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Original Article

Chinese rice wine polyphenol compounds inhibit vascular smooth muscle cell dedifferentiation and its mechanism

Liping Meng[1], Hui Lin[2], Sunlei Pan[2], Weiliang Tang[1], Changzuan Zhou[2], Xiaoya Zhai[1], Chengjian Jiang[1], Zheng Ji[1], Jufang Chi[1], Fang Peng[1], Hangyuan Guo[1,2]

[1]*Department of Cardiology, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing, Zhejiang, China;* [2]*The First Clinical Medical College, Wenzhou Medical University, Wenzhou, Zhejiang, China*

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Abstract: Objective: To verify whether Chinese rice wine polyphenol compounds (RWPC) could inhibit rat aortic vascular smooth muscle cells (VSMCs) dedifferentiation through mammalian target of rapamycin (mTOR)/P70S6K pathway. Methods: The primary culture and identification of rat VSMCs was conducted and VSMCs in passages 3-5 were used for the following experiments. Platelet derived growth factor-BB (PDGF-BB) was used to induce VSMCs dedifferentiation. Rapamycin and MHY-1485 were used to inhibit or activate mTOR/P70S6K pathway respectively. After incubation in different concentrations of RWPC, the proliferation and migration ability of the VSMCs were tested by MTT assay, Transwell chambers, and wound healing assay. Western blotting was employed to investigate the expressions of SM-actin, calponin, SM-MHC, OPN, mTOR, p-mTOR, P70S6K and p-P70S6K in VSMCs. Results: PDGF-BB improved the proliferation and migration ability of VSMCs and increased the expression of calponin, SM-MHC, p-mTOR, p-P70S6K. RWPC significantly inhibited the induction of these phenotypic changes induced by PDGF-BB, Meanwhile RWPC suppressed mTOR signaling in the VSMCs. Identical to RWPC, inhibiting the activity of mTOR signaling by rapamycin could also inhibit VSMC dedifferentiation. In contrast, up-regulation of mTOR signaling by MHY-1485 could reverse the RWPC-induced inhibition of VSMC dedifferentiation. Conclusion: RWPC inhibits VSMCs dedifferentiation by suppressing mTOR/P70S6K signaling pathway.

Keywords: Vascular smooth muscle cells, rice wine polyphenol compounds, dedifferentiation

Introduction

The cardiovascular protective effects of red wine are widely acknowledged according to the hypothesis of the 'Mediterranean diet model' and 'French paradox' [1, 2]. Researcher had already found that polyphenols such as resveratrol and catechin played the anti-atherosclerosis role in red wine [3]. As one of the oldest wines in China, rice wine is brewed from a mixture of spergillus oryzae, yeast, rice, and water. In the previous studies, we had demonstrated the beneficial effect of rice wine on atherosclerosis (AS) in vivo and vitro experiments [4, 5]. Recently, we further found that rice wine polyphenol compounds (RWPC) could decrease atherosclerotic plaque area in LDL-receptor-knockout mice [6]. However, the mechanism by which RWPC inhibit AS remains unknown.

Excessive proliferation and migration of vascular smooth muscle cells (VSMCs) plays an important role in the pathogenesis of AS [7, 8]. Unlike skeletal or cardiac muscle cells that have undergone terminal differentiation, VSMCs of adult animals retain plasticity. The mature, quiescent, contractile phenotype VSMCs could dedifferentiate to the proliferative, synthetic phenotype in response to various physiological and pathological factors [9]. Differentiation status of VSMCs in vitro can be measured by testing the proliferation, migration ability and investigating the expression of smooth muscle-specific phenotype marker proteins, including smooth muscle actin (SM-acitn), calponin, smooth muscle myosin heavy chain (SM-MHC) and osteopontin (OPN) [10]. Phenotypic changes of VSMCs are known to be critical in the origin of lots of cardiovascular diseases like ath-

erosclerosis, intimal hyperplasia, and restenosis [11].

Although several factors can induce VSMCs phenotype switching, platelet-derived growth factor-BB (PDGF-BB) is a primary regulator of VSMCs proliferation and has been shown to be one of the most robust phenotype-modulating agents [12]. Antibodies against PDGF or PDGF receptors, PDGF aptamers, or antisense oligonucleotides to PDGF receptors inhibit VSMCs accumulation in the intima after balloon injury [7, 13]. In addition, VSMCs lacking PDGF receptorβ show strikingly diminished neointimal accumulation after carotid artery ligation [14]. Moreover, pharmacological inhibition of PDGF signaling decreased VSMC proliferation, migration and dedifferentiation [15]. So, in this experiment, PDGF-BB was chosen as the inducer of VSMCs dedifferentiation.

Mammalian target of rapamycin (mTOR) plays an important role in regulating cell proliferation and autophagy in response to cellular environment [16]. Recently, the importance of this pathway in metabolism, diabetes, obesity, and cancer is becoming increasingly appreciated [16]. The mTOR signaling is activated by certain growth factors, such as PDGF, which influences the phenotype of VSMCs [17, 18]. In this study, we hypothesized that RWPC inhibited VSMCs dedifferentiation through suppressing mTOR signaling pathway.

Materials and methods

Animals used in this study were in compliance with the Guide for Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Shaoxing Hospital of Zhejiang University.

Materials

(1) Animals: SPF degree Sprague-Dawley rats (50 d, body weight 150-180 g), regardless of gender, were obtained from the animal center laboratory of Zhejiang Province Institute of Medicine. (2) Reagents: Rice wine polyphenols [19] were extracted from Chinese rice wine provided by National Engineering and Research Center for Traditional Chinese Medicine (Shanghai, China). Dulbecco's modified eagle medium (DMEM)-High Glucose, PBS, 0.25% trypsase-EDTA, Penicillin and streptomycin were purchased from JinuoBiotech Company (Hangzhou, China). PDGF-BB, rapamycin and MHY-1485 was purchased from Sigma (USA), dimethylsulfoxide (DMSO) from MP Biomedicals (USA), fetal calf serum (FBS) from GIBCO (USA), MTT from Emresco (USA), and DAPI from Rcohe (USA). Antibodies against SM-actin, calponin, OPN, SM-MHC and β-actin were purchased from Abcam (Cambridge, MA, USA), and horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit IgG antibody were purchased from Jackson Immuno Research Laboratories (USA). Gelatin was purchased from Abcam (Cambridge, MA, USA). The other reagents for immunoblot assay were purchased from Beyotime (Jiangsu, China).

Cell culture

VSMCs were primarily cultured using tissue-sticking method from rat thoracic aorta as reported in our previous researches [20]. We identified VSMCs through morphology and immunofluorescence detection of SM-actin and ensured their purity through multiple fluorescent staining with DAPI and SM-actin antibody. Cells between 3 to 5 generations were used in the following experiments.

Cells were seeded onto six-well plates and grown to 75-85% confluence and serum-starved in DMEM containing 0.1% fetal calf serum (FBS) for 24 h. Rapamycin (20 nmol/L) or MHY-1485 (10 μmol/L) was used to inhibit or activate mTOR signaling pathway [21, 22]. Then, PDGF-BB (20 ng/ml) was added to induce VSMCs dedifferentiation. After incubation in PDGF-BB for 12 h, VSMC were treated with vehicle (ethanol) or RWPC as indicated in the figure legends. (The concentration of polyphenol compounds in the rice wine is about 50 mg/L. So, we chose 10 mg/L, 50 mg/L, and 200 mg/L RWPC in this study).

MTT assay

About 6×10^3 cells were seeded onto 96-well plates per well. After 24 h incubation with non-serum DMEM and 12 h with PDGF-BB, cells were treated with each intervention factors and incubated at 37°C under 5% CO_2. 12 h, 24 h, and 48 h later, 20 ul MTT solution was added to each well, followed by 4-6 h incubation at 37°C. The supernatant was removed with a pipette, and 150 μl dimethylsulfoxide (DMSO) was

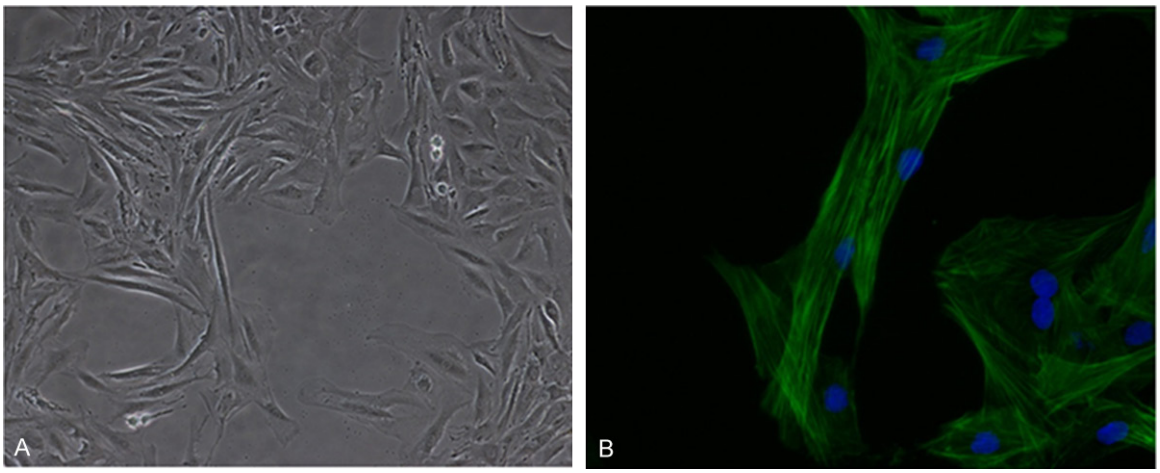

Figure 1. Identification of the VSMCs by morphology (×100) and immunocytochemistry (×400). A: VSMCs exhibited a typical spindle-shaped appearance with a characteristic 'hill and valley' pattern; B: VSMCs were SMα-actin positive showed by immunofluorescent staining.

added to each well. After 10 min of incubation at room temperature, plates were read on a microplate reader (Anthos, Austria) at 490 nm. Values were normalized using the control value.

Wound-healing assay

The 3-5 passages of VSMCs were digested with 0.25% trypsase and suspended with DMEM containing 10% FBS and cells were seeded onto 6-well plates. When grew up to 80% confluence, they were cultured in serum-free medium containing hydroxyurea for 24 h to synchronize cells and suppress cells proliferation. We created wounds by manually scraping the cell monolayer with a 100 ul pipet tip. After being washed with PBS for three times to remove the isolated cells, corresponding intervention factor was added to each group. Multiple photographs of the wounds were then taken at 0 and 18 h post wounding under an inverted Nikon microscope (Nikon Corporation, Tokyo, Japan) at a×200 magnification. The migration area was analyzed with Image-pro plus 6.0 (Media Cybernetics Inc., Bethesda, MD, USA) and the migration activity was expressed as the number of cells migrating into the wound in each field.

Transwell migration assay

After being synchronized by serum free medium, cells were seeded onto the upper chamber of the Transwell inserts (2×10^4 cells per well) (Corning, St. Lowell, MA). Cells were subsequently allowed to migrate for 12 h at 37°C. The cells on the upper side of the inserts were softly scraped off. Cells that migrated to the lower side of the inserts were fixed with 4% paraformaldehyde and stained with DAPI (2 ug/ml), and then the cells from nine independent, randomly chosen visual fields were counted under an immunofluorescence microscope (×200 magnification) for quantification of cells.

Immunohistochemistry analysis

VSMCs were cultured on glass coverslips. Cells were washed with PBS and fixed in 4% paraformaldehyde and then permeabilized by 0.1% Triton. Subsequently, VSMCs were blocked with 10% goat serum in PBST and incubated with SMα-actin antibodies in PBST for 1 h. After washing, anti-mouse fluorescein isothiocyanate (FITC) conjugated second antibody was incubated for 1 h and washed. Coverslips were then processed for immunofluorescent microscopy.

Western blot analysis

After incubation with corresponding intervention factors, cellular protein was obtained with the radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Haimen, China) for Western blot analysis. BCA method (BCA Protein assay kit, Beyotime Company, China) was used to detect the protein concentrations of the supernatant. The supernatants were then separated on SDS-PAGE (10%) and trans-

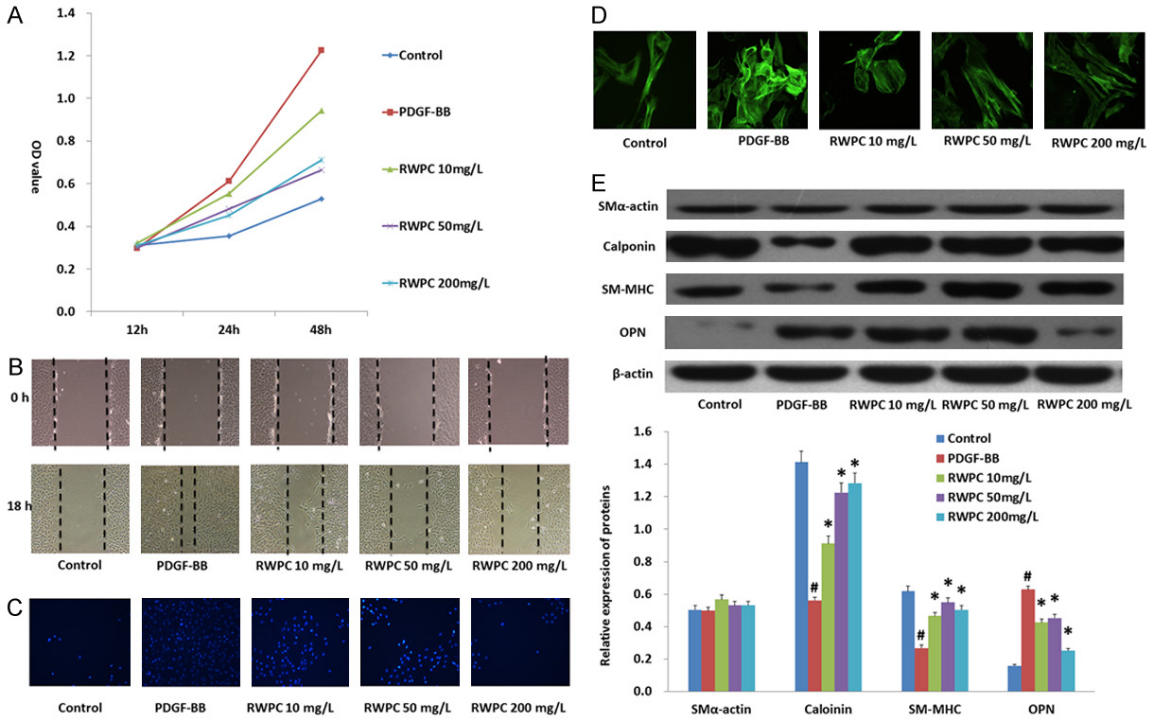

Figure 2. RWPC inhibited PDGF-BB induced VSMCs dedifferentiation. A: MTT assay showed that RWPC inhibited VSMCs proliferation in a dose-dependent manner. B: Wound healing assay demonstrated that RWPC inhibited VSMCs migration in a dose-dependent manner. C: Transwell chambers revealed that RWPC inhibited VSMCs invasion in a dose-dependent manner. D: Immunocytochemistry showed that RWPC restored the regular distribution of SMα-actin in VSMCs. E: RWPC decreased the expression of OPN and increased the expression of SM-MHC and Calponin. RWPC: Rice wine polyphenol compounds. #P < 0.01 compared with control, *P < 0.01 compared with PDGF-BB.

ferred to polyvinylidene fluoride membranes. After that, the membranes were blocked with blocking buffer for 30 mins at room temperature and then incubated with the rabbit anti-SMα-actin, calponin, OPN, SM-MHC, mTOR, p-mTOR, P70S6K and p-P70S6K monoclonal antibody (1:1,000 dilution), and mouse anti-β actin monoclonal antibody (1:10,000 dilution) overnight at 4°C. TBST was used to wash the membranes (3 times for 10 min), and the membranes were incubated with goat anti-rabbit IgG-HRP (1:10,000 dilution), or goat anti-mouse IgG-HRP (1:10,000 dilution) for 1 h at room temperature. The standard chemical luminescence method (Beyotime Company, China) was used to detect the antigen by exposing the membranes to Kodak X-Omat AR film. The resultant films were scanned on a gel imaging and analysis system and analyzed by Quantity One 4.4 (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All experiments were repeated three times. All statistical analysis were performed using SPSS 20.0. Student's T-test was used for the comparisons between two different groups. One-way analysis of variance (ANOVA), followed by Tukey's post-hoc analysis was performed to compare between multiple experimental groups. $P < 0.05$ was considered statistically significant.

Results

RWPC inhibited VSMCs dedifferentiation

The primary culture of confluent VSMCs exhibited a typical spindle-shaped appearance with a characteristic "hill-and-valley" pattern (**Figure 1A**), and immunofluorescence showed 98% positive SMα-actin staining (**Figure 1B**).

To explicit the mechanisms regulating the phenotypic switch of VSMCs, we first measured changes in cell functions and molecular markers of contractile and synthetic VSMC phenotypes. For this, VSMCs were serum-starved in DMEM containing 0.1% FBS for 24 h to induce cell-cycle arrest. The cells were then incubated

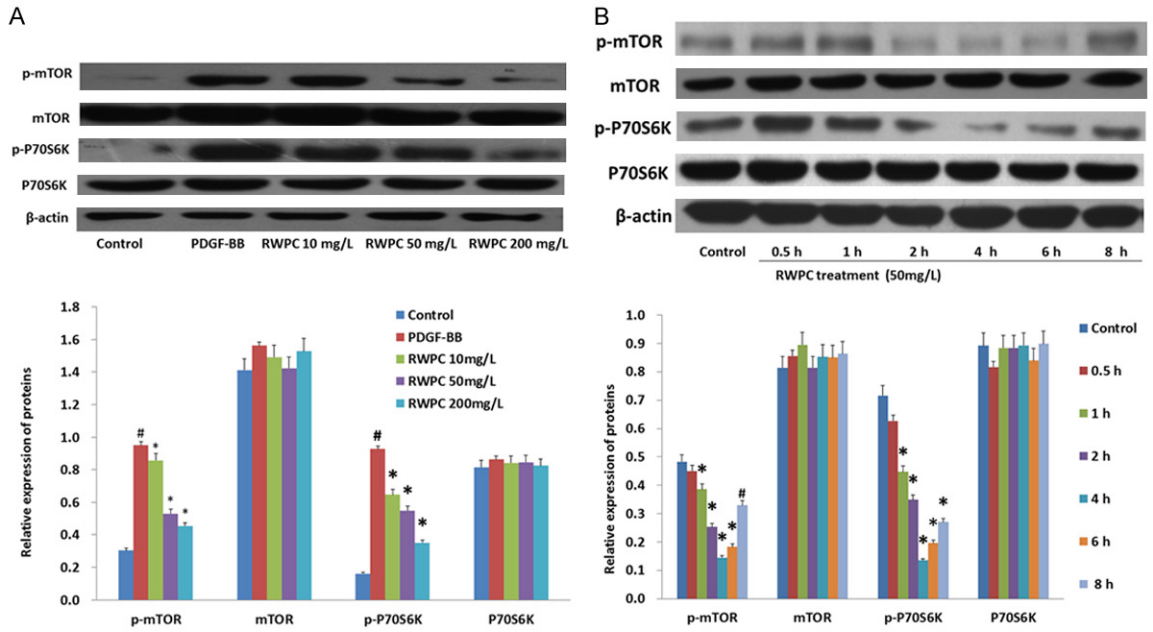

Figure 3. RWPC inhibited PDGF-BB induced activation of mTOR/P70S6K signaling pathway in VSMCs. A: RWPC inhibited the expression of p-mTOR and p-P70S6K after 4 h. B: The expression of p-mTOR and p-P70S6K decreased from 2 h to 6 h after treatment with RWPC (50 mg/L). RWPC: Rice wine polyphenol compounds. #P < 0.01 compared with control, *P < 0.01 compared with PDGF-BB.

with PDGF-BB (20 ng/ml) or vehicle (ethanol) for 12 h to induce dedifferentiation. Then the cells were incubated with different concentrations of RWPC or vehicle.

MTT assay were performed to explore the function of RWPC in VSMCs proliferation. As shown in the **Figure 2A**, PDGF-BB significantly increased the proliferation of VSMCs and RWPC could dramatically inhibit the proliferation ability of VSMCs in a concentration-dependent manner.

Images of the scratches were captured at 0 and 18 h after RWPC were added. We found that after stimulating for 18 h, RWPC markedly inhibited PDGF-BB induced migration of VSMCs in a dose-dependent manner (**Figure 2B**, P < 0.01). In agreement, transwell chamber assay revealed that RWPC could also decrease cell invasion ability induced by PDGF-BB after 12 h in a dose-dependent manner (**Figure 2C**).

As observed by immunofluorescence imaging, the SMα-actin showed marked regular distributed after treatment with RWPC and this was accompanied by marked changes in cell morphology (**Figure 2D**).

Western blot analysis showed that PDGF-BB decreased the expression of SM-MHC and calponin, meanwhile, increased the expression of OPN. In contrast, RWPC significantly suppressed the expression of OPN and increased the expression of SM-MHC, calponin (**Figure 2E**, P < 0.05). Taken together, these results indicated that RWPC inhibited PDGF-BB induced VSMCs dedifferentiation.

RWPC inhibited the mTOR/P70S6K signaling in VSMCs

Whether RWPC influenced mTOR/P70S6K signaling during differentiation of VSMCs was further investigated. Western blot analysis showed that the expression of p-mTOR and p-P70S6K were decreased after treatment with RWPC for 4 h (**Figure 3A**, P < 0.05), and it was further found that the expression of p-mTOR and p-P70S6K decreased from 2 h to 6 h after treatment with RWPC (50 mg/L) (**Figure 3B**, P < 0.05). These data suggested that RWPC inhibited the mTOR/P70S6K signaling in VSMCs.

Inhibition of mTOR suppressed VSMCs dedifferentiation

To determine the role of the mTOR signaling in dedifferentiation of VSMCs, rapamycin (20

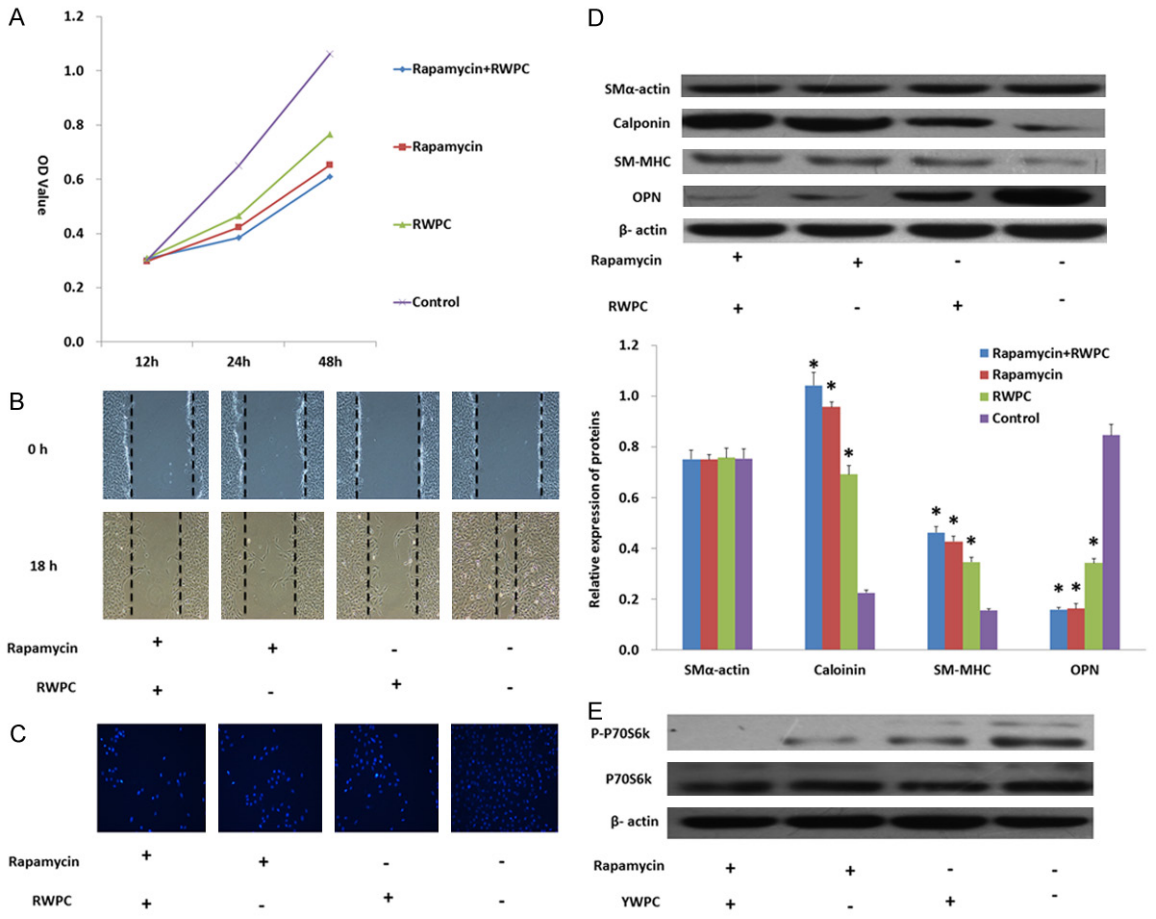

Figure 4. Rapamycin inhibited PDGF-BB induced VSMCs' dedifferentiation. A: MTT assay showed that rapamycin inhibited VSMCs proliferation. B: Wound healing assay demonstrated that rapamycin inhibited VSMCs migration. C: Transwell chambers revealed that rapamycin inhibited VSMCs invasion. D: Rapamycin decreased the expression of OPN and increased the expression of SM-MHC and Calponin. E: Rapamycin and RWPC inhibited the expression of p-P70S6K. RWPC: Rice wine polyphenol compounds. *P < 0.01 compared with control.

nmol/L) was used to block the mTOR pathway. Then, all the VSMCs were incubated with PDGF-BB to induce dedifferentiation. After that, the cells were treated with RWPC or vehicle. We found that VSMCs pretreated with rapamycin (20 nmol/L) showed a notable decrease in p-mTOR and p-P70S6K (**Figure 4A**). Identical to RWPC, inhibition of mTOR signaling by rapamycin could also decrease VSMCs proliferation and migration (**Figure 4B-D**, P < 0.05). In agreement, western blot analysis revealed that, the same as RWPC, rapamycin suppressed the expression of OPN and increased the expression of SM-MHC, calponin (**Figure 4E**, P < 0.05). These data showed that inhibition of the mTOR pathway suppressed VSMC dedifferentiation.

Activation of mTOR reversed the RWPC-induced inhibition of VSMC dedifferentiation

To further verify the role of the mTOR signaling in dedifferentiation of VSMCs, MHY-1485 (10 μmol/L) was used to activate the mTOR pathway. Then, all the VSMCs were incubated with PDGF-BB to induce dedifferentiation. After that, the cells were treated with RWPC or vehicle. We found that VSMCs pretreated with MHY-1485 (10 μmol/L) showed a notable increase in p-mTOR and p-P70S6K (**Figure 5A**). In addition, pretreatment with MHY-1485 (10 μmol/L) suppressed the RWPC-induced inhibition of VSMC proliferation, migration and expression of OPN (**Figure 5**, P < 0.05). These results further verified that the mTOR signaling pathway was

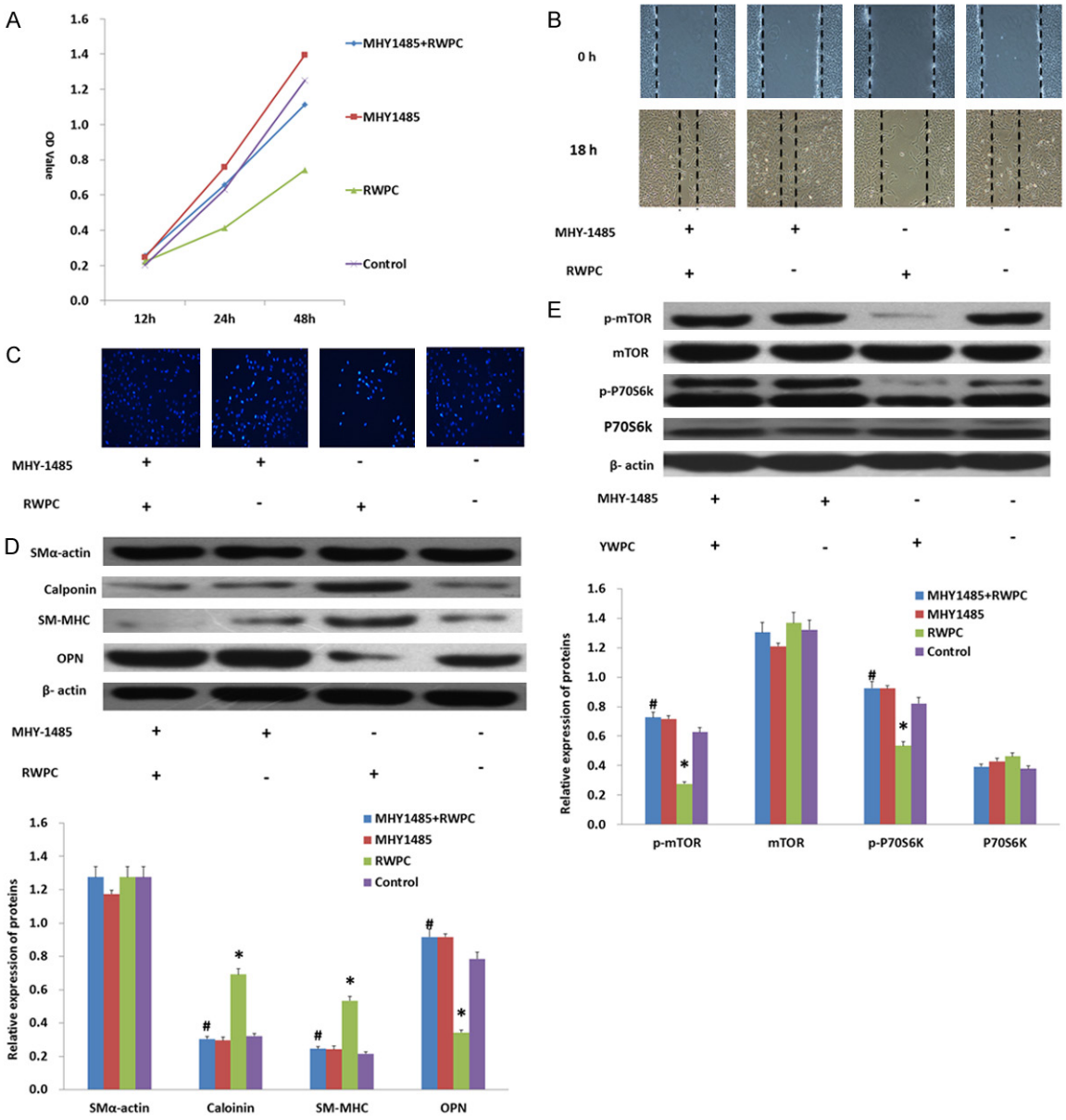

Figure 5. MHY-1485 reversed RWPC-induced inhibition of VSMCs dedifferentiation. A: MTT assay showed that MHY-1485 reversed RWPC-induced inhibition of VSMCs proliferation. B: Wound healing assay demonstrated that MHY-1485 reversed RWPC-induced inhibition of VSMCs migration. C: Transwell chambers revealed that MHY-1485 reversed RWPC-induced inhibition of VSMCs invasion. D: MHY-1485 increased the expression of OPN and decreased the expression of SM-MHC and Calponin. E: MHY-1485 inhibited RWPC-induced decrease of p-mTOR and p-p70S6K in VSMCs. RWPC: Rice wine polyphenol compounds. *P < 0.01 compared with control; #P < 0.01 compared with RWPC.

involved in RWPC-induced inhibition of VSMC dedifferentiation.

Discussion

It has been widely acknowledged that regular and moderate drinking of red wine is associated with a decreased risk of cardiovascular disease, and experimental evidence has indicated that polyphenols in the red wine are the key components responsible for the wine's cardiovascular protective effects. Similarly, Chinese rice wine, which is brewed from a mixture of spergillus oryzae, yeast, rice, and water, is also rich in polyphenols (about 50 mg/L) such as catechinic acid and gallic acid [23]. Based on our previous founding which demonstrated that

Chinese rice wine had a beneficial impact on the cardiovascular system [4-6, 24], this study further confirmed that RWPC could protect the cardiovascular system by inhibiting VSMCs' dedifferentiation.

VSMCs, locating in the medial layer of the healthy arteries where they express contractile proteins which help to regulate the vessel tone and blood flow, are essential regulators of vascular function [25]. During atherogenesis and arterial restenosis, VSMCs dedifferentiate from a contractile phenotype to a synthetic phenotype. Although there are likely many alternative phenotypic states of VSMCs, in general VSMCs phenotypic switching is characterized by markedly reduced expression of VSMC-selective differentiation marker genes and increased VSMC proliferation, migration, and synthesis of extracellular matrix components required for vascular repair [26]. These phenotype changes in VSMCs appear to be common and necessary in the development in a large number of major cardiovascular diseases, including atherosclerosis, hypertension, and restenosis [27, 28].

Inhibition of the VSMCs dedifferentiation is the mechanism by which lots of drugs paly their cardiovascular protective role [29]. Wagner et al. demonstrated that in addition to its lipid-lowering effect, lovastatin could also suppress VSMC dedifferentiation through inhibition of Rheb [30]. Meanwhile, Kaimoto et al. revealed that nifedipine, a calcium antagonist, inhibited VSMC dedifferentiation in injured arteries and suppressed neointimal thickening after balloon injury by modulation of the akt signaling [28]. In agreement, researchers found that resveratrol, a component of the red wine polyphenols, could also inhibit phenotypic switching of neointimal VSMCs after balloon injury through blockade of Notch pathway or by stimulation of SirT1 and AMPK [31, 32]. What's more, Lee et al. found that resveratrol inhibited VSMCs dedifferentiation and proliferation rate by interruption of the balance of Akt, 42/44MAPK, and p38MAPK pathway activation which were induced by PDGF-BB [12]. Consistent with these experimental findings, we demonstrated that RWPC could decrease the proliferation and migration ability of VSMCs and make the SMα-actin distributed regular. Meanwhile, RWPC could also increase the expression of VSMC-selective differentiation marker genes and decrease the expression of OPN. All these data suggested that RWPC could inhibit VSMCs dedifferentiation, and this maybe the mechanism by which RWPC paly its anti-atherosclerosis role.

MTOR is a protein kinase which is ubiquitously expressed in the cells. Through the effectors P70S6K1 and 4E-BP1/eIF4E, mTOR regulates translation initiation of specific growth-related mRNA subsets. The mTOR signaling pathway regulates protein synthesis in response to amino acids, and its effectors integrate signals from mTOR with growth factor signals via PI3K to coordinately regulate cell cycle progression, protein synthesis, cell proliferation and migration [33]. Since Martin et al. first found that mTOR/P70S6K signaling pathway was involved in the dedifferentiation of VSMCs, a lot of studies had demonstrated the key role of mTOR signaling during the drug induced VSMCs differentiation. Grundmann et al. found that miRNA-100 regulates neovascularization by suppression of mTOR in VSMCs [34]. Additionally, Wagner et al. revealed that lovastatin induced VSMC differentiation through inhibition of Rheb and mTOR [30]. Besides, Thompson et al. demonstrated that high dose resveratrol, which was extracted from red wine, stimulated VSMCs differentiation through AMPK-mediated inhibition of the mTOR pathway [32]. In agreement, Lee et al. recently found that Mesoglycan could also attenuate VSMC proliferation through activation of AMP-activated protein kinase and mTOR [35]. Consistent with these experimental findings, this study demonstrated that RWPC inhibited PDGF-induced increases in p-mTOR and p-P70S6K expression. We further used rapamycin to block the mTOR signaling of VSMCs and found that the same as RWPC, rapamycin inhibit PDGF-induced VSMCs dedifferentiation. What's more, we found that MHY1485, a new activator of mTOR [21, 22], reversed the RWPC-induced inhibition of VSMC dedifferentiation. These findings suggest that RWPC inhibits PDGF-induced VSMCs dedifferentiation by suppressing mTOR signaling pathway.

Based on our previous researches that proved the anti-atherosclerosis effect of Chinese rice wine, this experiment further confirmed that RWPC could inhibit VSMCs dedifferentiation by suppressing mTOR signaling pathway. These finding provides new insights into the mechanisms underlying the beneficial effects of Chinese rice wine in the prevention of cardiovascular diseases.

However, our experiment still had limitations. For instance, RWPC we used in this experiment is a mixture of different monomer components and which one in the RWPC that plays the role is still unknown.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Hangyuan Guo, Department of Cardiology, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 312000, Zhejiang, China. Tel: +86-575-88228888; Fax: +86-575-88228899; E-mail: ghangyuan@hotmail.com

References

[1] Li H and Forstermann U. Red wine and cardiovascular health. Circ Res 2012; 111: 959-961.
[2] Bertelli AA and Das DK. Grapes, wines, resveratrol, and heart health. J Cardiovasc Pharmacol 2009; 54: 468-476.
[3] Mullin GE. Red wine, grapes, and better health--resveratrol. Nutr Clin Pract 2011; 26: 722-723.
[4] Guo HY, Liu LB, Shi YF, Sun AJ, Xu FK, Chi JF and Huang DL. Chinese Yellow Wine and Red Wine Inhibit Matrix Metalloproteinase-2 and Improve Atherosclerotic Plaque in LDL Receptor Knockout Mice. Cardiovasc Ther 2010; 28: 161-168.
[5] Guo HY, Wang P, You BQ, Xing YB and Lee JD. Chinese yellow wine inhibits production of homocysteine-induced extracellular matrix metalloproteinase-2 in cultured rat vascular smooth muscle cells. Clin Nutr 2007; 26: 348-354.
[6] Zhai X, Chi J, Tang W, Ji Z, Zhao F, Jiang C, Lv H and Guo H. Yellow wine polyphenolic compounds inhibit matrix metalloproteinase-2, -9 expression and improve atherosclerotic plaque in LDL-receptor-knockout mice. J Pharmacol Sci 2014; 125: 132-141.
[7] Yu K, Zheng B, Han M and Wen JK. ATRA activates and PDGF-BB represses the SM22alpha promoter through KLF4 binding to, or dissociating from, its cis-DNA elements. Cardiovasc Res 2011; 90: 464-474.
[8] Davis-Dusenbery BN, Chan MC, Reno KE, Weisman AS, Layne MD, Lagna G and Hata A. down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-beta and bone morphogenetic protein 4. J Biol Chem 2011; 286: 28097-28110.
[9] Owens GK, Kumar MS and Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004; 84: 767-801.
[10] Owens GK. Molecular control of vascular smooth muscle cell differentiation and phenotypic plasticity. Novartis Found Symp 2007; 283: 174-191; discussion 191-173, 238-141.
[11] Chistiakov DA, Orekhov AN and Bobryshev YV. Vascular smooth muscle cell in atherosclerosis. Acta Physiologica (Oxford, England) 2015; 214: 33-50.
[12] Lee MH, Kwon BJ, Seo HJ, Yoo KE, Kim MS, Koo MA and Park JC. Resveratrol inhibits phenotype modulation by platelet derived growth factor-bb in rat aortic smooth muscle cells. Oxid Med Cell Longev 2014; 2014: 572430.
[13] Aikawa M, Rabkin E, Voglic SJ, Shing H, Nagai R, Schoen FJ and Libby P. Lipid lowering promotes accumulation of mature smooth muscle cells expressing smooth muscle myosin heavy chain isoforms in rabbit atheroma. Circ Res 1998; 83: 1015-1026.
[14] Han XB, Zhang HP, Cao CJ, Wang YH, Tian J, Yang XL, Yang AN, Wang J, Jiang YD and Xu H. Aberrant DNA methylation of the PDGF gene in homocysteinemediated VSMC proliferation and its underlying mechanism. Mol Med Rep 2014; 10: 947-954.
[15] Zheng B, Han M, Bernier M, Zhang XH, Meng F, Miao SB, He M, Zhao XM and Wen JK. Kruppel-like factor 4 inhibits proliferation by platelet-derived growth factor receptor beta-mediated, not by retinoic acid receptor alpha-mediated, phosphatidylinositol 3-kinase and ERK signaling in vascular smooth muscle cells. J Biol Chem 2009; 284: 22773-22785.
[16] Munson MJ and Ganley IG. MTOR, PIK3C3, and autophagy: Signaling the beginning from the end. Autophagy 2015; 11: 2375-2376.
[17] Stepanenko AA, Andreieva SV, Korets KV, Mykytenko DO, Baklaushev VP, Chekhonin VP and Dmitrenko VV. mTOR inhibitor temsirolimus and MEK1/2 inhibitor U0126 promote chromosomal instability and cell type-dependent phenotype changes of glioblastoma cells. Gene 2016; 579: 58-68.
[18] Wang W, Liu J, Ma A, Miao R, Jin Y, Zhang H, Xu K, Wang C and Wang J. mTORC1 is involved in hypoxia-induced pulmonary hypertension through the activation of Notch3. J Cell Physiol 2014; 229: 2117-2125.

[19] Li Y, Lu LL, Zheng XX and Ding CX. Study on the separation of peptides from chinese rice wine by macroporous resin. Chemical Research and Application 2010; 22: 1272-1277.
[20] Meng LP, Jiang CJ, Zhao F, Guo Y, Chi JF and Guo HY. An improvement in primary culture of rat vascular smooth muscle cells and its identification. Journal of Wenzhou University 2015; 45: 593-596.
[21] Cheng Y, Kim J, Li XX and Hsueh AJ. Promotion of ovarian follicle growth following mTOR activation: synergistic effects of AKT stimulators. PLoS One 2015; 10: e0117769.
[22] Choi YJ, Park YJ, Park JY, Jeong HO, Kim DH, Ha YM, Kim JM, Song YM, Heo HS, Yu BP, Chun P, Moon HR and Chung HY. Inhibitory effect of mTOR activator MHY1485 on autophagy: suppression of lysosomal fusion. PLoS One 2012; 7: e43418.
[23] Xie GF, Dai J and Zhao GA. Scientifically understanding of the health-protect function of rice wine. China Brewing 2004; 13: 30-31.
[24] Zhao F, Ji Z, Chi J, Tang W, Zhai X, Meng L and Guo H. Effects of Chinese yellow wine on nitric oxide synthase and intercellular adhesion molecule-1 expressions in rat vascular endothelial cells. Acta Cardiol 2016; 71: 27-34.
[25] Salabei JK, Cummins TD, Singh M, Jones SP, Bhatnagar A and Hill BG. PDGF-mediated autophagy regulates vascular smooth muscle cell phenotype and resistance to oxidative stress. Biochem J 2013; 451: 375-388.
[26] Alexander MR and Owens GK. Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. Annual Rev Physiol 2012; 74: 13-40.
[27] Liu R, Leslie KL and Martin KA. Epigenetic regulation of smooth muscle cell plasticity. Biochim Biophys Acta 2014; 24: 567-579.
[28] Kaimoto T, Yasuda O, Ohishi M, Mogi M, Takemura Y, Suhara T, Ogihara T, Fukuo K and Rakugi H. Nifedipine inhibits vascular smooth muscle cell dedifferentiation via downregulation of Akt signaling. Hypertension 2010; 56: 247-252.
[29] Shi N and Chen SY. Mechanisms simultaneously regulate smooth muscle proliferation and differentiation. J Biomed Res 2014; 28: 40-46.
[30] Wagner RJ, Martin KA, Powell RJ and Rzucidlo EM. Lovastatin induces VSMC differentiation through inhibition of Rheb and mTOR. Am J Physiol Cell Physiol 2010; 299: C119-127.
[31] Zhang J, Chen J, Xu C, Yang J, Guo Q, Hu Q and Jiang H. Resveratrol inhibits phenotypic switching of neointimal vascular smooth muscle cells after balloon injury through blockade of Notch pathway. J Cardiovasc Pharmacol 2014; 63: 233-239.
[32] Thompson AM, Martin KA and Rzucidlo EM. Resveratrol induces vascular smooth muscle cell differentiation through stimulation of SirT1 and AMPK. PLoS One 2014; 9: e85495.
[33] Martin KA, Rzucidlo EM, Merenick BL, Fingar DC, Brown DJ, Wagner RJ and Powell RJ. The mTOR/p70 S6K1 pathway regulates vascular smooth muscle cell differentiation. Am JPhysiol 2004; 286: C507-517.
[34] Grundmann S, Hans FP, Kinniry S, Heinke J, Helbing T, Bluhm F, Sluijter JP, Hoefer I, Pasterkamp G, Bode C and Moser M. MicroRNA-100 regulates neovascularization by suppression of mammalian target of rapamycin in endothelial and vascular smooth muscle cells. Circulation 2011; 123: 999-1009.
[35] Lee KY, Lee DH and Choi HC. Mesoglycan attenuates VSMC proliferation through activation of AMP-activated protein kinase and mTOR. Clin Hypertens 2015; 22: 2.