Original Paper

Nogo-B Promotes Angiogenesis in Proliferative Diabetic Retinopathy via VEGF/PI3K/Akt Pathway in an Autocrine Manner

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Key Words

Nogo-B • Angiogenesis • Proliferative diabetic retinopathy

Abstract

Background/Aims: Nogo-B, a conservative protein of endoplasmic reticulum, is a member of the reticulon family of proteins. Proliferative diabetic retinopathy (PDR) is the major concerning problem of diabetic retinopathy. This study explored the role of Nogo-B in the regulation of angiogenesis in PDR patients and primary human retinal endothelial cells (HRMECs). **Methods:** Nogo-B was down-regulated through the use of Lentivirus-NogoB-RNAi, the effects of Nogo-B on angiogenesis under high glucose stimulation were evaluated via CCK-8 assay, wound closure assay, transwell assay, and tube formation assay. Expression of Nogo-B, VEGF, PI3K and Akt were determined by western blotting, immunofluorescence, enzyme-linked immunosorbent assay (ELISA). Co-culture system was used to explore cell communication. **Results:** Nogo-B was highly enriched in ocular tissues of PDR patients and in HRMECs exposed to high glucose. Down-regulation of Nogo-B attenuated high glucose induced cell migration and tube formation in HRMECs. Mechanistically, in comparison with the negative control group, Lentivirus-NogoB-RNAi group had exhibited reduced VEGF secretion, weakened PI3K and Akt activation. Besides, high glucose treatment promoted the secretion of Nogo-B and presented as a "long-term memory". **Conclusions:** These data collectively indicated that Nogo-B promoted angiogenesis in HRMECs via VEGF/PI3K/Akt pathway in an autocrine manner.

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Introduction

Diabetes mellitus is one of the most common metabolic diseases and is associated with long-term damage of various organs [1, 2]. Despite the well-developed diagnosis and medical care, the global prevalence of diabetes will increase to 366 million by 2030 estimated by the World Health Organization [3]. Diabetic retinopathy (DR) is a severe complication of diabetes and remains one of the leading cause of blindness among working-age adults worldwide [4]. The major vision-threatening form of DR is proliferative diabetic retinopathy (PDR), the advanced stage of DR with characteristics of neovascularization [5, 6]. Population-based studies reported an annual incidence PDR rates of 1.5% in participants of type 1 diabetes and 4-year incidence PDR rates of 2-7% in those of type 2 diabetes [7-9].

At present, the standard management of PDR is laser photocoagulation and vitreoretinal surgery, nevertheless, both of the treatments are focused on the advanced stage of DR and carry potential side effects [5, 10]. Hence a wide range of new approaches for DR are investigated including anti-inflammatory agents, G-protein coupled receptor agonists and antagonists, neuroprotectants [4, 11-14]. However, most of the clinical trials of new agents to inhibit progression of DR have been far more disappointing [3, 15]. Thus, further studies to explore novel pharmacological targets to manage DR are still pressing needed.

The reticulon (RTN) family, which have been identified in most eukaryotic cells, is a transmembrane protein sharing the common C-terminal region encodes the reticulon-homology domain [16]. There are four reticulon genes (RTN1, RTN2, RTN3, RTN4) in mammalian, among which RTN4 (also known as Nogo) has recently been the focus of intensive investigation [16]. Nogo-A, Nogo-B, Nogo-C are the major protein isoforms translated from RTN4 gene, and they share an identical C-terminal domain (Nogo-66) [17]. Despite Nogo-A and Nogo-C are found in the central nervous system, with Nogo-C being additionally detected in skeletal muscle, Nogo-B is widely expressed in most tissues [18, 19]. Much known is about Nogo-A because of its function as neurite outgrowth inhibitor while the other two isoforms remains largely elusive [20]. This study is concentrated on the second homologues Nogo-B. Previous studies reported that Nogo-B was associated with vascular remodeling upon vascular injury [21]. More recently, it was demonstrated that Nogo-B regulated sphingolipid homeostasis to control blood pressure [22, 23]. However, little attention was paid to the function of Nogo-B in diabetic vascular pathology especially in PDR.

In the current study, Nogo-B was found to be highly enriched in ocular tissues of PDR patients and in primary human retinal endothelial cells (HRMECs) treated with high glucose. To explore the role of Nogo-B in angiogenesis in PDR and evaluate its potential function as a therapeutic target for the treatment of PDR, we downregulated the expression of Nogo-B using lentivirus in HRMECs, and multiple assays associated with angiogenesis, as well as the expressions of related genes were investigated.

Materials and Methods

Cell culture

Primary HRMECs were purchased from Angio-Proteomie (Oxfordshire, UK) and cultured in endothelial cell medium supplemented with 5% fetal bovine serum (ScienCell, Carlsbad, CA). Cells from passage 3 to 10 were used and incubated with normal D-glucose (5.5 mM), high D-glucose (25 mM) and L-glucose (25 mM, as osmotic control).

Human surgical samples

All procedures in this study were approved by the Ethics Committee of the Renji Hospital of Shanghai Jiaotong University School of Medicine and adhered to the tenets of the Declaration of Helsinki. All experiments were performed in accordance with relevant guidelines. Vitreous fluids (500-1300 mL) were collected from 17 eyes of 17 patients who had undergone vitrectomy. Among which, non-diabetic macular hole (MH) and rhegmatogenous retinal detachment (RRD) patients were served as control group ($n_{MH} = 5$, $n_{RRD} = 5$, $n_{PDR} =$

7). Samples with obvious bleeding were excluded. Vitreous samples were immediately centrifuged at 15,000g for 20 min at 4°C and the supernatants were stored at -80°C until use. Six fibrovascular samples were surgically removed from the PDR eyes and were used in immunohistochemistry and Immunofluorescence.

Immunohistochemistry

Fibrovascular samples were fixed in 4% paraformaldehyde and embedded in paraffin. The fixed sections were stained with Nogo-B antibody (abcam, Cambridge, USA). Nuclei were counterstained with hematoxylin and sections were visualized with light microscopy.

ELISA assay for Nogo-B and VEGF

Concentrations of Nogo-B in vitreous humor was measured using human soluble Nogo-B elisa kit (Biolegend, San Diego, CA) according to the manufacturer's instruction as previously described [24]. Concentrations of in the cell supernatant was measured by human VEGF ELISA kit (R&D, Minneapolis, USA). Assays were performed in three replicated wells and each experiment was repeated three times independently.

Immunofluorescence

Paraffin sections of fibrovascular samples were deparaffinised and hydrated by exposure to xylene and graded alcohols followed by water. And microwave-based antigen retrieval was performed in 10 mmol/l citrate buffer (pH = 6). Then sections were probed with Nogo-B (abcam, Cambridge, USA) and CD31 (abcam, Cambridge, USA). The secondary antibodies used for fluorescence detection were Alexa Fluor 488 and 555 (Invitrogen, Carlsbad, USA). Sections were visualised under microscopy.

Lentiviral vector transduction

Both Lenti-NogoB-RNAi and Lenti-NC were constructed and produced by Genechem (Shanghai, China). 2×10^5 HRMECs were plated in each well of a 6-well-plate. When the cells reached 20% to 30% confluence, lentiviral vector expressing siRNA-NogoB (5'-3': CACAGAAUCUAUGGACUGAAU) and negative control (5'-3': UUCUCCGACGUGUCACGUTT) was added (MOI=10) with 50 µg/mL Polybrene (Genechem, Shanghai, China). After incubation for 8 hours, the media replaced with complete culture media. Transduced cell clones were selected with 3 µg/mL puromycin (Genechem, Shanghai, China) for 2 weeks, and cells were expanded in complete culture medium.

CCK-8 assay

HRMECs (2×10^4 cells/well) were seeded into 96-well plates. After attachment, cells were incubated with different glucose for 48 hours. Cell viability was determined by CCK-8 (Dojindo, Rockville, USA). The optical density values at 450 nm were quantified by a microplate reader (BioTek, Winooski, USA). Assays were performed in three replicated wells and each experiment was repeated three times independently.

Wound closure assay

Confluent monolayers of HRMECs were wounded using a micropipette tip (200 µL tip), rinsed with PBS to remove detached cells, and incubated with cell culture medium without fetal bovine serum. Wound closure was monitored after 24 hours and photographed under inverted microscope.

Cell migration assay

HRMECs (1×10^5 cells/well) were seeded into the upper chamber of the transwells, and then high D-glucose (25 mM) or L-glucose (25 mM) was added into the lower chamber. After 12 hours, the non-migrated cells were wiped off with a cotton swab, and the filters were fixed with 4% paraformaldehyde for 20 minutes before stained by crystal violet (Beyotime, Shanghai, China). Images were pictured under the inverted microscope.

Tube formation assay

HRMECs (1×10^5 cells/well) were starved in endothelial cell medium containing 0.1% fetal bovine serum for 8 hours, and then incubated with different glucose for 48 hours. Cells were seeded into 24-well plates precoated with matrigel (BD Biosciences, Maryland, USA), and treated in different glucose for another 6 hours. Images were pictured under the inverted microscope.

Western-blot analysis

Protein samples were separated by SDS-PAGE and blots were probed with an appropriate combination of primary and secondary antibodies. The bands were identified by enhanced chemiluminescence kits and visualized using the Amersham Image 600 System. Primary antibodies were antibodies to Nogo-B (Abcam, Cambridge, USA), PI3K p85 (Cell Signaling Technology, Danvers, USA), PI3K p-p85 (Cell Signaling Technology, Danvers, USA), Akt (Cell Signaling Technology, Danvers, USA), p-Akt (Cell Signaling Technology, Danvers, USA), GAPDH (Santa Cruz, Dallas, USA). All the second antibodies were obtained from Calbiochem.

Co-culture system

First, HRMECs infected by lentivirus were cultured on transwell inserts (NC and LV group), and D-glucose (25 mM) was added to both of the inserts. After 48 hour treatment, the cell supernants was collected, and fresh complete growth medium with normal D-glucose was added for further culture. Then normal HRMECs were seeded to the bottom wells of chamber for additional 24 hours to test their ability of angiogenesis and both the supernants and cells were collected for subsequently examination (symbolled NC-f and LV-f group).

Statistical analysis

Statistical analyses were performed using the SPSS version 20.0. Data were expressed as means $\pm$ standard error of the mean or median with interquartile range. The significance of differences between groups was evaluated by two-tailed student's t-test, non-parametric tests and one-way ANOVA test, and $P < 0.05$ was considered as statistically significant differences.

Results

Nogo-B was highly expressed in human PDR tissues

Since no studies on Nogo-B expression in human ocular tissue have been reported, first, using assays of immunohistochemistry, we confirmed the expression of Nogo-B in fibrovascular tissues surgically excised from human eyes (Fig. 1a). Next, ELISA analysis on vitreous humor suggested that the expression of Nogo-B in PDR patients was considerably higher than MH and RRD patients (Fig. 1b). Further, doublestaining experiments of fibrovascular tissue samples demonstrated colocalization of Nogo-B signal with CD31, a vascular endothelial cell marker, showing that Nogo-B protein could be produced by vascular endothelial cells (Fig. 1c).

Fig. 1. Nogo-B was highly expressed in human PDR tissues. (a) Nogo-B immunohistochemistry in fibrovascular membranes of patients with PDR, the scale bar was 50 μ m (b) ELISA tests of Nogo-B in human vitreous fluid samples, the data were represented as median with interquartile range, $n_{MH} = 5$, $n_{RRD} = 5$, $n_{PDR} = 7$. **, $P < 0.01$ by Mann-Whitney U test (c) Triple labelling of Nogo-B (green), CD31 (red) and DAPI (blue) in the fibrovascular tissues of patients with PDR. The boxed area indicated higher power magnifications. Scale bar was 50 μ m.

Fig. 2. High glucose induced Nogo-B expression and angiogenesis in HRMECs. Left panel: Nogo-B protein expression in HRMECs exposed to different glucose for 48 hours. Right panel: Representative blots for Nogo-B, $n = 3$ in each group (a). The cell viability was quantified and presented as a histogram by CCK-8 assay in HRMECs exposed to different glucose for 48 hours, $n = 3$ in each group (b). The migration rate of HRMECs, cultured under various glucose conditions, was determined in scratch wound assay, $n = 3$ in each group (c) and transwell assay, $n = 3$ in each group (d). The tube formation ability was assessed by plating the cells on matrigel, $n = 3$ in each group (e). Quantitative assessment of the data was presented in the right. Data were shown as mean $\pm$ SEM, *, $P < 0.05$, **, $P < 0.01$ by Tukey HSD test.

High glucose induced Nogo-B expression and angiogenesis in HRMECs.

Angiogenesis, a critical hallmark of pathological changes in PDR patients, is a complicated process involves the proliferation, migration and tubulogenesis of endothelial cells [25, 26]. In this study, treatment of high D-glucose (25 mM) considerably promotes Nogo-B protein expression compared with osmotic control L-glucose (25 mM) and normal D-glucose control (5.5 mM) (Fig. 2a). To verify whether D-glucose induces angiogenesis, multiple assays were applied in HRMECs. Exposure to D-glucose (25 mM) resulted in markedly enhancement of proliferation in HRMECs as indicated from CCK-8 assay (Fig. 2b). To detected the migration of HRMECs, scar assay and transwell assay were examined. High D-glucose exhibited faster wound closure rate than L-glucose and normal D-glucose control, which is consistent with the transwell results (Fig. 2c, d). Tube formation assay was used to further investigate potential effects of high glucose on tubulogenesis, another critical step responsible for angiogenesis. Formation of tubular-like network was more profound in the presence of high D-glucose,

Fig. 3.

3. Downregulation of Nogo-B attenuated high glucose-induced angiogenesis in HRMECs. HRMECs were infected by lentivirus-siNogoB (LV group) and lentivirus negative control (NC group), then treated with D-glucose (25 mM). The cell viability was quantified and presented as a histogram by CCK-8

assay after treatment with D-glucose (25 mM) for 48 hours, $n = 3$ in each group (a). The migration rate was determined in scratch wound assay, $n=3$ in each group (b, c) and transwell assay, $n=3$ in each group (d, e). The tube formation ability was assessed by plating the cells on matrigel, $n = 3$ in each group (f, g). The expression of Nogo-B infected by lentivirus was detected by western blot (h). Data were shown as mean $\pm$ SEM, **, $P < 0.01$ by student's t-test.

with a few tubes of disconnected networks observed in L-glucose and normal D-glucose control (Fig. 2e).

Downregulation of Nogo-B attenuated high glucose-induced angiogenesis in HRMECs

As high D-glucose promoted Nogo-B expression, we studied the effects of Nogo-B in high glucose-induced angiogenesis using lentivirus downregulating Nogo-B (LV-siNogoB) in HRMECs. Compared with negative control of lentivirus (NC group), downregulating of Nogo-B partially reversed high D-glucose (25 mM) induction of migration and tube formation in HRMECs as indicated from scar assays, transwell assay and tube formation assay (Fig. 3b-g). However, downregulation of Nogo-B didn't affect the proliferation rate of HRMECs from CCK-8 assay (Fig. 3a).

Downregulation of Nogo-B blocked high glucose-induced activation of VEGF/PI3K/Akt pathway in HRMECs

Previous studies revealed that activation of the PI3K/Akt pathway was crucial for VEGF induced angiogenesis, including cell survival, migration and tube formation [27-30]. To elucidate the underlying mechanisms for Nogo-B to affect angiogenesis of HRMECs, we

Fig. 4. Down-regulation of Nogo-B blocked high glucose-induced activation of VEGF/PI3K/Akt pathway in HRMECs. (a) Protein concentrations of VEGF in the cell supernatant were measured by ELISA. Left panel: HRMECs were exposed to different glucose for 48 hours. Right panel: Lentivirus infected HRMECs were exposed to high D-glucose (25 mM) for 48 hours. n = 3 in each group. (b) Akt and PI3K p85

was activated after treatment with high D-glucose (25 mM) for 48 hours by using wetern blot, n = 3 in each group. Quantitative assessment of the data was presented in the right. (c) The glucose-induced activation of PI3K p85 and Akt was blunted after downregulation of Nogo-B, n = 6 in each group. Quantitative assessment of the data was presented in the right. Data were shown as mean $\pm$ SEM, **, $P < 0.01$ by student's t-test.

assessed VEGF secretion in lentivirus affected HRMECs cultured in high D-glucose (25 mM). As evidenced by Fig. 4a, high D-glucose (25 mM) treatment stimulated VEGF secretion in the supernatant to a great extent, which was blunted after down-regulation of Nogo-B; Further, high D-glucose was shown to activate PI3K p85 and Akt, which was blocked by down-regulation of Nogo-B, demonstrated that the action of Nogo-B in angiogenesis could correlated with VEGF/PI3K/Akt pathway (Fig. 4b, c).

Recently Nogo-B was shown to inhibit the sphingolipid de novo biosynthesis [22]. Ceramide, a central metabolite of this pathway, has been shown to decrease the activation of the Akt pathway [31]. So we used myriocin to inhibit sphingolipid biosynthesis in Nogo-B knock down HRMECs. It was shown that the rate of migration and tube formation, as well as Akt activation in LV group was increased after myriocin pretreatment (Fig. 5).

High glucose treatment promoted the secretion of Nogo-B and presented as a "long-term memory"

Since western blotting of cell extracts from high D-glucose treatment indicated the elevated expression of Nogo-B, we next tested whether high glucose induced upregulation of Nogo-B could be released. ELISA analysis of cell supernants reflected that HRMECs exposed to high D-glucose for 48 hours induced notable secretion of Nogo-B, compared with L-glucose groups and normal D-glucose control (Fig. 6a). Interestingly, after 48 hour treatment of high D-glucose followed by fresh complete growth medium with normal D-glucose of additional 24 hours, Nogo-B was still apparently detectable and was much more sereted in the high D-glucose group (Fig. 6b). Next, we tested whether this phenomenon could be observed

Fig. 5. Myriocin partially rescued the impairment of angiogenesis and Akt activation in Nogo-B knock down HRMECs. HRMECs were infected by lentivirus-siNogoB (LV group) and lentivirus negative control (NC group). And cells were exposed to D-glucose (25 mM) after treatment with 100nmol/L myriocin (Myr) or vehicle for 3 hours. The migration rate of HRMECs was determined in scratch wound assay (a) and transwell assay (b). The tube formation ability was assessed by plating the cells on matrigel (c). The expression of Akt and p-Akt was detected by western blot (d). $n = 3$ in each group. Quantitative assessment of the data was presented in the right. Data were shown as mean $\pm$ SEM, **, $P < 0.01$.

in HRMECs infected by lentivirus. Similarly, 48 hour exposure to high D-glucose implied a considerable release of Nogo-B in the supernant of NC group and a relative paucity of Nogo-B in LV group (Fig. 6c). Besides, after medium replaced by fresh cell culture medium with normal D-glucose for additional 24 hours, the release of Nogo-B still persisted (Fig. 6d).

Nogo-B promoted angiogenesis through PI3K/Akt activation in co-culture system

Futhermore, we established co-culture system as described below. First, we cultured HRMECs infected by lentivirus on transwell inserts (NC and LV group) with high D-glucose treatment for 48 hours, then replaced with fresh complete growth medium with normal D-glucose for further culture, and seeded normal HRMECs to the bottom wells of chamber,

Fig. 6. High glucose treatment promoted the secretion of Nogo-B and presented as a “long-term memory”. (a) ELISA analysis of Nogo-B in the supernatant of HRMECs exposed to different glucose for 48 hours, n = 6 in each group, (b) After treatment of different glucose for 48 hours, the supernatant was discarded and replaced by fresh cell culture medium with normal glucose for further 24 hours, Elisa results of the supernants were presented as a histogram, n = 6 in each group. (c) ELISA analysis of Nogo-B in the supernatant of lentivirus infected HRMECs exposed to D-glucose (25mM) for 48 hours, n = 6 in each group. (d) After treatment of D-glucose for 48 hours, the supernatant was discarded and replaced by fresh cell culture medium with normal glucose for further 24 hours, Elisa results of the supernants were presented as a histogram, n = 6 in each group. **, P < 0.001.

the lower cells under LV group (LV-f group) showed blunted ability of migration and tube formation compared with the downside cells under NC group (NC-f group). However, the proliferation rate was not affected (Fig. 7a-g). Besides, PI3k p85 and Akt was activated in NC-f group compared with LV-f group (Fig. 7h, i).

Discussion

PDR, the stage of abnormal newly formed blood vessels grow from the retina, results in subsequently tractional retinal detachment and hemorrhage, is the major concerning problem of DR [32, 33]. Although the underlying mechanisms of aberrant angiogenesis in PDR is less than completely understood, lines of evidence shows that hyperglycemia is the most influential factor that contributes to the angiogenesis [34, 35]. Nogo-B, one of the evolutionary conservative proteins of endoplasmic reticulum that primarily play a role in promoting membrane curvature and tubules maintenance, was reported as a regulator of vascular remodeling and blood pressure, suggesting its role in vasvluar function [21, 22, 36, 37]. However, the role and function of Nogo-B in angiogenesis, especially in PDR have not been established yet. Therefore, we initially evaluated the presence of Nogo-B in the human PDR tissues and HRMECs *in vitro*. Strikingly, Nogo-B was enriched in both the vitreous and fibrovascular tissue samples of PDR patients. And stimulating of HRMECs with high glucose

Fig. 7. Nogo-B promoted angiogenesis through PI3K/Akt activation in co-culture system. We established co-culture system as described in methods, after fresh cell culture medium with normal glucose replacement, the lower cells under HRMECs infected by lentivirus were symbolized as LV-f group and NC-f group. The cell viability was quantified and presented as a histogram by CCK-8 assay after treatment, $n = 3$ in each group (a). The migration rate was determined in scratch wound assay, $n = 3$ in each group (b, c) and transwell assay, $n = 3$ in each group (d, e). The tube formation ability was assessed by plating the cells on matrigel, $n = 3$ in each group (f, g). Activation of PI3K p85 and Akt was detected using western blot (h, i). Data were shown as mean $\pm$ SEM, **, $P < 0.01$ by student's t-test.

triggered the upregulation of Nogo-B. Therefore, we hypothesized that Nogo-B could likely be associated with angiogenesis in PDR.

To get insights into the participation of Nogo-B in angiogenesis of PDR, we use high glucose stimulation of HRMECs, an established cell culture model [5, 38]. The cell proliferation assay, cell migration assay and tube formation assay are mature methods to imitate three

critical steps of angiogenesis process. High glucose stimulation of HRMECs, which is the major target of hyperglycemia for its location of inner wall in blood vessel [39], resulted in considerable promotion of proliferation, migration and tube formation in HRMECs. And high glucose-induced angiogenesis was partially reversed through downregulation of Nogo-B. The results suggested a novel aspect for the role of Nogo-B in high glucose condition for HRMECs as a positive regulator of angiogenesis, which was consistent with the previous reports demonstrated that Nogo-B promoted migration of HUVECs [21].

The underlying molecular mechanism of diabetic retinopathy is complex and remains largely elusive, which includes PI3K/Akt, p38 MAP kinase, calcium/calmodulin dependent protein kinase α , NOTCH/Wnt signalling [40-42]. Among them, an active VEGF/PI3K/Akt pathway has been dominated linked to pathological angiogenesis in PDR [28, 43, 44]. To analysis the molecular mechanisms and mediators of Nogo-B in regulation of angiogenesis, we examined VEGF secretion, as well as PI3K and Akt activation in lentivirus infected HRMECs. Our results showed that HRMECs exposed in high glucose elicited substantially activation of PI3K and Akt, and stimulated VEGF secretion, which was in consistent with accumulating previous reports [27, 28, 43]. In addition, downregulation of Nogo-B considerably blocked high glucose induced VEGF secretion, PI3K and Akt activation in HRMECs, indicating that Nogo-B may exert its angiogenesis actions through VEGF/PI3K/Akt pathway. Recently Nogo-B was reported to inhibit the sphingolipid biosynthesis through weakening the activity of serine palmitoyltransferase, the rate-limiting enzyme of the pathway [22]. Ceramide, a central metabolite of this pathway, was significantly upregulated in the absence of Nogo-B and has been shown to induce an inhibition of Akt activation [22, 31, 45]. Our results showed that myriocin, an inhibitor of sphingolipid biosynthesis, partially rescued the impairment of angiogenesis and Akt activation in Nogo-B knock down HRMECs. However, the underlying mechanisms remained largely speculative and we will investigate further in our future study.

Considering the cell surface location of Nogo-B in endothelial cells and the extracellular domains exposed and released upon injury [21], we examined whether Nogo-B could be released to cell culture medium upon high glucose stimulation. Surprisingly, the relatively detectable of Nogo-B after exposure to high glucose was found in the present study. Besides, after a subsequent 24 hours of normal glucose culture, it was still apparently detectable in the culture medium. To verify the role of Nogo-B in this phenomenon, we used co-culture system with the upper chamber cultured with lentivirus infected HRMECs and normal HRMECs in the lower wells afterward, and added high glucose to the inserts. We found the secretion of Nogo-B persisted after replacement of fresh medium for 24 hours, and the elevated Nogo-B in supernatants could still promote angiogenesis of normal HRMECs in the downside chamber. These data indicated that HRMECs affected by high glucose could communicate with its neighboring normal cells through Nogo-B, which may contribute to the signalling cascade amplification of hyperglycemia in DR.

Conclusion

We reported that Nogo-B was highly enriched in ocular tissues of PDR patients and in HRMECs exposed to high glucose, and Nogo-B could be secreted upon high glucose exposure and acted as a positive regulator of angiogenesis in HRMECs partially through VEGF/PI3K/Akt pathway, and the cell to cell communication between impaired HRMECs and neighboring normal cells could be ascribed to "the high glucose-induced elevation of Nogo-B memory". Therefore, elucidation of novel ways to downregulate the retinal level of Nogo-B or prevent its release can be a feasible therapeutic strategy for treating angiogenesis of PDR.

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Disclosure Statement

The authors declare that they have no Disclosure Statement.

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