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Nitrosative stress induces downregulation of ribosomal protein genes *via* MYCT1 in vascular smooth muscle cells

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Abstract. – OBJECTIVE: In our previous genomic studies in human intracranial aneurysms, we observed downregulations in the expression of a number of ribosomal protein genes and the c-Myc-related gene MYC target 1 (MYCT1). So far there is no information about the roles of MYCT1 in vascular cells. Our study aims to investigate the functional roles of MYCT1 in vascular smooth muscle cells (SMCs).

MATERIALS AND METHODS: Primary SMCs were isolated from rat thoracic aorta and cultured *in vitro*. The mRNA and protein expressions were determined by real-time PCR and western blot respectively. Apoptosis was detected by measuring caspase 3/7 activity. Collagen production was determined with ELISA.

RESULTS: Using PCR, we validated our previous genomic data showing that the expressions of MYCT1 and ribosomal protein genes were decreased in human aneurysm tissues. In vascular SMCs, we showed that nitrosative stress downregulated the expression of both MYCT1 and ribosomal proteins. Knockdown of MYCT1 mimicked the effects of nitrosative stress on ribosomal protein expressions, whereas overexpression of MYCT1 blunted the effects of nitrosative stress. MYCT1-dependent downregulation of ribosomal proteins compromised the protein translational capacity of the cells for collagen production. Moreover, the endogenously expressed MYCT1 in vascular SMCs was involved in maintaining normal cellular functions including survival, proliferation and migration.

CONCLUSIONS: MYCT1-dependent gene regulation may, at least partly, explain the downregulated expressions of ribosomal proteins observed in human intracranial aneurysms. It is suggested that MYCT1 may represent a novel molecular target for counteracting the decreased activity of aneurysmal SMCs for tissue repairmen/regeneration.

Key Words:
MYCT1, Vascular smooth muscle cell, Gene regulation, Ribosomal proteins, Intracranial aneurysm.

Introduction

Intracranial aneurysm (IA) is a cerebrovascular disorder characterized by local dilatation of intracranial arteries. The prevalence of unruptured IAs in the general population is approximately 3-5%[1]. Rupture of IAs results in subarachnoid haemorrhage (SAH), which is associated with high mortality and morbidity[2]. The pathogenic mechanisms of IA formation remain poorly understood. Several risk factors, including genetic backgrounds, aging, hypertension, smoking and arteriosclerosis, have been identified in IA patients, all of which may contribute to IA formation and development[3].

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Our previous genomic studies have suggested that there are dysregulations of the protein translation/synthesis pathway in human IA tissues[4,5]. Specifically, a number of genes encoding ribosomal proteins were significantly downregulated in IA tissues[4]. However, the mechanisms of this phenomenon are not clear. It is well established that the transcription factor c-Myc is a master regulator of ribosome biogenesis[6]. Evidence has shown that c-Myc drives the transcription of ribosomal RNAs and the expression of ribosomal proteins[6,7]. Nevertheless, our study did not detect a significant change in c-Myc expression in IAs[4]. Interestingly, we found a significant downregulation of a c-Myc-related gene, MYC target 1 (MYCT1), in IAs[4].

MYCT1 was first cloned in myeloid cells[8]. It is a direct transcriptional target gene of c-Myc. Interestingly, it has been shown that MYCT1 *per se* can regulate the expression of various c-Myc target genes thereby recapitulating many c-Myc functions in cells [8-10]. Previous studies on the cellular functions of MYCT1 were mainly carried out in cancer cells. Hitherto, whether MYCT1 has any functional role in vascular cells, especially in vascular smooth muscle cells (SMCs), remains to be clarified. Based on our previous results [4], here we hypothesize that MYCT1 may be involved in regulating the expression of ribosomal proteins in vascular SMCs.

Materials and Methods

Animal Tissues Collection

Use of animals in the present study was reviewed and approved by the Animal Ethics Committee of Shandong University. Normal adult male Sprague-Dawley rats were purchased from Vital River Laboratories (Beijing, China), and maintained on standard chow diet and water ad libitum. Animals were handled in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Rats were euthanized with overdose of pentobarbital sodium. Cerebral blood vessels (including the basilar artery, middle and posterior cerebral artery, and circle of Willis, all pooled together) and carotid arteries were dissected out, frozen in liquid nitrogen, and stored at -80°C. Thoracic aortae were removed under sterile conditions, and used immediately for SMC isolation.

Studies with Human Tissues

Human IA tissue samples and normal superficial temporal arteries (controls) used in this study were the leftovers from our previous study[5], which were preserved in -80°C freezer. The original study was approved by the Institutional Human Ethics Committee (#10051), with completed informed consents being obtained from the subjects or their first-degree relatives, in accordance with the Declaration of Helsinki. The IA tissues were obtained during aneurysmal clipping surgery; the control tissues were obtained from trauma patients undergoing craniotomy treatments[5].

Culture of Rat Primary Aortic SMCs

Rat aortic SMCs were isolated from thoracic aorta as previously described [11]. Briefly, the vessel was pre-digested with a mixture containing collagenase I (1 mg/ml), elastase (0.5 mg/ml) and trypsin (1.25 mg/ml) (all from Sigma-Aldrich, St. Louis, MO, USA). The adventitia was peeled off using fine forceps. The remaining tissue was cut into small blocks and further digested using the same enzyme mixture. The released cells were collected by centrifugation and cultured in Dulbecco's Modified Eagle Medium (DMEM) (M&C Gene Technology, Beijing, China) supplemented with 20% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA, USA), in 5% CO_2 at 37°C. After passage 3, cells were changed to and maintained in DMEM supplemented with 10% FBS. Cells between passages 5 - 10 were used for the experimentation. For in vitro treatments, NOC-18 was purchased from BioVision (Mountain View, California, USA); TGF-β1 was from Thermo Fisher Scientific, Waltham, MA, USA.

MYCT1 Overexpression and Knockdown

An original clone of human wild type MYCT1 was purchased from OriGene Technologies (#SC305235) (Rockville, MD, USA), and subcloned into a mammalian expression lentiviral vector system (Lenti-MYCT1) (GenePharma, Shanghai, China). A vector expressing GFP was used as control. Lentiviruses expressing shRNA constructs targeting rat MYCT1 were obtained from GenePharma. Three different shRNA sequences were designed and named shRNA-126, shRNA-215 and shRNA-420 respectively (**Supplementary Table I**). Transfection was performed by incubating SMCs with lentiviruses at 150 Multiplicity of Infection for Lenti-MYCT1 or at 100 Multiplicity of Infection for shRNAs. After 24 hours of incubation, the cells were washed and changed to fresh medium, and further cultured for different time intervals as indicated.

Real-Time Quantitative Polymerase Chain Reaction (qPCR)

Total RNA was extracted using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA was reverse transcribed to cDNA using PrimeScript RT Reagent Kit from Takara Bio (Shiga, Japan). The real-time PCR reaction was carried out using UltraSYBR Mixture (from Beijing ComWin Biotech, Beijing, China) for SYBR green chemistry, or Taqman Master Mix (from Thermo Fisher Scientific, Waltham, MA, USA) for Taqman chemistry. GAPDH or 18S was used as the house keeping gene. The raw data were processed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for SYBR green detection were listed in **Supplementary Table II**. Other genes not listed in the table were amplified using the inventory TaqMan primer-probe sets (purchased from Thermo Fisher Scientific, Waltham, MA, USA).

Western Blot Analysis

Total protein was extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) at 4°C. The protein concentration was determined using BCA Protein Assay Kit (Beyotime). The proteins were separated by SDS-PAGE and transferred onto PVDF membranes (Merck KGaA, Darmstadt, Germany). The membranes were probed overnight with primary antibodies diluted in 5% non-fat milk at 4°C, followed by incubation with horseradish peroxidase-conjugated secondary antibodies. The membrane was developed with an ECL Chemiluminescence substrate (Merck), and visualized using a Chemi-Doc XRS+ imaging system (BioRad, Hercules, CA, USA). Densitometry analysis was performed using ImageJ software bundled with Java (version 1.8.0_112) (NIH, Bethesda, Maryland, USA). The following primary antibodies were used: anti-RPS6 (#2217S, 1:1000), anti-RPL26 (#2065S, 1:1000) (both from Cell Signaling Technology, Beverley, MA, USA), anti-RPS7 (WH0006201M3, 1:1000), anti-RPL8 (SAB2500882, 1:1000), anti-RPL10A (WH0004736M1, 1:1000), anti-RPL36A (WH0006173M2, 1:1000) (all from Sigma-Aldrich).

Detection of Apoptosis

Cell apoptosis was detected with Caspase-Glo® 3/7 kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. The luminescence signal was detected using a Gemini XPS microplate reader (Molecular Devices, San Jose, CA, USA).

ELISA

The level of collagen I in the cell homogenate was determined using an ELISA kit from R&D Systems (Minneapolis, MN, USA), according to the manufacturer's instructions. Absorption at 450 nm was measured using an EMax Plus microplate spectrophotometer (Molecular Devices).

Cell Counting Kit-8 (CCK-8) Assay

Cell proliferation was measured by CCK-8 assay (kit from Beyotime)[12]. Cells were seeded in a 96-well plate at a density of 2×10^3 cells per well. At the end point, 10 µl of CCK-8 reagent was added in each well and incubated at 37°C for 4 hr. The O.D. values were read at 450 nm using EMax Plus microplate spectrophotometer.

EdU Pulse Labeling

Cell proliferation was also assessed by EdU incorporation using a kit from RiboBio (Guangzhou, China). Cells were pulse-labeled with 50 mM of EdU for 2 hr. After washing, cells were fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.5% Triton X-100. EdU staining was performed using the 1X Apollo® dye. The fluorescence was detected using flow cytometry at an emission wavelength of 643 nm (FACScalibur from Becton Dickinson, Franklin Lakes, NJ, USA).

Cell Migration

Cell migration was evaluated by the wound healing assay. Cells were maintained in 6-well plates. A wound (strip of scratch) was created on the cell monolayer using a 200-µl pipette tip. The wound was photographed under a light microscope at 0, 24 and 72 hr, and the average width of the wound was measured using ImageJ. The rate of cell migration was expressed as the % reduction of the wound at 24 and 72 hr.

Immunofluorescence

Immunofluorescence was performed as described previously[13]. Cells were fixed in 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. After blocking with 1% BSA in PBS, the cells were incubated overnight with polyclonal anti-MYCT1 antibody (#PA5-109999 from Thermo Fisher, 1:100). The signal was detected with Alexa Fluor 594-conjugated secondary an-

tibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Nuclei were counterstained with DAPI for 15 min. Images were taken using an upright fluorescence microscope (Nikon Eclipse Ni-U, Nikon, Tokyo, Japan).

Statistical Analysis

All quantitative data were expressed as mean ± standard error of the mean (SEM). Differences among multiple groups were detected with one-way analysis of variance (ANOVA) followed by *post-hoc* Tukey's test (two-tailed). Differences between two groups were detected with unpaired *t*-test (two-tailed). SPSS software version 20 (IBM, Armonk, NY, USA) was used for statistical analyses. GraphPad Prism version 6 (GraphPad Software, San Diego, CA, USA) was used for creation of data graphs. A p-value of < 0.05 was considered to be statistically significant.

Results

Downregulated Expressions of MYCT1 and Ribosomal Protein Genes in Human IA Tissues

Our previous genomics data (GEO accession #GSE26969) have revealed a trend of downregulation of ribosomal protein genes in human IA tissues[4] (**Supplementary Figure 1**). To validate this finding, we performed qPCR assays using human IA samples. For this purpose, we selected 5 ribosomal protein genes, namely RPS3, RPS7, RPS15, RPL8 and RPL19. The results demonstrated that all of these ribosomal protein genes were significantly downregulated in human IA tissues, as compared to normal superficial temporal arteries as controls (Figure 1A). To exclude the possibility that intracranial and extracranial blood vessels have distinct phenotypes in ribosomal protein gene expressions, we compared the mRNA levels of RPS15, RPL8 and RPL19 between the cerebral artery and carotid artery from normal rats. As shown in Figure 1B, there were no significant differences in the expression of these ribosomal protein genes between intracranial and extracranial blood vessels. Furthermore, our previous microarray data showed that the expression level of MYCT1 was also downregulated in IAs (**Supplementary Figure 2**). In the present study, we also confirmed this change in MYCT1 expression with qPCR (Figure 1C).

Intracellular Distribution of MYCT1 Protein in Vascular SMCs

Since SMC is the most abundant cell type in the arterial wall, in the following experiments we

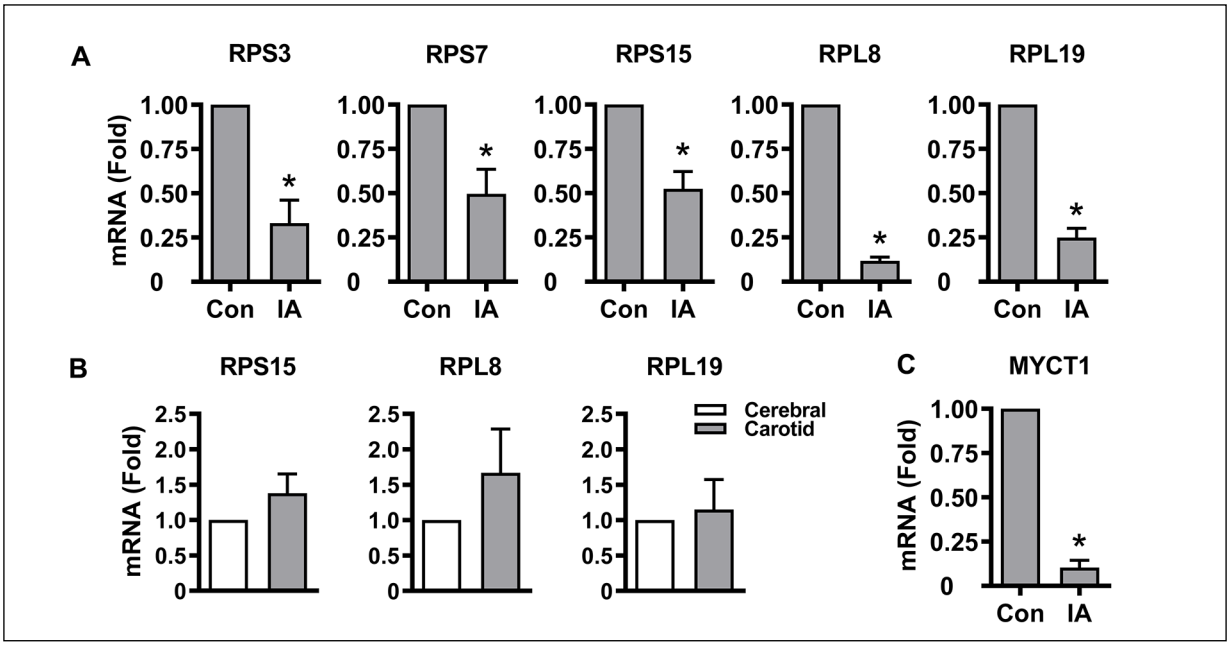

Figure 1. Downregulated expressions of ribosomal protein genes and MYCT1 in human intracranial aneurysm (IA) tissues. **A,** Real-time qPCR results showing the expression levels of various ribosomal proteins in human IAs and normal superficial temporal arteries (Con). **B,** qPCR results showing that there were no significant differences in the expression of ribosomal protein genes between rat cerebral and carotid arteries. **C,** qPCR results showing that MYCT1 expression was reduced in human IAs. Data are expressed as mean ± SEM. *$p < 0.05$ *vs.* Con, unpaired *t*-test ($n = 4$ for A, 3 for B, and 4 for C).

used primary vascular SMCs to study the interrelationship between MYCT1 and the expression of ribosomal protein genes. Currently there is no data about MYCT1 in vascular cells, we therefore first detected the intracellular expression pattern of MYCT1 in SMCs. Immunofluorescence labeling demonstrated that the majority of MYCT1 was located in the nucleus (**Supplementary Figure 3**), which is consistent with the observation in myeloid cells[8].

Nitrosative Stress Downregulates the Expression of Both MYCT1 and Ribosomal Proteins in Vascular SMCs

There is evidence suggesting that nitrosative stress induced by over-production of nitric oxide (NO) in the cerebral vasculature may contribute to the pathogenesis of IA[14-16]. Hence, in the following experiments we examined whether nitrosative stress could affect the expression of ribosomal proteins in vascular SMCs. Nitrosative stress was mimicked by treating cells with the long-lasting NO donor NOC-18. Concentration-response and time course experiments demonstrated that treatment with NOC-18 at 100 mM for 24 hr consistently reduced the expression of RPS6, RPS7 and RPL19 genes (Figure 2A). Using this treatment condition, we confirmed that NOC-18 significantly downregulated the expression levels of RPS3, RPS14, RPS15, RPL8, RPL18 and RPL36 (Figure 2B). Next, we demonstrated that NOC-18 treatment also significantly downregulated the expression of MYCT1 (Figure 2C).

Knockdown of MYCT1 Expression Mimics the Effects of NOC-18 on Ribosomal Proteins

To clarify whether there was a direct link between MYCT1 and ribosomal protein gene expressions, we treated the cells with three different MYCT1 shRNA constructs. Validation experiments showed that shRNA-420, but not shRNA-126 or shRNA-215, exhibited a potent silencing effect on MYCT1 expression (**Supplementary Figure 4**). Using shRNA-420, we demonstrated that knockdown of MYCT1 expression mimicked the inhibitory effects of NOC-18 on the expression of ribosomal proteins (tested with RPS14, RPS15, RPL8, RPL18 and RPL36) (Figure 3A). To further confirm the effects of MYCT1 silencing, we performed western blotting, and found that shRNA-420, but not shRNA-126, significantly decreased the protein levels of RPS6, RPS7, RPL8, RPL10A, RPL26 and

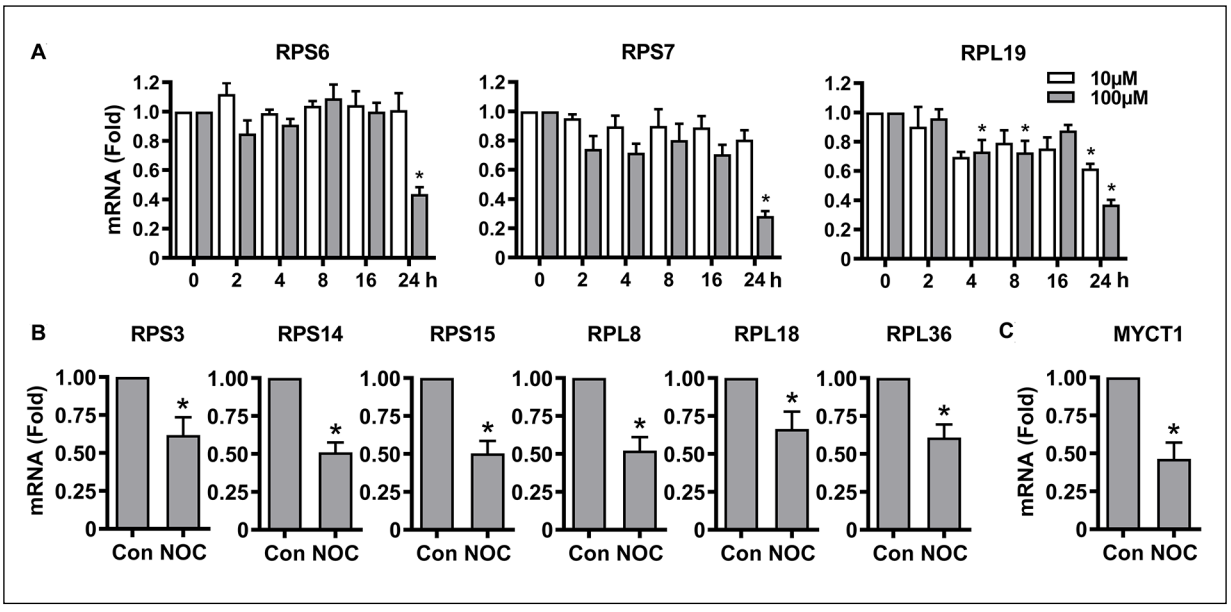

Figure 2. Effects of nitrosative stress induced by NOC-18 on the expression of ribosomal proteins and MYCT1 in rat primary vascular smooth muscle cells. **A,** qPCR results showing time- and concentration-dependent effects of NOC-18 on the expression of RPS6, RPS7 and RPL19. **B,** qPCR results showing the downregulation of various ribosomal protein genes after treatment with NOC-18 (100 mM for 24 hr). **C,** qPCR results showing that NOC-18 (100 mM for 24 hr) downregulated the expression of MYCT1. Data are mean ± SEM. *$p < 0.05$ *vs.* control (0 hr or Con groups), unpaired *t*-test or one-way ANOVA as appropriate ($n = 3$ for A, 4 for B, and 3 for C).

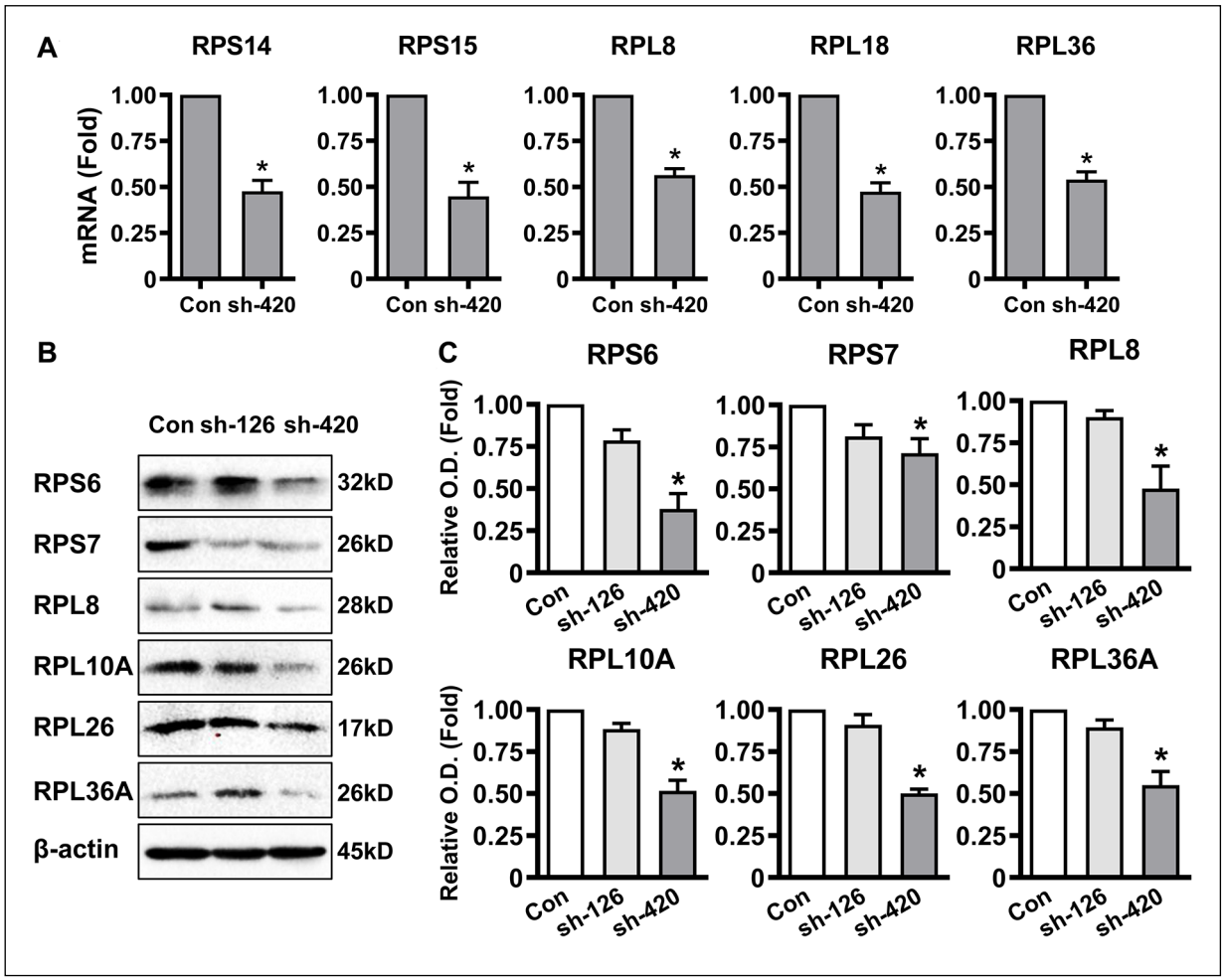

Figure 3. Effects of MYCT1 gene silencing on the expression of ribosomal proteins. **A,** qPCR results showing the effects of MYCT1 silencing by lentiviral shRNA-420 (treatment for 6 days) on the expression of various ribosomal proteins. **B,** and **C,** Western blots and quantitative densitometry data showing the effects of shRNA-126 and shRNA-420 on the protein levels of various ribosomal proteins. Data are mean ± SEM. *$p < 0.05$ *vs.* control shRNA (Con), unpaired *t*-test or one-way ANOVA as appropriate ($n = 5$ for A and 3 for B).

RPL36A (Figure 3B). The tested genes in qPCR and western blot experiments were not entirely identical, because some ribosomal proteins did not have suitable antibodies for western blotting.

Overexpression of MYCT1 Blunts the Effects of NOC-18 on Ribosomal Protein Expressions

Treatment with Lenti-MYCT1 did not change the basal mRNA levels of ribosomal proteins (Figure 4). However, Lenti-MYCT1 partially reversed the inhibitory effects of NOC-18 on ribosomal protein expressions (Figure 4). These data suggest that there is a causal relationship between MYCT1 and altered ribosomal protein gene expressions in SMCs under stress conditions.

Knockdown of MYCT1 Expression Limits the Protein Translation of Collagen I

Downregulated ribosomal protein expressions might limit ribosomal biogenesis, thereby decreasing the efficiency of protein synthesis. Given the importance of defects in extracellular matrix components in the development of IA[17], we tested whether knockdown of MYCT1 could affect the cellular capacity for collagen synthesis. ELISA (for procollagen α1) and qPCR (for COL1A1 gene) were conducted simultaneously in control and MYCT1-silenced cells; these experiments were to compare differential changes between the protein and mRNA levels of collagen I. Interestingly, MYCT1 silencing resulted in opposite changes in the mRNA and protein levels of col-

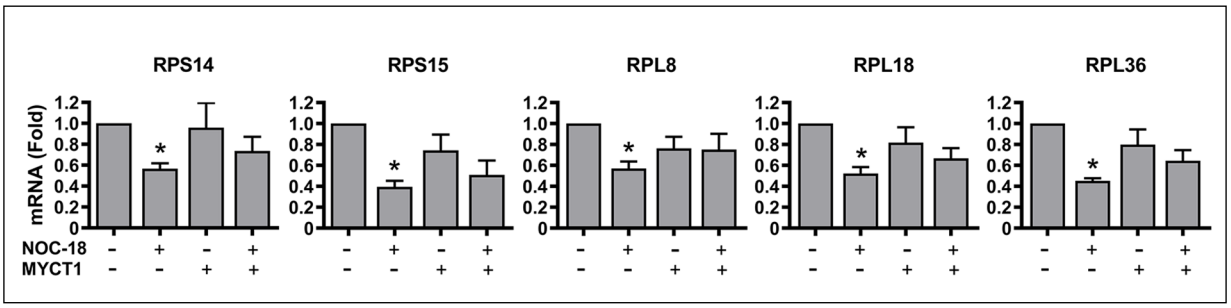

Figure 4. qPCR results showing that MYCT1 overexpression partially reversed the inhibitory effects of NOC-18 on ribosomal protein gene expressions. Lentivirus infection was carried out 6 days before NOC-18 treatment. Data are mean ± SEM. * $p < 0.05$ *vs.* control, one-way ANOVA ($n = 5$).

lagen I, with mRNA being upregulated whereas protein being decreased (Figure 5A). To further confirm this finding, we repeated the experiments in the presence of the pro-fibrotic factor TGF-β. TGF-β consistently stimulated collagen I mRNA and protein expressions in both control and MYCT1-silenced cells. However, in the presence of TGF-β, MYCT1 silencing still produced opposite effects on the mRNA and protein levels of collagen I (Figure 5B). It is not clearly understood why MYCT1 silencing upregulates the mRNA expression of collagen I, nevertheless these data indicate that reduced MYCT1 expression in SMCs may potentially result in a decrease in the translational efficiency of extracellular matrix proteins.

Impacts of MYCT1 on SMC Proliferation, Migration and Apoptosis

CCK-8 assays demonstrated that MYCT1 overexpression with Lenti-MYCT1 had no significant effect on SMC proliferation, while MYCT1 knockdown with shRNA-420 reduced cell proliferation (Figure 6A). We also performed EdU-labeling and flow cytometry assays, which confirmed the inhibitory effect of MYCT1 silencing on SMC proliferation (Figure 6B). MYCT1 overexpression did not affect SMC apoptosis, whereas MYCT1 silencing significantly increased cell apoptosis (Figure 6C). Finally, we showed that MYCT1 silencing also significantly inhibited the migration function of SMCs (Figure 6D).

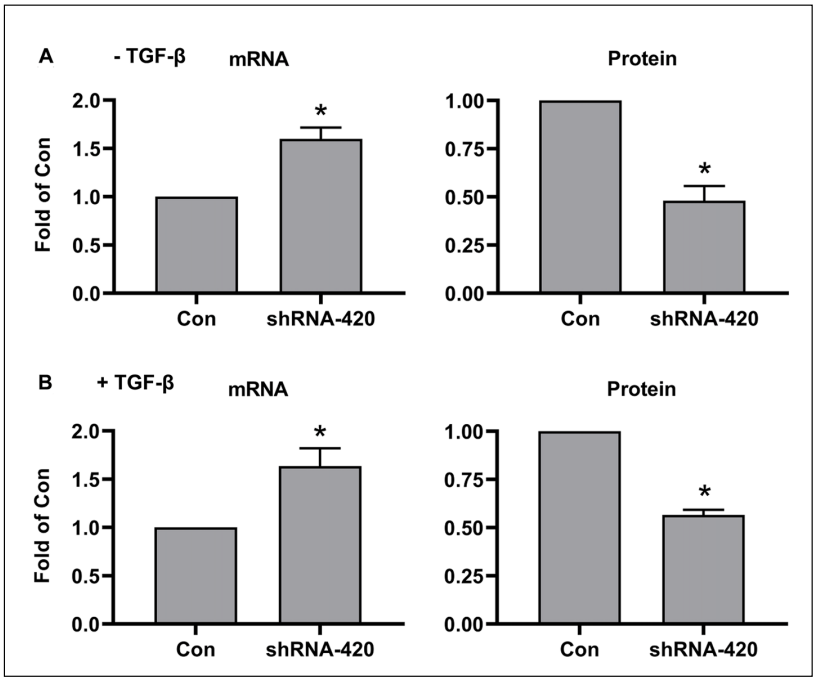

Figure 5. Differential effects of MYCT1 gene silencing on the mRNA and protein levels of collagen I in (**A**) unstimulated cells and (**B**) cells stimulated with TGF-β (10 ng/ml), as measured by qPCR and ELISA assays respectively. Data are mean ± SEM. *$p < 0.05$ *vs.* Con, unpaired *t*-test ($n = 4$ for **A** and 3 for **B**).

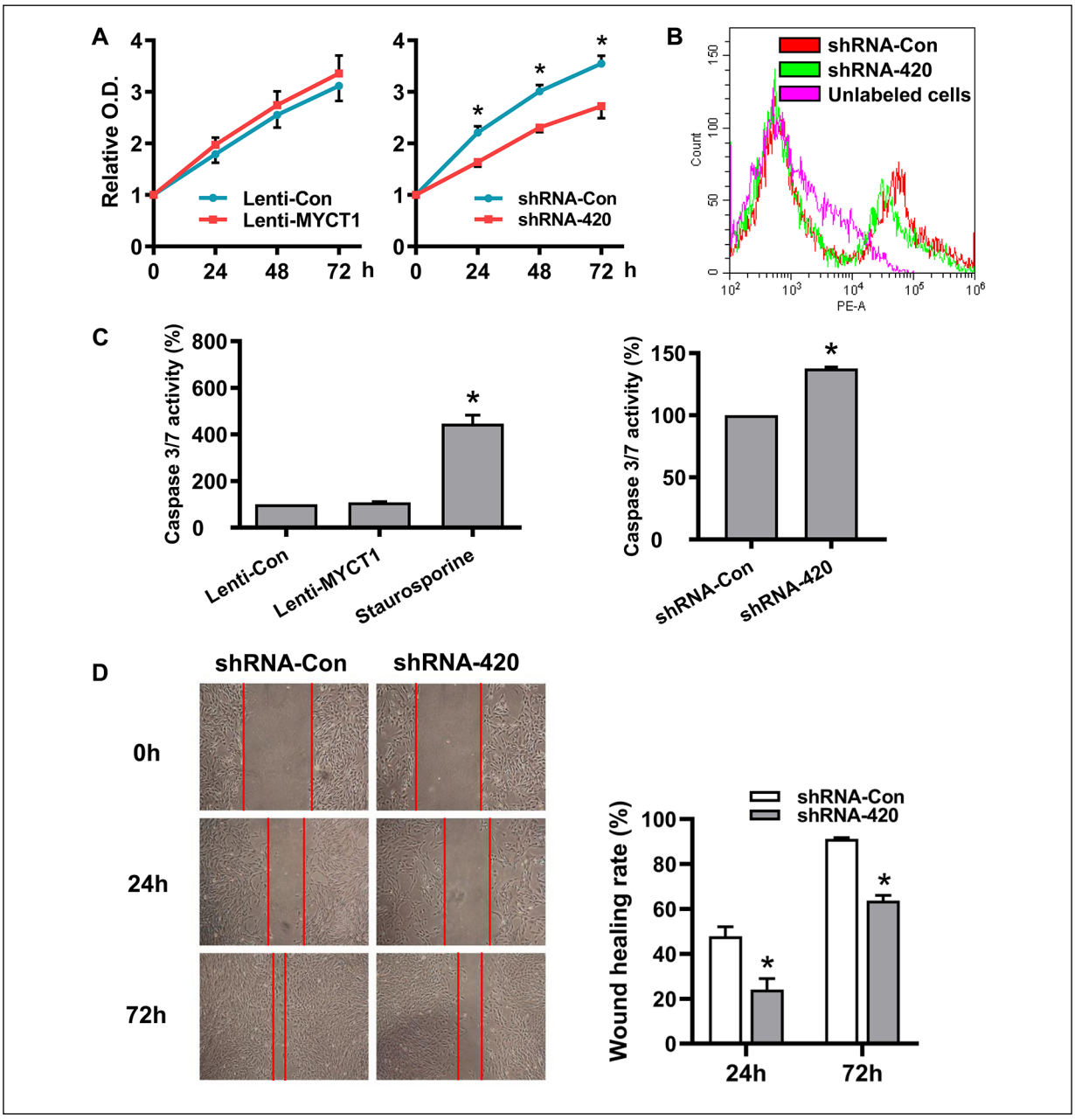

Figure 6. Impacts of MYCT1 expression on different cellular functions in vascular smooth muscle cells. **A,** Proliferation curves in control (Con), MYCT1-overexpressing (left panel) and MYCT1-silenced (right panel) cells, as measured with CCK-8 assays. **B,** Flow cytometry detection of EdU incorporation in control (red) and MYCT1-silenced (green) cells. **C,** Apoptosis in control, MYCT1-overexpressing (left panel) and MYCT1-silenced (right panel) cells, as detected with caspase 3/7 activity assays. Staurosporine (0.1 mM) was used as a positive control. **D,** Representative images (magnification 40×) and quantitative data of monolayer wound healing assays showing the effect of MYCT1 gene silencing on cell migration. Red lines indicate the border between cell monolayer and the denuded area. *$p < 0.05$ *vs.* Con, unpaired *t*-test or one-way ANOVA ($n = 3$ for all experiments).

Discussion

In the present study, we have characterized the cellular functions of MYCT1 in vascular SMCs, and provided evidence suggesting that MYCT1 is a regulator of the expression of ribosomal protein genes. Under nitrosative stress, SMCs exhibit reductions in the expression of both MYCT1 and ribosomal proteins. We argue that the MYCT1-dependent regulation of ribosomal protein expres-

sions may provide a plausible explanation to the downregulation of ribosomal protein genes observed in human IA tissues. Supporting our data, reanalysis of a data set published recently (#GSE75436) by Wang et al[18] has also revealed that MYCT1 gene expression is downregulated in human IAs. Conventionally, regulation of the gene expression of ribosomal proteins is mainly studied in yeast as a model platform, and it is well documented that the transcription of ribosomal protein genes is sensitive (downregulated) to cellular stressors[19-21]. There is only limited evidence, however, showing that ribosomal protein genes are also downregulated in mammalian cells under stress conditions[22]. Hence, our data have provided further support to these early findings. Indeed, the phenomenon of regulated gene expression for ribosomal proteins in mammalian cells is theoretically conceivable, because ribosomal biogenesis is the most resource-consuming process in eukaryotes[19].

MYCT1 has been shown to act as a transcription factor in the nucleus, where it may have reciprocally redundant roles with c-Myc in regulating gene expressions[10]. Early studies on MYCT1 have mainly focused on the cellular effects following MYCT1 gain-of-function, showing that MYCT1 overexpression may induce tumorigenic transformation of normal cells as does c-Myc[8,9]. By using gene silencing experiments, in contrast, more recent studies have revealed that the endogenously expressed MYCT1 has important roles in maintaining normal cellular functions. For example, Holmfeldt et al[23] have demonstrated that knockdown of MYCT1 expression causes an impairment of the repopulating function in hematopoietic stem cells. Here, our study has provided the first evidence suggesting that MYCT1 may be a novel regulator of gene expressions in vascular SMCs. Interestingly, we have observed that although MYCT1 knockdown decreases ribosomal protein gene expressions, overexpression of MYCT1 does not further increase their expressions, indicating that the endogenous MYCT1 is necessary but not sufficient for driving the expression of ribosomal proteins in normal SMCs.

Currently, the pathophysiological importance of the downregulation of MYCT1 and ribosomal protein genes in IA formation remains to be elucidated. Increased turnover of extracellular matrix proteins, especially collagens, is a hallmark of IA[24]. On one hand, collage degradation is also accelerated because of increased production of matrix metalloproteinases[25]. Indeed, the transcription of collagen genes appears to be upregulated in IAs as revealed by previous genomic studies[4,18,26]. However, this favorable response may be counter balanced by disruption of the protein translation process under pathological conditions[27], leading to inefficient tissue repair in the vessel wall. In the present study, we have shown that knockdown of MYCT1 results in a dissociation of the mRNA and protein levels of collagen, indicating that the process of collagen protein translation is compromised. Based on these results, we suggest that the downregulations of MYCT1 and ribosomal proteins in IAs may contribute to the deficiency in extracellular matrix contents in the IA vessel wall.

In addition to the regulation of ribosomal protein genes, our data indicate that MYCT1 may also affect SMC proliferation, apoptosis, and migration. Interestingly, previous studies in tumor cells have reported conflicting results regarding the MYCT1 effects on cell apoptosis and proliferation[28,29]. These data together suggest that MYCT1 modulates cell apoptosis and proliferation in a cell type-specific manner. In vascular SMCs, knockdown of MYCT1 increases apoptosis and represses proliferation, supporting a pro-survival and pro-growth role of the endogenously expressed MYCT1. In comparison, MYCT1 overexpression exhibits little effects on these cellular functions, suggesting that endogenous MYCT1 activity is functionally maximum in normal SMCs. Moreover, consistent with the finding in tumor cells [30], MYCT1 knockdown attenuates the migration activity of vascular SMCs. Overall, our data suggest that MYCT1 has a non-redundant role in maintaining SMC functions, which are critical for efficient tissue repairment in the arterial wall. However, it should be noted that the effects of MYCT1 on SMC proliferation, apoptosis, and migration might not be directly related to changes in ribosomal biogenesis.

Conclusions

MYCT1 is expressed in primary vascular SMCs and is downregulated under nitrosative stress. MYCT1 downregulation causes reductions in the expression of ribosomal proteins, leading to compromised protein translation efficiency for optimal collagen production in SMCs. MYCT1-dependent gene regulation may, at least partly, explain the downregulat-

ed expressions of ribosomal proteins observed in human IAs. Moreover, the endogenously expressed MYCT1 is involved in maintaining normal cellular functions including survival, proliferation and migration. Taken these together, we suggest that MYCT1 may represent a novel molecular target for counteracting the decreased activity of aneurysmal SMCs for tissue repairmen/regeneration.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Data Statement

The raw data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' Contribution

F.Y. and J.W. conducted experiments, analyzed data, and wrote the manuscript draft. X.W. and X.L. conducted experiments and analyzed data. Y.W., W.C. and X.C. collected and analyzed data. X.G. and F.J. conceived the study, supervised the project, revised and finalized the manuscript. All authors have read and approved the final version of manuscript.

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