

Osteoglycin knockdown promotes vascular smooth muscle cell proliferation and migration in aortic dissection via the VEGF/VEGFR2 axis

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Abstract. Extracellular matrix (ECM) proteins serve a major role in the pathogenesis of aortic dissection (AD). The aim of the present study was to investigate the effect of osteoglycin (OGN), an ECM proteoglycan, on aortic dissection (AD), as well as the underlying mechanism. Thoracic aortic tissues from 20 patients with AD and healthy thoracic aortic tissue from 5 patients undergoing coronary artery bypass grafting were collected to detect OGN expression levels. Following OGN knockdown in rat aortic smooth muscle cells, cell proliferation was detected by performing Cell Counting Kit-8 and BrdU assays, cell migration was assessed by performing the wound healing assay, cell invasion was detected by performing the Transwell assay, and VEGFR/AKT signaling pathway-related protein expression levels were measured via western blotting. The results demonstrated that OGN expression was significantly downregulated in patients with AD compared with healthy controls. Compared with the si-negative control (NC) group, OGN knockdown promoted RASMC proliferation and migration. Compared with the si-NC group, OGN knockdown also significantly enhanced the phosphorylation of the downstream signaling molecules of VEGFR, including AKT and ERK1/2, in VEGF-stimulated RASMCs. Collectively, the present study indicated that OGN knockdown facilitated RASMC proliferation and migration by activating AKT and ERK1/2 signaling. Therefore, OGN may serve as a novel therapeutic target for AD.

Introduction

Aortic dissection (AD) is a major complication of thoracic aortic disease, which is initiated by tears in the aortic intima and media. The tears allow blood to enter into the media and separate the medial layer along the long axis of the aorta, thus leading to the formation of a false lumen (1,2). Multiple factors, including poorly controlled hypertension, older age, male gender, smoking, genetic conditions, pre-existing aortic diseases, aortic instrumentation or surgery, and immune/inflammatory diseases, are associated with an increased risk of AD (3-5). Although AD is an uncommon disease with an estimated annual incidence of 5-30 cases per million individuals (6), the disease displays high mortality and is ranked among the most lethal vascular diseases worldwide (7-9). Despite advances in therapeutic strategies, the cellular and molecular mechanisms underlying AD are not completely understood and require further investigation.

AD is pathologically characterized by the degeneration of the aortic media, which includes depletion of smooth muscle cells, destruction of elastic fibers and disruption of the extracellular matrix (ECM) network (10,11). ECM components in the aortic wall not only provide structural support for vascular cells, but also integrate extracellular signals and modulate cellular responses (12). Alterations in ECM components serve critical roles in the pathogenesis of AD (13). Increasing evidence has suggested that an increase in the expression of proteoglycans, a major group of nonfibrillar ECM components, is a crucial event in AD and is closely associated with the degeneration of the aortic media (12,14). Therefore, exploring the expression profiles of proteoglycans and their functional effects on medial smooth muscle cells is important for understanding the development of AD.

In our previous study, 99 aortic tissue samples from patients with AD were collected, and the genomic profiles were analyzed (15). A total of 3,425,873 SNPs, 685,245 insertion-deletions and 1,177 copy number variations were identified. By performing disease correlation analysis, 20 candidate genes were identified. A number of identified genes, such as myosin heavy chain 11, fibrillin 1 and actin α 2, smooth muscle, were consistent with previous studies (16-18), whereas,

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to the best of our knowledge, MAX dimerization protein MLX, DAB2 interacting protein, E1A binding protein p300, zinc finger FYVE-type containing 9, PML nuclear body scaffold, protein kinase C- δ and osteoglycin (OGN) were identified as AD-associated genes for the first time in our previous study.

OGN, which belongs to cluster III of the small leucine-rich proteoglycans (SLRP), is an ECM component that modulates various biological processes, including cell proliferation, inflammation and collagen fibrillogenesis (19-21). OGN is involved in numerous pathological conditions, including bone disease, eye disease, neurological damage and cancer (22). Plasma OGN expression levels are lower in patients with coronary artery disease with complex atherosclerotic lesions compared with patients with coronary artery disease without lesions (23). Proteomic analysis has demonstrated decreased OGN expression levels in calcified abdominal aortic aneurysm tissues compared with healthy adjacent aortic tissues (24). Although emerging evidence has revealed the effects of OGN on vascular diseases, the exact role of OGN in AD formation requires further investigation.

The present study aimed to investigate the effect of OGN, an ECM proteoglycan, on AD, as well as the underlying mechanism. Therefore, OGN expression profiles in thoracic aortic tissues from patients with AD and healthy thoracic aortic tissues from control subjects were determined. The effect of OGN on cellular proliferation and migration was determined in cultured rat aortic smooth muscle cells (RASMCs). RASMCs are typically used to study alterations in cellular and molecular biology during the progression of vascular disease under the influence of internal and external factors, and are a good model for studying mechanisms *in vitro* (25). The present study investigated the possible mechanisms underlying OGN in the pathological process of AD.

Materials and methods

Cell culture. RASMCs (Procell Life Science & Technology Co., Ltd.) were cultured in DMEM (Procell Life Science & Technology Co., Ltd.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin in a humidified incubator at 37°C with 5% CO₂. RASMCs at passage 3-6 were used for subsequent experiments. Following transfection, cells were treated with recombinant human VEGF protein (10 ng/ml; R&D Systems China Co., Ltd.) for 5, 15 or 30 min at 37°C.

Sample collection. In the present study, all volunteers were recruited from Fuwai Hospital Chinese Academy of Medical Sciences Shenzhen, although volunteers were from residence across China. Pathological aortic tissues from 20 patients with AD (age, 45-62 years; 16 male patients and 4 female patients) and healthy aortic tissues from 5 patients who underwent coronary artery bypass grafting surgery (age, 55-74 years; 3 male patients and 2 female patients) were collected between October 2018 and October 2019. The following inclusion criteria were used in the present study: Pain symptoms for <48 h; and diagnosed with acute AD after aorta computed tomography angiography examination. The following exclusion criteria were used in the present study: Heart failure, acute myocardial infarction, connective tissue disease or tumor disease.

The present study was approved by the Research Ethics Committee of Fuwai Hospital Chinese Academy of Medical Sciences Shenzhen (approval no. SP2019004). Written informed consent was obtained from each patient.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from each sample using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using PrimeScript™ RT Master Mix [cat. no. RR036A; Takara Biomedical Technology (Beijing) Co., Ltd.] according to the manufacturer's protocol. Subsequently, qPCR was performed using SYBR green fluorescence [Takara Biomedical Technology (Beijing) Co., Ltd.]. The following primers were used for qPCR: OGN forward, 5'-TCTACACTTCTCCTGTTACTGCT-3' and reverse, 5'-GAGGTGGTGGTGTT-ATTGCCTCA-3'; and GAPDH forward, 5'-GGCAGTGATGGCATGGACTGT-3' and reverse, 5'-CCTTCATTGACCTCAACTACA-3'. The following thermocycling conditions were used for qPCR: 95°C for 2 min; followed by 40 cycles of 95°C for 5 sec, 60°C for 30 sec and 72°C for 60 sec. mRNA expression levels were quantified using the 2^{- $\Delta\Delta C_q$} method (26) and normalized to the internal reference gene GAPDH.

Western blotting. Total protein was extracted from tissues or cultured cells using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.). Protein concentration was determined using a BCA Protein Assay kit (Beijing Solarbio Science & Technology Co., Ltd.). Equal amounts of protein (30 μ g) were separated via 10% SDS-PAGE and transferred to PVDF membranes. Following blocking with 5% skimmed milk in TBST (0.05% Tween-20) for 1 h at room temperature, the membranes were incubated at 4°C overnight with the following primary antibodies: anti-GAPDH (cat. no. 5174; 1:10,000; Cell Signaling Technology, Inc.), anti-Tubulin (cat. no. 2148; 1:10,000; Cell Signaling Technology, Inc.), anti-OGN (cat. no. ab211456; 1:2,000; Abcam), anti-phosphorylated (p)-AKT (cat. no. 4060; 1:2,000; Cell Signaling Technology, Inc.), anti-total AKT (cat. no. 9272; 1:2,000; Cell Signaling Technology, Inc.), anti-p-ERK1/2 (cat. no. 9102; 1:1,000; Cell Signaling Technology, Inc.), anti-total ERK1/2 (cat. no. 9101; 1:1,000; Cell Signaling Technology, Inc.), anti-p-VEGFR2 (cat. no. 2478; 1:2,000; Cell Signaling Technology, Inc.) and anti-total VEGFR2 (cat. no. 9698; 1:2,000; Cell Signaling Technology, Inc.). After washing with TBST for three times for 5 min each, the membranes were incubated with an anti-rabbit IgG (cat. no. 7074; 1:10,000; Cell Signaling Technology, Inc.) secondary antibody for 1 h at room temperature. Following washing with TBST for three times for 5 min each, protein bands were visualized using SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Inc.) and an iBright bioimaging system (Invitrogen; Thermo Fisher Scientific, Inc.). Protein expression levels were semi-quantified using Quantity One Analysis software (version 4.0; Bio-Rad Laboratories, Inc.) with GAPDH or Tubulin as the loading control.

Transfection of OGN-targeting small interfering (si) RNA. At 80% confluence, RASMCs were transfected with 50 nM OGN-specific siRNA or control siRNA using

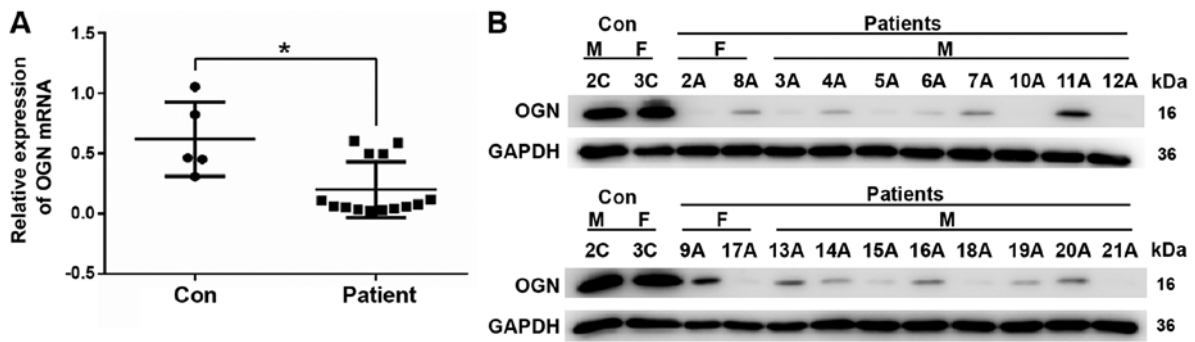


Figure 1. OGN expression levels in patients with AD and healthy control subjects. Thoracic aortic tissues from 20 patients with AD and healthy thoracic aortic tissues from 5 control subjects were collected. In two of the healthy thoracic aortic tissue samples, total protein extraction and OGN protein expression analysis failed. OGN (A) mRNA and (B) protein expression levels. * $P < 0.05$. OGN, osteoglycin; AD, aortic dissection; Con, control; M, male; F, female; C, control; A, AD.

Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 48 h. The OGN-specific siRNA (si-OGN; forward, 5'-GUGCCCACCAAGAAAGAAATT-3' and reverse, 5'-UUUCUUUCUUGGUGGGCACTT-3') and control siRNA [si-negative control (NC); non-specific; forward, 5'-ACAACGACAAGCGAACAACA-3' and reverse, 5'-UGUUGUUCGCUUGUUCGUUGU-3'] were synthesized by Sangon Biotech Co., Ltd. Cells were divided into three groups: i) Blank, cells were left untreated; ii) NC, cells were transfected with si-NC; and iii) OGN (marked as OGN-Rat-401), cells were transfected with si-OGN. At 48 h post-transfection, cells were used for subsequent experiments.

Cell proliferation assay. The effect of OGN knockdown on RASMC viability was assessed by performing a Cell Counting Kit-8 (CCK-8) assay. Briefly, RASMCs were seeded (2.5×10^3 cells/well) into 96-well plates. At 48 h post-transfection, platelet-derived growth factor-BB (PDGF-BB; final concentration, 60 $\mu\text{g/l}$; Cyagen Biosciences, Inc.) was added to the culture medium to stimulate cell proliferation for 24 h at 37°C. Subsequently, 10 μl CCK-8 reagent (Dojindo Molecular Technologies, Inc.) was added to each well and incubated in the dark for 1.5 h at 37°C. The optical density was measured at a wavelength of 450 nm using a microplate reader (Promega Corporation).

A BrdU immunofluorescence assay was performed on OGN-knockdown RASMCs. RASMCs were seeded (2×10^4 cells/well) into 6-well plates. At 48 h post-transfection, cells were incubated with 50 μM BrdU for 12 h at 37°C in humidified incubator with 5% CO_2 . Cells were fixed with 4% paraformaldehyde at room temperature for 20 min. Subsequently, cells were washed with PBS and desaturated with 2 M HCl at 37°C for 10 min. H_3BO_3 (pH 8.5) was used for renaturation at room temperature for 10 min. Samples were washed three times with PBS for 5 min. Cells were permeabilised using 0.5% Triton X-100 at room temperature for 15 min. Cells were washed three times with PBS for 5 min, blocked with non-immunized goat serum (Beijing Dingguo Changsheng Biotechnology Co., Ltd.; 1:1,000) at 37°C for 30 min and incubated with a BrdU primary antibody (cat. no. ab8152; 1:100; Abcam) overnight at 4°C. Following washing three times with PBS for 5 min, cells were incubated with the secondary antibody (cat. no. 8890; 1:1,000; Cell Signaling Technology, Inc.) at 37°C for 2 h. Samples were washed three times with PBS and

then were incubated with DAPI (1:100) at room temperature for 5 min. Following two washes with PBS for 5 min, samples were washed with distilled water. The slides were dried and sealed with 50% glycerin. Stained sections were observed in five fields of view using an LH-M100CB inverted fluorescence microscope (Nikon Corporation; magnification, $\times 200$).

Wound healing assay. For the wound healing assay, RASMCs were seeded into 6-well plates. At 95% confluence, the cell monolayer was mechanically scraped using a sterile pipette tip to create a single scratch. Cells were maintained in serum-free medium. At 0, 24 and 48 h, the wound was observed using a light optical microscope (magnification, $\times 40$). The results are presented as the distance of wound healing, which was measured using ImageJ software (version 1.8.0.112; National Institutes of Health).

Cell invasion assay. RASMCs were seeded (5×10^4 cells/well) into the upper chamber of the Matrigel-coated Transwell plate (pore size, 8 μm). DMEM supplemented with 10% FBS was plated into the lower chambers. Following incubation for 48 h at 37°C, invading cells were fixed with 4% paraformaldehyde at room temperature for 20 min, and stained with 0.5% crystal violet solution at room temperature for 10 min. Invading cells were visualized in five randomly selected fields of view using a light microscope (magnification, $\times 200$).

Statistical analysis. Statistical analyses were performed using GraphPad Prism software (version 6; GraphPad Software, Inc.). Comparisons among multiple groups were analyzed using one-way ANOVA followed by Dunnett's post hoc test. Comparisons between two groups were analyzed using an unpaired Student's t-test. Data are presented as the mean \pm SD of three independently repeated experiments. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

OGN is downregulated in aortic tissues from patients with AD. OGN mRNA expression levels were significantly lower in the thoracic aortic tissues of 20 patients with AD compared with the healthy thoracic aortic tissues of 5 control subjects (Fig. 1A). The western blotting results demonstrated that the protein expression levels of OGN in the thoracic aortic tissues

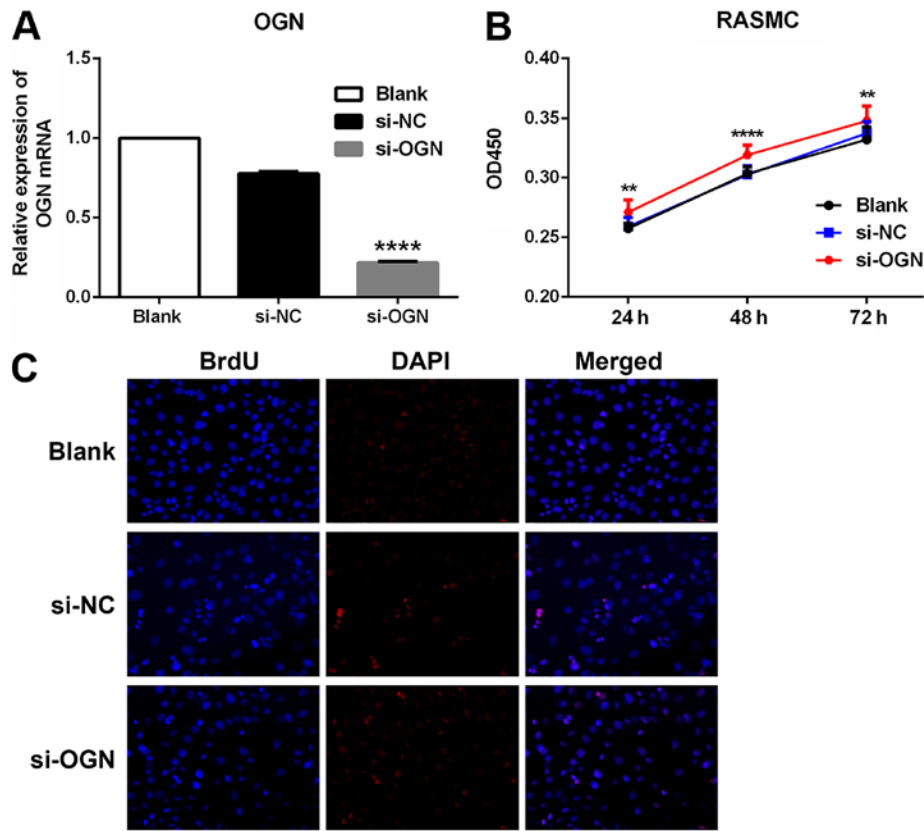


Figure 2. Effect of OGN knockdown on RASMC proliferation. Cells were divided into three groups: i) Blank, cells were left untreated; ii) NC, cells were transfected with si-NC; and iii) OGN (marked as OGN-Rat-401), cells were transfected with si-OGN. Transfected RASMCs were seeded into 96-well plates and stimulated with 60 $\mu\text{g/l}$ platelet-derived growth factor-BB. (A) Transfection efficiency of si-OGN. (B) Cell proliferation was determined by performing the Cell Counting Kit-8 assay. (C) Cell proliferation was also assessed by performing BrdU immunofluorescence staining (magnification, x200). ** $P < 0.01$ and **** $P < 0.0001$ vs. si-NC. OGN, osteoglycin; RASMC, rat aortic smooth muscle cell; NC, negative control; si, small interfering RNA; OD, optical density.

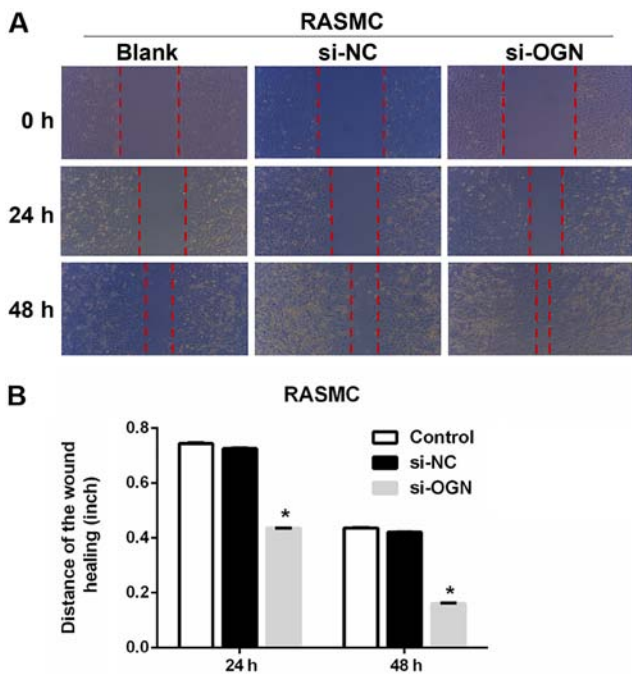


Figure 3. Effect of OGN knockdown on RASMC migration. si-OGN-transfected RASMCs were seeded into 6-well plates to form confluent monolayers. A sterile pipette was used to form a single scratch in the cell monolayer. The wound was observed at 0, 24 and 48 h using an optical microscope. Cell migration was (A) determined by performing a wound healing assay (magnification, x40) and (B) quantified. * $P < 0.05$ vs. si-NC. OGN, osteoglycin; RASMC, rat aortic smooth muscle cell; si, small interfering RNA; NC, negative control.

from 20 patients with AD were markedly reduced compared with the thoracic aortic tissues from 3 out of the 5 control subjects (Fig. 1B).

OGN knockdown enhances RASMC proliferation and migration. Following transfection with si-OGN, OGN mRNA expression levels were significantly decreased compared with the si-NC group (Fig. 2A). The CCK-8 assay was performed to investigate whether OGN knockdown affected PDGF-BB-induced cell proliferation and survival. OGN knockdown significantly enhanced RASMC proliferation at 24, 48 and 72 h compared with the si-NC group (Fig. 2B). The immunofluorescence assay displayed similar results (Fig. 2C); cell proliferation was also notably increased in the si-OGN group compared with the si-NC group.

To assess the effect of OGN on RASMC migration, wound healing and Transwell invasion assays were performed. Compared with the si-NC group, OGN knockdown significantly decreased the width of the wound by 41.1 and 61.9% at 24 and 48 h, respectively (Fig. 3A and B). Moreover, the number of invading RASMCs was decreased by ~30% in the si-OGN group compared with the si-NC group (Fig. 4A and B). The results indicated that OGN knockdown was associated with increased RASMC proliferation and migration, and decreased invasion.

OGN knockdown further increases the phosphorylation of AKT and ERK1/2 in VEGF-treated RASMCs. In mouse models

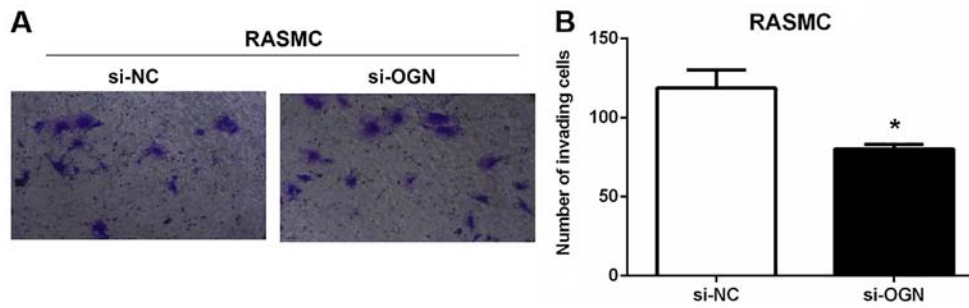


Figure 4. Effect of OGN knockdown on RASMC invasion. si-OGN transfected RASMCs were seeded (5×10^4 cells/well) into the upper chamber of the Transwell plate. DMEM supplemented with 10% FBS was plated into the lower chambers. Following incubation for 48 h, invading cells were fixed with cold methanol and 4% paraformaldehyde, and stained with 0.5% crystal violet solution. Invading cells were observed in five randomly selected fields of view. Cell invasion was (A) determined by performing a Transwell invasion assay (magnification, $\times 200$) and (B) quantified. * $P < 0.05$ vs. si-NC. OGN, osteoglycin; RASMC, rat aortic smooth muscle cell; si, small interfering RNA; NC, negative control.

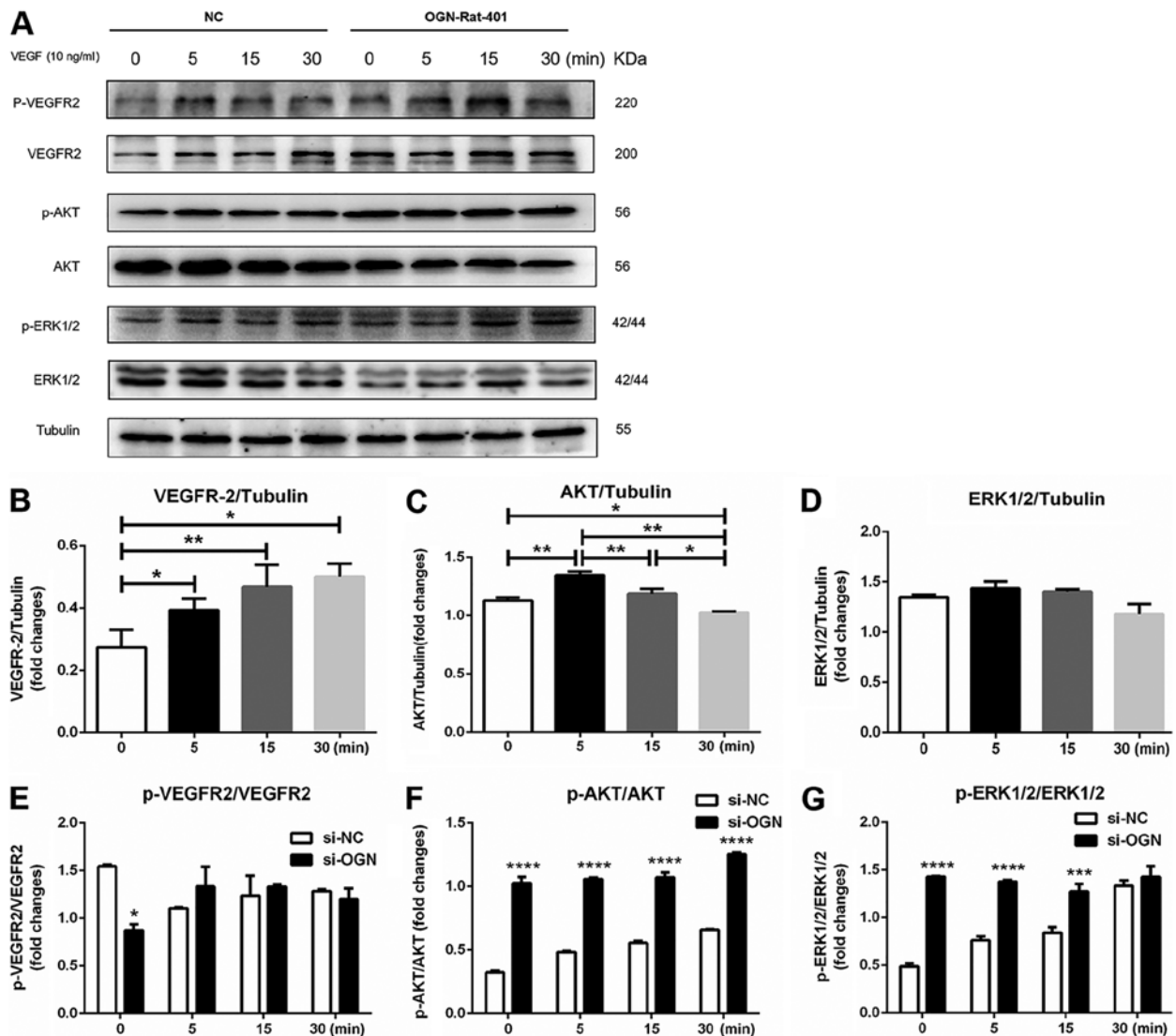


Figure 5. OGN regulates the VEGFR/AKT signaling pathway. Cells were transfected with si-OGN or si-NC for 48 h. Subsequently, cells were treated with VEGF (10 ng/ml) for 5, 15 or 30 min. Protein expression levels were (A) determined via western blotting and semi-quantified for (B) VEGFR2, (C) AKT, (D) ERK1/2, (in si-NC-transfected cells) (E) p-VEGFR2/VEGFR2, (F) p-AKT/AKT and (G) p-ERK1/2/ERK1/2. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ vs. si-NC. OGN, osteoglycin; si, small interfering RNA; NC, negative control; p, phosphorylated.

of limb ischemia, increased expression levels of OGN are closely related to inhibition of the VEGF/VEGFR2 signaling pathway (27,28). In the present study, OGN-knockdown

RASMCs displayed significantly reduced VEGFR2 phosphorylation compared with si-NC-transfected RASMCs (Fig. 5A and E). VEGF-mediated stimulation of

si-NC-transfected RASMCs significantly increased the levels of VEGFR2, and increased the expression level of AKT at 5 min compared with 0 min, which returned to the baseline level at 30 min (Fig. 5A, B and C). The protein expression levels of ERK1/2 were not significantly altered among the different groups (Fig. 5A and D). In si-NC-transfected RASMCs, VEGF stimulation notably increased the phosphorylation levels of ERK1/2 and AKT in a time-dependent manner (Fig. 5A, F and G). Moreover, ERK1/2 and AKT phosphorylation levels were significantly increased in si-OGN-transfected RASMCs compared with si-NC-transfected RASMCs, although a significant difference in ERK1/2 phosphorylation between the si-NC and si-OGN groups was not observed following 30 min VEGF stimulation (Fig. 5A, F and G). The results suggested that OGN knockdown facilitated VEGF-induced activation of AKT and ERK1/2.

Discussion

In the present study, the results demonstrated that OGN expression levels were significantly downregulated in patients with AD compared with healthy controls. Furthermore, the results suggested that OGN expression was negatively associated with cell proliferation and migration. Inspired by our previous work and other previous studies, the present study indicated that OGN may regulate the downstream signaling molecules AKT and ERK1/2 via the VEGF/VEGFR2 signaling pathway, thereby affecting cell proliferation, migration and angiogenesis.

Increasing evidence has demonstrated that the altered expression of proteoglycans is associated with degeneration of the aortic wall in AD (29). Versican, a large chondroitin sulfate proteoglycan, is required for RASMC proliferation and migration, and its degradation leads to fragmentation of elastin and predisposition to AD (30,31). Genetic depletion of biglycan, a member of the class I family of SLRPs, results in spontaneous AD and rupture (32). Alterations in the expression levels of OGN, which was originally identified as a modulator of bone formation, have been implicated in atherosclerosis, myocardial fibrosis, schamaemia-induced angiogenesis and other vascular diseases (28,33,34). The expression levels of OGN in aortas are increased in adult rats at 2 weeks post-balloon injury and in 2-week-old neonatal rats (33). OGN mRNA expression levels are downregulated in rat RASMCs stimulated with basic fibroblast growth factor, TGF β , PDGF and angiotensin II (33). Similarly, OGN expression levels are decreased in the hypertrophic aortas of sinoaortic-denervated rats (35). Consistent with the aforementioned studies, the present study demonstrated that OGN expression levels were significantly downregulated in the thoracic aortic tissues of patients with AD compared with healthy controls, suggesting that OGN might be involved in the pathological progression of AD.

Aortic vascular smooth muscle cells, the predominant cell type in the medial layer of the aortic wall, serve an important role in maintaining structural integrity and regulating vascular tone (36). RASMC proliferation and migration occur in response to various vascular injuries, and contribute to the development of pathological remodeling and vascular diseases (37). Mutations in genes encoding proteins required for SMC contraction leads to the occurrence of thoracic aortic aneurysms and dissections (38). The switch from a contractile

RASMC phenotype to a synthetic, migratory and proliferative RASMC phenotype is a pivotal contributor to the development of AD (39). The present study indicated that compared with si-NC-transfected RASMCs, OGN-knockdown RASMCs displayed enhanced proliferation and migration, which may facilitate the development of AD. Previous studies have indicated that OGN is closely related to proliferation and migration in a number of different cell types (40,41). OGN overexpression reduces proliferation and inhibits invasion in human colon cancer cell lines (20,42). In the present study, compared with si-NC, OGN knockdown increased RASMC migration but decreased invasion. Therefore, it was hypothesized that RASMCs might respond to multiple growth factors, inflammatory cytokines and vasoactive substances, leading to OGN downregulation, which promotes cell proliferation and migration, and thereby modulating AD progression. The different effects of OGN knockdown on the invasion abilities of RASMCs and colon cancer cells may be attributed to the distinct extracellular stimuli and intracellular signaling pathways of the two cell types.

The VEGF/VEGFR axis is involved in numerous physiological and pathological processes, including embryologic development, normal growth, tissue repair and tumorigenesis (43,44). In the process of angiogenesis, VEGF binds to VEGFR on endothelial cells, resulting in endothelial cell proliferation and migration (45). OGN competitively binds to VEGFR2 in cultured human umbilical vein endothelial cells (HUVECs) (46), and a coimmunoprecipitation assay confirmed the direct interaction between OGN and VEGFR2. Wu *et al* (28) further reported that OGN negatively modulates the activation of VEGFR2 and its downstream signaling pathways. In the present study, no significant alterations in the levels of phosphorylated VEGFR2 after exposure to VEGF for 5-30 min were observed between si-NC- and si-OGN-transfected RASMCs; however, VEGF-induced activation of AKT and ERK1/2 was significantly enhanced in OGN-knockdown cells compared with si-NC-transfected cells. AKT and ERK1/2 are important downstream signaling molecules that are required for the VEGFR2-induced proliferation and migration of lymphatic endothelial cells and human brain RASMCs (47,48). The results of the present study indicated that compared with the si-NC group, OGN knockdown significantly enhanced the phosphorylation of AKT and ERK1/2 in RASMCs, which triggered cell proliferation and migration. The results of the present study were consistent with the finding that OGN knockdown in HUVECs resulted in enhanced AKT and ERK1/2 phosphorylation in response to VEGF (46).

Other VSMC-related mechanisms may exist in AD. ECM softening serves a pivotal role in regulating the VSMC phenotype switch and provides a potential target for treating VSMC dysfunction and AD disease, which has been reported in Cardiovascular Toxicology (49). The aforementioned study focused on synthetic phenotype-related genes and ECM softening phenotype, whereas the present study focused on the role of OGN in VSMC proliferation and migration, providing two possible mechanisms underlying AD. The two identified mechanisms may display cross-talk and share certain targets and factors, but further investigation is required. Synthetic phenotype-related genes, including osteopontin, matrix

metalloproteinases and inflammatory cytokines, are upregulated in VSMCs (49). On the other hand, OGN is a component of the vascular extracellular matrix and may also influence the vascular system (50). Therefore, it was hypothesized that OGN may contribute to ECM softening in the regulation of the VSMC phenotype switch.

The present study had a number of limitations. First, the number of samples included in the present study was limited due to the low incidence of AD. The RASMC model used in the present study is a common tool used in AD research (25), but cell proliferation and migration can occur in a number of other vascular diseases, such as atherosclerosis and vascular neointima formation (51,52). In addition, the function of OGN in AD may be related to the process of angiogenesis. However, no angiogenesis-related genes were analyzed in the AD tissues or in the RASMC model in the present study. If angiogenesis is the target of OGN, further research in vascular endothelial cells rather than vascular smooth muscle cells is required. Therefore, future studies should investigate the effect of OGN on angiogenesis in AD to reveal the possible underlying mechanisms.

In conclusion, the present study suggested that OGN knockdown facilitated the stimulatory effect of PDGF-BB on RASMC proliferation and migration. The results indicated that OGN regulated the VEGF/VEGFR2 axis and the downstream signaling molecules AKT and ERK1/2, thus affecting the biological activity of RASMCs. Therefore, OGN may serve as a novel therapeutic target for AD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZW and MW performed the literature review, wrote the initial draft and analyzed the data. XZ and BC performed the experiments and collected the data. MW provided ideas and recommendations, designed the research and reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All the procedures involving human participants were performed in accordance with the ethical standards of the institutional and the 1964 Declaration of Helsinki and its later

amendments or comparable ethical standards. The present study was approved by the Ethics Committee of Fuwai Hospital Chinese Academy of Medical Sciences Shenzhen (approval no. SP2019004). All patients signed an informed consent form, which was approved by the Institutional Review Board.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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