK, Dll4, and VEGF contribute to the development of RNV [6-9]. While various therapeutic approaches have been developed and used to treat pathologic angiogenesis and RNV in the clinic [10], the exact underlying molecular mechanisms for these disorders remain unclear. Recent studies have shown that microRNAs contribute to the process of angiogenesis, which has gained more attention from investigators who study RNV progression.

MicroRNAs (miRNAs or miRs) comprise a group of small (19-25 nucleotides) and ubiquitously distributed endogenous non-coding single-stranded RNAs. MiRNAs post-transcriptionally modulate gene expression by binding to the 3' untranslated regions of their target mRNA molecules. This binding causes degradation of the target mRNAs and blocks production of their encoded proteins [11]. MiRNAs are implicated in various physiological processes and pathological conditions, including cell proliferation, differentiation, apoptosis, inflammatory responses, angiogenesis, cardiovascular diseases, and cancer [11-15].

Several recent studies have identified some miRNAs that mediate angiogenesis and play a

crucial role in RNV progression. These miRNAs are termed "angiomiRs", and include miR-23, miR-21, and miR-24. Fish et al and Wang et al showed that miR-126 is involved in both angiogenesis and maintenance of vascular integrity [16-18]. Therefore, it is important to gain a greater understanding of how microRNAs help regulate pathogenic angiogenesis and RNV, if new therapeutic approaches are to be developed for these disorders.

MiR-126 is a recently identified novel microRNA that is expressed by endothelial cells found in all blood vessels, and has been shown to play functional roles in cancer, diabetes, allergic asthma, and cystic fibrosis [19-22]. MiR-126 mainly functions in the regulation and control of angiogenesis by targeting critical angiogenetic factors such as EGFL7 and CRK [22, 23]. However, the mechanism by which miR-126 mediates the angiogenesis process during the development of RNV is remains under investigation. In the present study, we used pluripotent stem cells (IPSs) induced from peripheral blood mononuclear cells obtained from patients with RNV as an in vitro model. Our results revealed that the levels of miR126 and VEGF-C expression gradually decreased, while the level of spred-1 expression significantly increased during the induction process. MiR-126-binding sites in spred-1 mRNA were predicted by computational algorithms and verified by the luciferase reporter assay. The use of miR-126 mimics revealed reduced spred-1 levels and increased VEGF levels. When using spred-1 3'UTR miRNA, the resultant reduced levels of spred-1 significantly enhanced VEGF expression. This study showed that miR126 enhances VEGF expression in IPS cells by inhibiting spred-1 expression, which contributes to angiogenesis during the progression of RNV. Our results suggest that *spred-1*, the target gene of miR126, might serve as a novel target for treating RNV-related *ocular diseases.*

Materials and methods

Patients and sample collections

Twenty patients with RNV were included in this study, and provided the blood samples used for analysis. Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque 1077 reagents kits according to the manufacturer's instructions. Biopsy results were interpreted according to the Updated Banff 07 criteria by H.R. The protocols used in the present study were approved by Shenzhen Eye Hospital, and a signed written informed consent was obtained from each enrolled subject.

Cell culture and induction

CD34+ cells were isolated using an EasySep™ Human CD34 Positive Selection Kit according to the manufacturer's protocol. The cells were cultured in StemPro™34 SFM supplemented with SCF (100 ng/mL), FLT3 (100 ng/mL), IL-3 (20 ng/mL), and IL-6 (20 ng/mL) for 4 days after isolation. The cells were incubated in a 5% CO_2 incubator at 37°C, and then transduced using a CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Virus was removed after 24 hours of transduction, and the cells were plated onto rh-Vitronectin (VTN) matrix at 3 days after transduction. At 7 days after transduction, the medium was changed to Essential 8™ Medium. To induce neural differentiation, cell differentiation was induced by switching the cell culture medium from mTESR1 to retinal induction medium (RIM) containing DMEM/F12, N2 and B27 serum-free supplements, 100 units/mL penicillin, 100 μg/mL streptomycin (Life Technology, Carlsbad, CA, USA), 0.45% glucose (Sigma, St. Louis, MO, USA), 20 μg/mL human insulin (Roche, Basal Switzerland), and 50 ng/mL human Noggin (Peprotech, Rocky Hill, NJ. USA). On day 5, the RIM medium was switched to neural differentiation medium plus (NDM+), containing Neurobasal, N2 and B27 serum-free supplements, 100 units/mL penicillin, 100 μg/mL streptomycin, Glutamax, MEM non-essential amino acids (Life Technology), 0.45% glucose, and 50 ng/mL human Noggin. Cells were harvested for use in analytical procedures at each of the following 10 time points: 1) PBMCs were isolated from peripheral blood; 2) CD34+ cells were isolated from PBMCs; 3) two weeks the induction of iPS cells; 4) iPS cells were generated; 5) second passage of the iPS cells; 6) third passage of the iPS cells; 7, 8, 9, 10) day 1, day 3, day 5, and day 7 of the retinal neural stem cell induction process.

Immunocytochemical staining

Cells were fixed by incubation with 4% paraformaldehyde for 2 minutes, and then washed

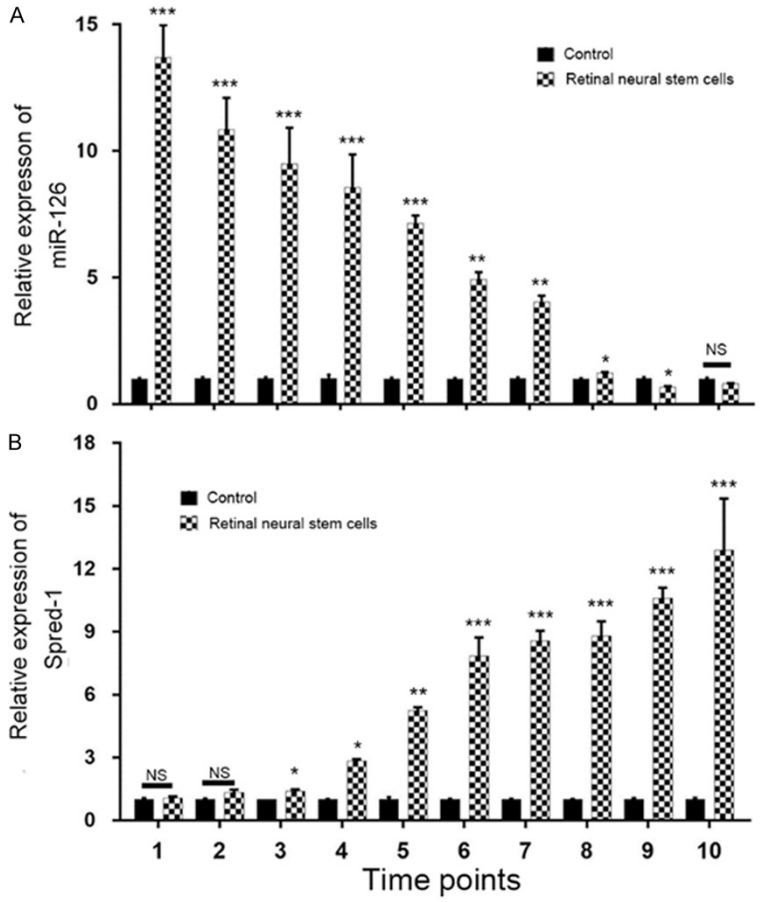

Figure 1. Relative expression of miR-126 and spred-1 during the induction of iPSs. A. Expression of miR-126 at 10 time points during induction of iPSs; B. Expression of spred-1 at 10 time points during induction of iPSs. *P < 0.05, **P < 0.01, ***P < 0.001, NS, not significant.

three times with PBS. The cells were then permeabilized by incubation with 0.1% Triton X-100 for 5 minutes, and washed with PBS. Next, the cells were treated for 30 minutes with a solution composed of 5% bovine serum albumin and 5% normal goat serum, and incubated overnight at 4°C with the primary antibody: mouse anti-human nestin (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Following incubation, excess primary antibody was removed by washing the cells five times with PBS; after which, the cells were incubated with secondary antibodies (fluorescein-conjugated goat anti-mouse IgG and rhodamine-conjugated goat anti-mouse IgG, [Santa Cruz Biotechnology]) at 37°C for 1 hour. After washing with PBS, the cell nuclei were stained with 4',6-diamidoino-2-phenylindole. Finally, the cells were overlaid with glycerol, visualized, and photographed with a laser scanning microscope (Nikon, Japan).

Transfection with miR-126 mimics

MiR-126 mimics and negative controls were chemically synthesized by Suzhou Jima. Cells were seeded into 6-well or 24-well cell culture plates that contained fresh medium without antibiotics. When the cells reached 80% confluence, they were transfected with oligonucleotides. All transfections were performed using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

RNA extraction and real-time PCR

Total cellular RNA was extracted with Trizol reagent. First-strand cDNA was synthesized from 1 μg of total RNA by using a Reverse Transcription System Bestar qPCR RT Kit according to the manufacturer's instructions. Real-time PCR was performed on an ABI 7500 Real-Time PCR System. Each assay was performed in triplicate, and β-actin served as an endogenous control gene. The primer sequences used were as follows: spred-1, 5'-CAGCCAGGCTTGGACATTCA-3' (forward) and 5'-TGGGACTTTAGGCTTCCACAT-3' (reverse); miR-126, 5'-ACACTCCAGCTGGGTCGTACCGTGAGTAATAA-3' (forward) and 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCGCATT-3' (reverse); GAPDH, 5'-TGTTCGTCATGGGTGTGAAC-3' (forward) and 5'-ATGGCATGGACTGTGGTCAT-3' (reverse) (Sangon Biotech, Shanghai, China). The relative amounts of miR-126 and BCL11B synthesized were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized by using β-actin cDNA as an internal control.

Target prediction and luciferase assay

The putative targets of miR-126 were predicted by Target Scan Release 6.2 software. The human spred-1 wild-type and mutant 3'UTR reporter vectors were constructed by inserting

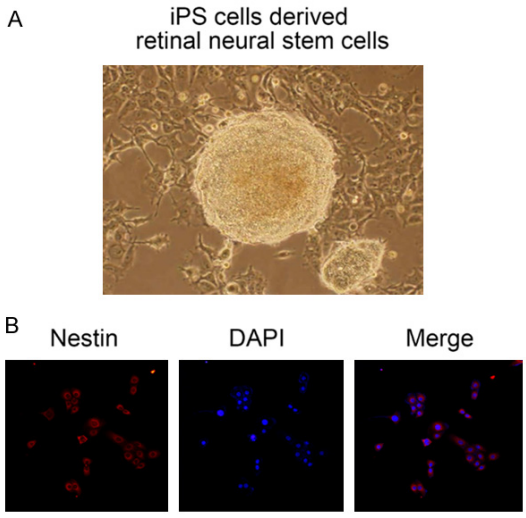

Figure 2. Verification of iPS-derived retinal neural stem cells. A. Neural spheres were formed after induction of IPSs. B. Positive results for nestin expression after induction. Editor Note: Please change the label of 2A to read: IPS cell-derived neural stem cells.

annealed oligonucleotides with tow flanking restriction sites into a pmirGLO vector. Firefly luciferase and Renilla luciferase signals were measured using a Dual-luciferase assay reporter kit and quantitated by a Lumat LB 9501 luminator.

Statistical analysis

All statistical calculations were performed using Prism 6 software (GraphPad, San Diego, California, USA). Results are presented as the mean ± SEM. Student's t test was used for comparisons between 2 groups, and 1-way ANOVA followed by the Tukey test was used for comparisons among multiple groups. A *P*-val ue < 0.05 was considered statistically significant.

Results

MiR-126 levels decreased during IPS cell induction

Quantitative real-time PCR (qPCR) was used to evaluate the level of miR-126 expression throughout the iPS cell induction period. MiR-126 expression in retinal neural stem cells derived from subjects with RNV was compared with that in neural stem cells derived from healthy control subjects. We found that miR-126 levels in the stem cells from RNV subjects showed a gradual significant decrease during the induction period when compared to the miR-126 levels in stem cells from the control subjects (**Figure 1A**). Spred-1 expression levels were also evaluated by qPCR. We found that spred-1 levels exhibited an opposite tendency by showing an increasing trend throughout the induction period (**Figure 1B**).

IPS cells differentiated into retinal neural stem cells via induction

In order to verify the successful induced differentiation of retinal neural stem cells, we tested the newly formed neural spheres (**Figure 2A**) for their expression of nestin (a retinal stem cell marker) by immunofluorescence (**Figure 2B**). We found that the neural spheres had the morphological appearance of retinal neural stem cells, and displayed positive immunostaining for nestin. These findings indicated that retinal neural stem cells had been successfully induced from iPS cells (**Figure 2**).

MiR-126 mimics enhanced VEGF expression by downregulating spred-1

To explore the functional role of miR-126 in iPS cell-derived retinal neural stem cells, miR-126 mimics or a negative control were transfected into human iPS cell-derived retinal neural stem cells obtained from the healthy subjects. We then used qPCR to evaluate the levels of miR-126, spred-1, and VEGF expression. Transfection with the miR-126 mimics significantly increased the levels of miR-126 and VEGF when compared to transfection with the negative control; however, spred-1 levels dramatically decreased (**Figure 3**). GAPDH RNA was used as an internal control for all qPCR analyses of miRNA expression.

Spred-1 as a target of miR-126

We used computational prediction (miRBase, TargetScan, PicTar, and MiRanda) to identify potential binding sites for spred-1 mRNA on miR-126. We found that the 3'UTR of spred-1 mRNA bears a miR-126-binding site that is conserved in mammals (**Figure 4A**). The effect of miR-126 on translation of spred-1 mRNA into protein was evaluated by the luciferase reporter assay. Transfection with the miR-126 mimics

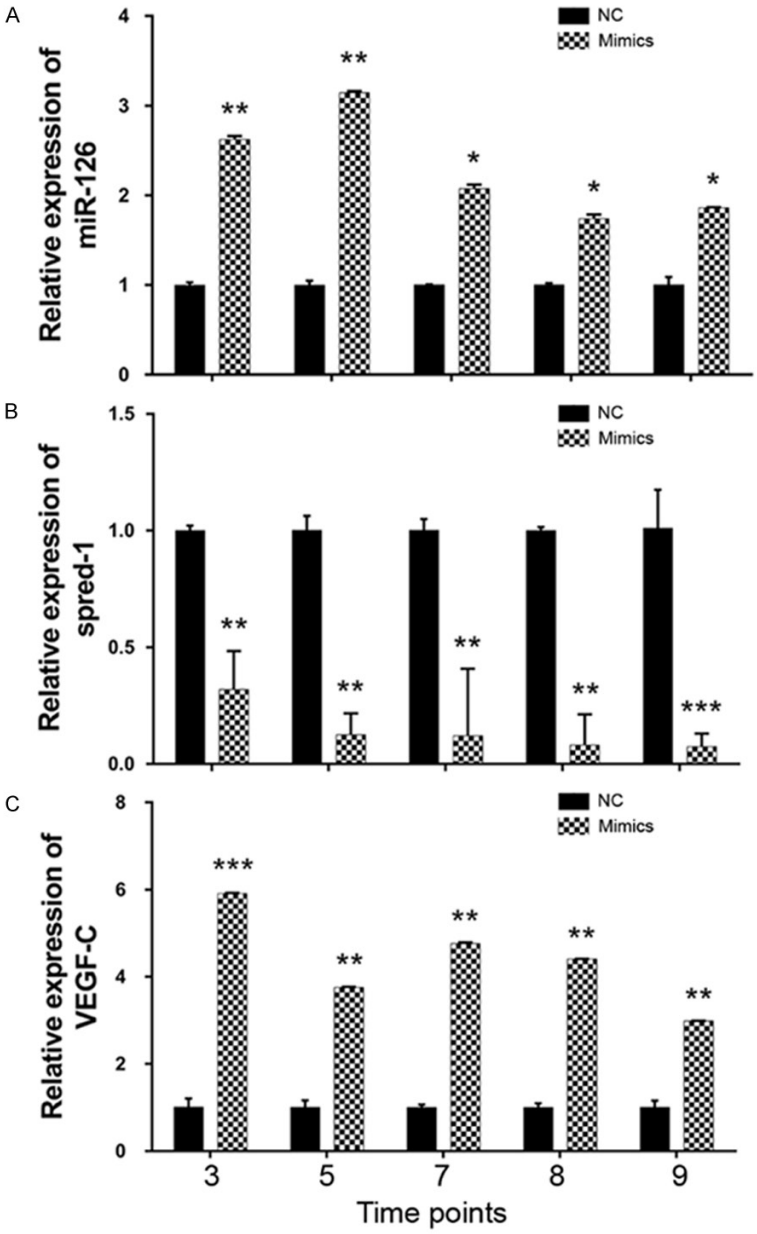

Figure 3. Relative expression of miR-126, spred-1, and VEGF-C during iPS induction relative to controls (PBMCs from healthy individuals).

significantly decreased the luciferase activity of the reporter gene with the wild-type spred-1 3'UTR when compared to transfection with the negative control. However, this regulatory effect of miR-126 was suppressed when the predicted miR-126-binding site in spred-1 mRNA was mutated (**Figure 4B**).

Inhibition of spred-1 increased VEGF expression

To further explore the relationship between spred-1 and VEGF-C in PBMCs obtained from healthy patients, the inhibition of spred-1 expression by spred-1-specific shRNA was evaluated by qPCR. Expression of spred-1 in cells transfected with shRNA was significantly reduced when compared with spred-1 expression in the negative control cells, while the levels of VEGF-C increased after inhibition of spred-1 expression (**Figure 5**).

Discussion

The development of retinal neovascularization (RNV) in the eye is a leading cause of vision loss among people of all ages. RNV underlies the pathology of conditions such as diabetic retinopathy, age-related macular degeneration, retinal vein occlusion, and external exudative retinopathy, which all have a negative impact on quality of life. The lack of an effective treatment for RNV has led to increased *incidence rates of ocular diseases caused by RNV*, and novel alternative therapeutic approaches are urgently needed. During the progression of RNV, the abnormal growth of new blood vessels within the choroid extends to the retina, and VEGF plays a critical role in this process. New treatments are available that target VEGF, and anti-VEGF agents such as Macugen, Eylea, and Lucentis are currently being used to treat ocular diseases caused by RNV [24-26]. However, anti-VEGF agents are only able to control RNV progression, and are not very efficient at improving vision. Therefore, the need for combination therapies is becoming increasingly imperative.

Studies have reported that a group of miRNAs contribute to angiogenesis; these include *miR-23, miR-21, and miR-24*. Therefore, the identification of miRNAs that serve as vital mediators of RNV progression might be on the critical path for developing novel therapeutic strate-

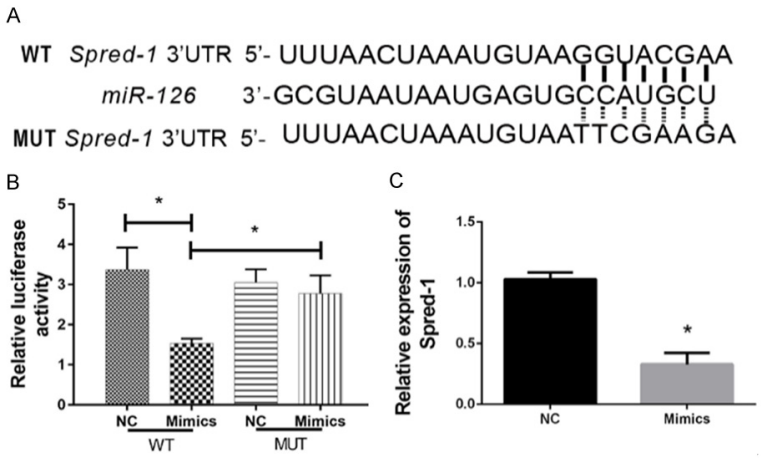

Figure 4. MiR-126 inhibited spred-1 expression by binding to the 3'UTR of spred-1 mRNA. A. MiR-126 targeted spred-1 by binding to the 3'UTR of spred-1 mRNA; B. Double luciferase assays showed that miR-126 could directly target spred-1 mRNA. C. MiR-126 inhibited spred-1 expression by direct targeting. *P < 0.05.

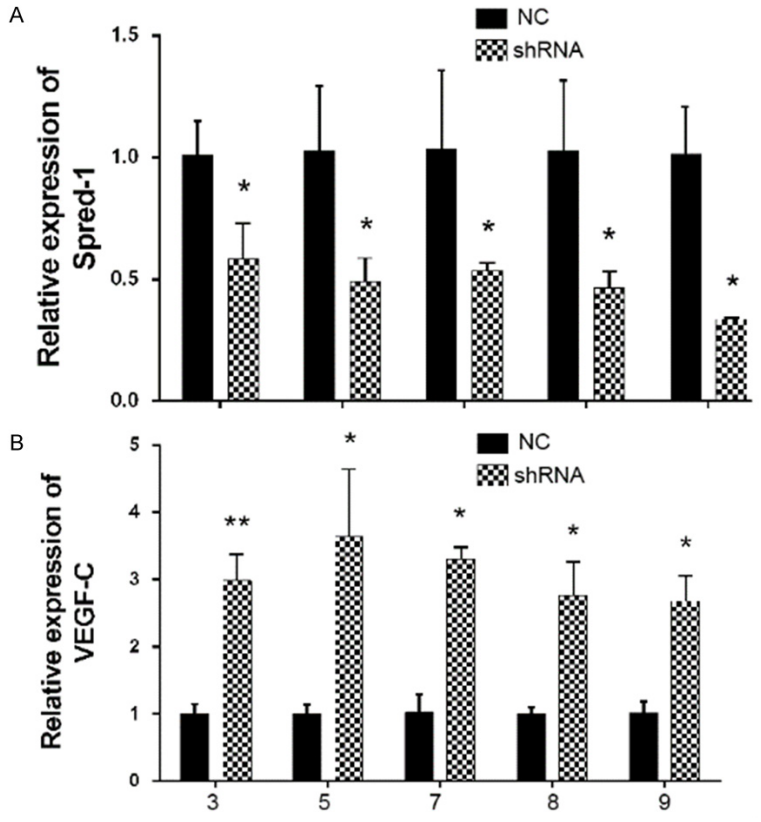

Figure 5. Spred-1 shRNA enhanced expression of VEGF-C.

gies. Zhuang and Qin et al [27] showed that downregulation of miRNA-155 attenuates RNV via the PI3K/Akt pathway [27]; Kong and Sun et al suggested that miR-155 regulates RNV via the Slit-Robo signaling pathway. In the present study, we showed that a novel miRNA, miR-126, helps mediate RNV progression via its ability to regulate VEGF expression, suggesting that miR-126 might play a role in modulating RNV. We found that transfection of miR-126 mimics increased VEGF levels, and that the levels of a neurofibromin recruitment factor (spred-1) decreased significantly after transfection. Conserved miR-126-binding sites were identified in spred-1 mRNA by computationalalgorithmsandverified by the luciferase reporter assay, suggesting that miR-126 had increased the levels of VEGF by inhibiting spred-1 expression.

Recently, spred-1 has been studied in several pathological conditions, including cancer and RNV, due to its sprouty-related function in angiogenesis. It has been shown that spred-1 is involved in regulating hepatocellular carcinoma and age-related macular degeneration [28, 29]. Moreover, several studies have identified spred-1 mRNA as a binding target for various miRNAs (miR-132, miR212, miR-206 miR-21, and miR-126) [30-32]. Our results demonstrated that inhibition of spred-1 expression in IPS cells remarkably increased the levels of VEGF in those cells, suggesting that spred-1 might downregulate VEGF expression. Moreover, our results also provide evidence that miR-126 mediates both angiogenesis and RNV by targeting spred-1.

Our study used pluripotent stem cells (IPSs) that had been induced from the peripheral blood mononuclear cells of patients with RNV as in vitro model. This in vitro model represents an innovative approach for studying human genetic diseases and various pathological conditions

such as neurogenetic diseases, muscular dystrophies, and cardiovascular diseases [33, 34]. IPS cells generated from primary patient samples can be used to provide information relevant to genomics, proteomics, and metabolomics, and support the development of novel therapeutic approaches. In our study, we successfully induced IPS cells from patient samples to differentiate into retinal neural stem cells, which were used to gain new insights into the underlying molecular mechanism of RNV progression.

In summary, this is the first study to use IPS cells from patients with RNV as *in vitro* model, and then show they could be induced to form retinal neural stem cells. We evaluated the miR-126 levels in our model cells, and identified spred-1 as a target for miR-126. This finding suggests that miR-126 enhanced VEGF expression in our induced pluripotent stem cell model by targeting spred-1. Our results provide a new insight into the mechanism of RNV progression, and can be used to develop new strategies for treating RNV-related ocular diseases.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Ishida S, Usui T, Yamashiro K, Kaji Y, Amano S, Ogura Y, Hida T, Oguchi Y, Ambati J, Miller JW, Gragoudas ES, Ng YS, D'Amore PA, Shima DT and Adamis AP. VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. J Exp Med 2003; 198: 483-489.

[2] Avunduk AM, Cetinkaya K, Kapicioglu Z and Kaya C. The effect of posterior vitreous detachment on the prognosis of branch retinal vein occlusion. Acta Ophthalmol Scand 1997; 75: 441-442.

[3] Moravski CJ, Kelly DJ, Cooper ME, Gilbert RE, Bertram JF, Shahinfar S, Skinner SL and Wilkinson-Berka JL. Retinal neovascularization is prevented by blockade of the renin-angiotensin system. Hypertension 2000; 36: 1099-1104.

[4] Campochiaro PA. Ocular neovascularization. J Mol Med (Berl) 2013; 91: 311-321.

[5] Das A and McGuire PG. Retinal and choroidal angiogenesis: pathophysiology and strategies for inhibition. Prog Retin Eye Res 2003; 22: 721-748.

[6] Guaiquil VH, Swendeman S, Zhou W, Guaiquil P, Weskamp G, Bartsch JW and Blobel CP. ADAM8 is a negative regulator of retinal neovascularization and of the growth of heterotopically injected tumor cells in mice. J Mol Med (Berl) 2010; 88: 497-505.

[7] Kroll J, Epting D, Kern K, Dietz CT, Feng Y, Hammes HP, Wieland T and Augustin HG. Inhibition of Rho-dependent kinases ROCK I/II activates VEGF-driven retinal neovascularization and sprouting angiogenesis. Am J Physiol Heart Circ Physiol 2009; 296: H893-899.

[8] Lobov IB, Renard RA, Papadopoulos N, Gale NW, Thurston G, Yancopoulos GD and Wiegand SJ. Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. Proc Natl Acad Sci U S A 2007; 104: 3219-3224.

[9] Ferrara N and Bunting S. Vascular endothelial growth factor, a specific regulator of angiogenesis. Curr Opin Nephrol Hypertens 1996; 5: 35-44.

[10] Tah V, Orlans HO, Hyer J, Casswell E, Din N, Sri Shanmuganathan V, Ramskold L and Pasu S. Anti-VEGF therapy and the retina: an update. J Ophthalmol 2015; 2015: 627674.

[11] Kloosterman WP and Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell 2006; 11: 441-450.

[12] Calin GA and Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.

[13] Krutzfeldt J and Stoffel M. MicroRNAs: a new class of regulatory genes affecting metabolism. Cell Metab 2006; 4: 9-12.

[14] Small EM and Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature 2011; 469: 336-342.

[15] Garofalo M and Croce CM. microRNAs: Master regulators as potential therapeutics in cancer. Annu Rev Pharmacol Toxicol 2011; 51: 25-43.

[16] Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, Ivey KN, Bruneau BG, Stainier DY and Srivastava D. miR-126 regulates angio-

genic signaling and vascular integrity. Dev Cell 2008; 15: 272-284.
[17] Kuhnert F, Mancuso MR, Hampton J, Stankunas K, Asano T, Chen CZ and Kuo CJ. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. Development 2008; 135: 3989-3993.
[18] Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, Richardson JA, Bassel-Duby R and Olson EN. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 2008; 15: 261-271.
[19] Liu B, Peng XC, Zheng XL, Wang J, Qin YW. MiR-126 restoration down-regulate VEGF and inhibit the growth of lung cancer cell lines in vitro and in vivo. Lung cancer 2009; 66: 169-175.
[20] Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, Shah A, Willeit J and Mayr M. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 2010; 107: 810-817.
[21] Mattes J, Collison A, Plank M, Phipps S and Foster PS. Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. Proc Natl Acad Sci U S A 2009; 106: 18704-18709.
[22] Oglesby IK, Bray IM, Chotirmall SH, Stallings RL, O'Neill SJ, McElvaney NG and Greene CM. miR-126 is downregulated in cystic fibrosis airway epithelial cells and regulates TOM1 expression. J Immunol 2010; 184: 1702-1709.
[23] Feng R, Chen X, Yu Y, Su L, Yu B, Li J, Cai Q, Yan M, Liu B and Zhu Z. miR-126 functions as a tumour suppressor in human gastric cancer. Cancer Lett 2010; 298: 50-63.
[24] Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY, Sy JP and Schneider S. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med 2006; 355: 1432-1444.
[25] Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY and Kim RY. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 2006; 355: 1419-1431.
[26] Zampros I, Praidou A, Brazitikos P, Ekonomidis P and Androudi S. Antivascular endothelial growth factor agents for neovascular age-related macular degeneration. J Ophthalmol 2012; 2012: 319728.
[27] Zhuang Z, Xiao Q, Hu H, Tian SY, Lu ZJ, Zhang TZ and Bai YL. Down-regulation of microRNA-155 attenuates retinal neovascularization via the PI3K/Akt pathway. Mol Vis 2015; 21: 1173-1184.
[28] Ji JS, Xu M, Song JJ, Zhao ZW, Chen MJ, Chen WQ, Tu JF and Yang XM. Inhibition of microRNA-126 promotes the expression of Spred1 to inhibit angiogenesis in hepatocellular carcinoma after transcatheter arterial chemoembolization: in vivo study. Onco Targets Ther 2016; 9: 4357-4367.
[29] Zhou Q, Anderson C, Hanus J, Zhao F, Ma J, Yoshimura A and Wang S. Strand and cell type-specific function of microRNA-126 in Angiogenesis. Mol Ther 2016; 24: 1823-1835.
[30] Lei Z, van Mil A, Brandt MM, Grundmann S, Hoefer I, Smits M, El Azzouzi H, Fukao T, Cheng C, Doevendans PA and Sluijter JP. MicroRNA-132/212 family enhances arteriogenesis after hindlimb ischaemia through modulation of the Ras-MAPK pathway. J Cell Mol Med 2015; 19: 1994-2005.
[31] Pitzler L, Auler M, Probst K, Frie C, Bergmeier V, Holzer T, Belluoccio D, van den Bergen J, Etich J, Ehlen H, Zhou Z, Bielke W, Poschl E, Paulsson M and Brachvogel B. miR-126-3p promotes matrix-dependent perivascular cell attachment, migration and intercellular interaction. Stem Cells 2016; 34: 1297-1309.
[32] Sharma SB, Lin CC, Farrugia MK, McLaughlin SL, Ellis EJ, Brundage KM, Salkeni MA and Ruppert JM. MicroRNAs 206 and 21 cooperate to promote RAS-extracellular signal-regulated kinase signaling by suppressing the translation of RASA1 and SPRED1. Mol Cell Biol 2014; 34: 4143-4164.
[33] Salani S, Donadoni C, Rizzo F, Bresolin N, Comi GP and Corti S. Generation of skeletal muscle cells from embryonic and induced pluripotent stem cells as an in vitro model and for therapy of muscular dystrophies. J Cell Mol Med 2012; 16: 1353-1364.
[34] Ebert AD, Liang P and Wu JC. Induced pluripotent stem cells as a disease modeling and drug screening platform. J Cardiovasc Pharmacol 2012; 60: 408-416.