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Inhibition of VRK1 suppresses proliferation and migration of vascular smooth muscle cells and intima hyperplasia after injury via mTORC1/β-catenin axis

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Characterized by abnormal proliferation and migration of vascular smooth muscle cells (VSMCs), neointima hyperplasia is a hallmark of vascular restenosis after percutaneous vascular interventions. Vaccinia-related kinase 1 (VRK1) is a stress adaption-associated ser/thr protein kinase that can induce the proliferation of various types of cells. However, the role of VRK1 in the proliferation and migration of VSMCs and neointima hyperplasia after vascular injury remains unknown. We observed increased expression of VRK1 in VSMCs subjected to platelet-derived growth factor (PDGF)-BB by western blotting. Silencing VRK1 by shVrk1 reduced the number of Ki-67-positive VSMCs and attenuated the migration of VSMCs. Mechanistically, we found that relative expression levels of β-catenin and effectors of mTOR complex 1 (mTORC1) such as phospho (p)-mammalian target of rapamycin (mTOR), p-S6, and p-4EBP1 were decreased after silencing VRK1. Restoration of β-catenin expression by SKL2001 and re-activation of mTORC1 by *Tuberous sclerosis* 1 siRNA (siTsc1) both abolished shVrk1-mediated inhibitory effect on VSMC proliferation and migration. siTsc1 also rescued the reduced expression of β-catenin caused by VRK1 inhibition. Furthermore, mTORC1 re-activation failed to recover the attenuated proliferation and migration of VSMC resulting from shVrk1 after silencing β-catenin. We also found that the vascular expression of VRK1 was increased after injury. VRK1 inactivation *in vivo* inhibited vascular injury-induced neointima hyperplasia in a β-catenin-dependent manner. These results demonstrate that inhibition of VRK1 can suppress the proliferation and migration of VSMC and neointima hyperplasia after vascular injury via mTORC1/β-catenin pathway. [BMB Reports 2022; 55(5): 244-249]

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INTRODUCTION

Percutaneous vascular intervention is an important method for treating coronary heart disease and other peripheral vascular diseases. However, vascular restenosis can badly harm the effectiveness of percutaneous intervention (1). Major causes of vascular restenosis include neointima hyperplasia and vascular remodeling, which involve endothelial dysfunction, abnormal proliferation, and migration of vascular smooth muscle cells (VSMCs) and inflammation (2). Although various treatments based on the above-mentioned mechanisms such as drug-eluting stent can reduce the incidence of restenosis, vascular restenosis has not been eradicated yet (3).

As a major component of blood vessel, VSMC exerts a crucial role in maintaining vascular function and structure (4). During mechanical injury such as angioplasty or stent deployment, VSMC undergoes phenotypic changing from a contractile/quiescent phenotype to a synthetic/proliferative phenotype (5). This phenotypic transition of VSMC involves excessive proliferation and migration (2). Therefore, inhibiting intimal VSMC proliferation and migration is an effective strategy to avoid vascular restenosis. However, current drugs inhibiting cell proliferation also affect re-endothelization and cause in-stent thrombosis (6). Thus, elucidating the etiology for the above-mentioned pathological transition of VSMC is crucial to prevent vascular restenosis.

Belonging to the ser/thr protein kinase family, vaccinia-related kinase 1 (VRK1) regulates various of transcription factors and protein molecules involved in tumor progress, cell proliferation, chromatin dynamics, and migration (7). VRK1 plays a role in coordinating basic cellular functions for adapting to pathological situations (7). VRK1 is characterized as a modulator of cell cycle. It is abundant in proliferative cells and tissues (8). Therefore, VRK1 can exert pro- or anti-proliferative role in various types of cancer cells and neuronal stem cells (7, 9). However, whether VRK1 is implicated in pathological proliferation, migration, and intima hyperplasia after vascular injury remains unclear.

In the current study, we revealed that VRK1 was elevated in proliferative VSMC and injured artery. Silencing VRK1 led to attenuated proliferation and migration of VSMC via reducing β-catenin expression. We further showed that mammalian target of rapamycin complex 1 (mTORC1) was responsible for

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VRK1-mediated regulation of β-catenin in VSMC. Additionally, we demonstrated that inhibiting VRK1 activity *in vivo* ameliorated injury-induced vascular neointima hyperplasia. Together, these data indicate that inhibiting VRK1 can attenuate proliferation and migration of VSMCs and intima hyperplasia via mTORC1/β-catenin pathway.

RESULTS

VRK1 induces proliferation and migration of VSMCs

To explore the potential role of VRK1 in the proliferation and migration of VSMCs, we firstly examined VRK1 expression in synthetic VSMCs. Platelet-derived growth factor (PDGF)-BB is known to promote phenotypic transition of VSMC (10). In the current study, VRK1 was upregulated by PDGF-BB stimulation in both dose-dependent manner (Fig. 1A) and time-dependent manner (Fig. 1B). We next used short hairpin RNA targeting *Vrk1* gene (shVrk1) to silence the protein expression of VRK1 and found that shVrk1 significantly reduced VRK1 expression (Fig. 1C). Using cell counting kit-8 (CCK-8) assay, we found that silencing VRK1 by shVrk1 reduced the cell number (Fig. 1D). We also examined cell proliferation via Ki-67 immunofluorescence staining. The number of Ki-67-positive VSMC was obviously decreased after shVrk1 transfection (Fig. 1E). Migration of VSMCs was monitored by transwell assay. VRK1 inhibition significantly suppressed the migration capacity of VSMCs (Fig. 1F). Besides, we found that proliferation (Supplementary Fig. S1A) and migration (Supplementary Fig. S1B) of VSMCs were both enhanced after transfection with adenovirus expressing *Vrk1* (Ad-Vrk1). Cell cycle analysis showed that the percentage of VSMCs in G0/G1 phase of cell cycle was significantly increased, whereas percentages of VSMCs in S and G2/M phases were significantly decreased after silencing VRK1 (Supplementary Fig. S2A), revealing the role of VRK1 in enhancing VSMC proliferative activity. We also determined cell apoptosis by measuring expression levels of apoptotic proteins, Caspase 3, and Caspase 9. Expression levels of Caspase 3 and Caspase 9 were not affected by VRK1 inhibition (Supplementary Fig. S2B), indicating that VRK1 did not affect apoptosis of VSMCs. These observations indicate that VRK1 can promote the proliferation and migration of VSMCs.

VRK1 promotes proliferation and migration of VSMC by upregulating β-catenin

β-catenin is a critical regulator of the proliferation and migration of VSMCs for vascular remodeling (11). A previous study has demonstrated that VRK1 can induce the proliferation and migration of gastric carcinoma cells by targeting β-catenin (12). We thus determined the role of β-catenin in VRK1-mediated regulation of VSMCs. Silencing VRK1 inhibited the expression of β-catenin (Fig. 2A). Such inhibition was rescued by SKL2001, an agonist of β-catenin (Fig. 2B). In addition, SKL2001 blocked the inhibitory effect of silencing VRK1 on VSMC proliferation based on Ki-67 (a marker of proliferation) immunofluorescence staining (Fig. 2C). Meanwhile, transwell assay evidenced that

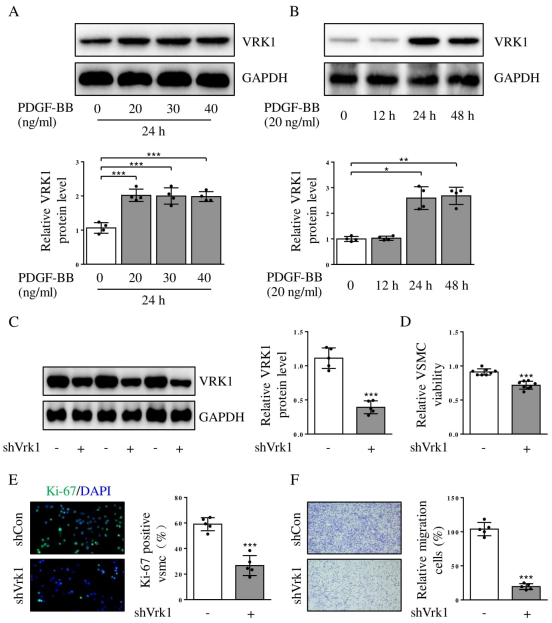

Fig. 1. Silencing Vaccinia-related kinase 1 (VRK1) inhibits proliferation and migration of vascular smooth muscle cells (VSMCs). (A) Relative expression level of VRK1 in VSMCs at 24 h after treatment (n = 4) with platelet derived growth factor (PDGF)-BB (0, 20, 30 and 40 ng/ml) was determined by immunoblotting. (B) Relative expression level of VRK1 in VSMCs at 0, 12, 24, and 48 h after PDGF-BB (20 ng/mL) treatment (n = 4) was determined by immunoblotting. (C) Relative expression level of VRK1 in VSMCs transfected with shCon or shVrk1 (n = 5) was determined by immunoblotting. (D) CCK-8 assay of VSMCs transfected with shCon or shVrk1. The absorbance at 450 nm was obtained (n = 8). (E) VSMCs were stained with Ki-67 (green) and DAPI (blue) after transfection with shCon or shVrk1. Representative images (left) and corresponding quantification of Ki-67-positive VSMCs (right) are shown (n = 5). Magnification at 400×. (F) VSMCs transfected with shCon or shVrk1 were assessed by transwell assay. Representative images (left) and corresponding quantification of migration cells (right) are shown (n = 5). Magnification at 100×. Data are shown as mean ± S.D. *P < 0.05, **P < 0.01 and ***P < 0.001 denote statistical comparison between the two marked groups.

VRK1 inhibition-mediated anti-migration effect was also abolished by the recovery of β-catenin expression (Fig. 2D). These results reveal that VRK1 can induce the expression of β-catenin to promote proliferation and migration of VSMCs.

VRK1 induces β-catenin expression in a mTORC1-dependent manner

We next investigated the upstream regulator of β-catenin driven by VRK1 in VSMCs. mTORC1 is a multifunctional atypical serine/threonine kinase. It is associated with higher activity of VSMC proliferation and migration (13). Translation of β-catenin during tumorigenesis is known to be enhanced after mTORC1 activation (14). Whether VRK1-mediated regulation of β-catenin expression in VSMC involves mTORC1 remains

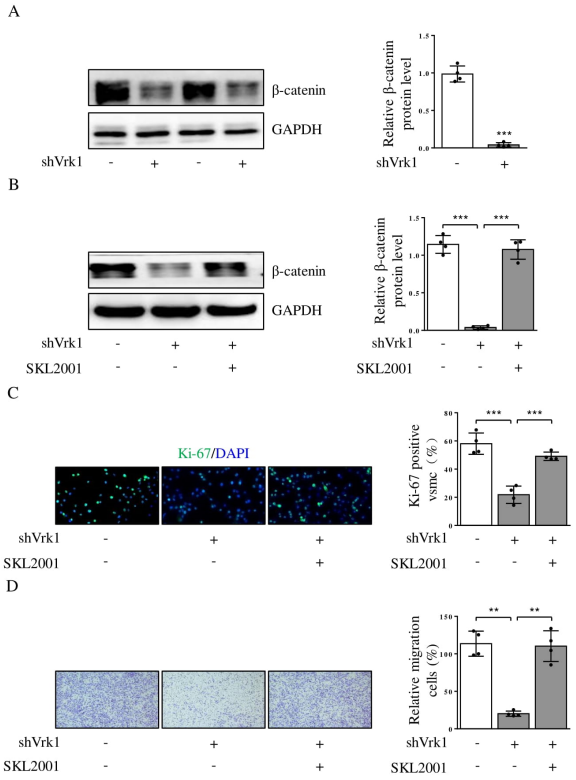

Fig. 2. VRK1 inhibition suppresses proliferation and migration of VSMCs by reducing β-catenin expression. VSMCs were transfected with shCon or shVrk1. (A) Relative expression of β-catenin in above-treated VSMCs (n = 4) was determined by immunoblotting. (B) Relative expression of β-catenin in above-treated VSMCs incubated with or without SKL2001 (40 μmol/L) for 6 h (n = 4) was determined by immunoblotting. (C) VSMCs treated as above mentioned were stained with Ki-67 (green) and DAPI (blue). Representative images (left) and corresponding quantification of Ki-67-positive VSMCs (right) are shown (n = 4). Magnification at 400×. (D) VSMCs treated as above mentioned were assessed by transwell assay. Representative images (left) and corresponding quantification of migration VSMCs (right) are shown (n = 4). Magnification at 100×. Data are shown as mean ± S.D. **P < 0.01 and ***P < 0.001 denote statistical comparison between the two marked groups.

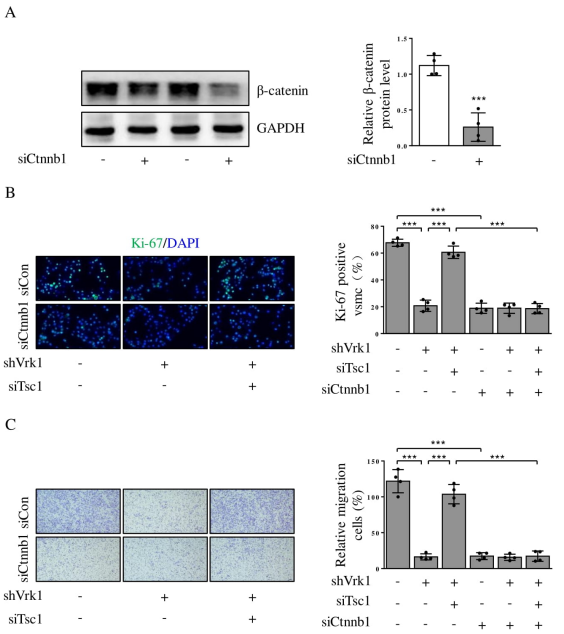

Fig. 3. Mechanistic target of rapamycin complex 1 (mTORC1)/β-catenin is required for VRK1-mediated regulation of VSMC phenotypic transition. (A) Relative expression of β-catenin in VSMCs incubated with or without siCtnnb1 (n = 4) was determined by immunoblotting. (B) VSMCs were transfected with shCon or shVrk1 and then incubated with siCon, siTsc1, or siCtnnb1. VSMCs treated as above mentioned were stained with Ki-67 (green) and DAPI (blue). Representative images (left) and corresponding quantification of Ki-67-positive VSMCs (right) are shown (n = 4). Magnification at 400×. (C) VSMCs treated as above mentioned were assessed by transwell assay. Representative images (left) and corresponding quantification of migration VSMCs (right) are shown (n = 4). Magnification at 100×. Data are shown as mean ± standard deviation (S.D.) ***P < 0.001 denotes statistical comparison between the two marked groups.

unclear. Western blotting showed that silencing VRK1 led to dephosphorylation of mTORC1 effectors including mTOR, S6, and 4EBP1 (Supplementary Fig. S3A). Tuberous sclerosis 1 (TSC1) is a classical negative regulator of mTORC1 (15). Noticeably, when we used *Tsc1* siRNA (siTsc1) to recover mTORC1 activity, the inhibitory effect of shVrk1 on β-catenin expression was abolished (Supplementary Fig. S3B).

To further explore the role of mTORC1/β-catenin in the proliferation and migration of VSMCs, we utilized *Ctnnb1* (the gene encoding β-catenin) siRNA (siCtnnb1) to transfect VSMCs and observed significantly declined protein level of β-catenin (Fig. 3A). Re-activation of mTORC1 by siTsc1 abolished the inhibitory effect of shVrk1 on the proliferation (Fig. 3B) and migration (Fig. 3C) of VSMCs. However, mTORC1 re-activation failed to affect the proliferation or migration of VSMCs after silencing β-catenin expression (Fig. 2B and 2C). Taken together, these findings suggest that VRK1 can promote the expression of β-catenin in an mTORC1-dependent manner.

Inactivation of VRK1 ameliorates neointima hyperplasia after vascular injury by reducing β-catenin expression

Since silencing VRK1 suppressed the proliferation and migration of VSMC *in vitro*, we next investigated the role of VRK1 in neointima hyperplasia after vascular injury *in vivo*. We conducted wire injury in the carotid artery of C57BL/6J mouse and measured neointima formation at day 28 after injury. The protein level of VRK1 was significantly upregulated after injury stimulation (Fig. 4A). To determine the crosstalk of VRK1 upregulation and vascular neointima hyperplasia, we used an antagonist of VRK1 named luteolin (16) to inhibit the activity of VRK1. Morphometric analysis revealed that injury of the left carotid artery developed obvious vascular wall thickening, leading to

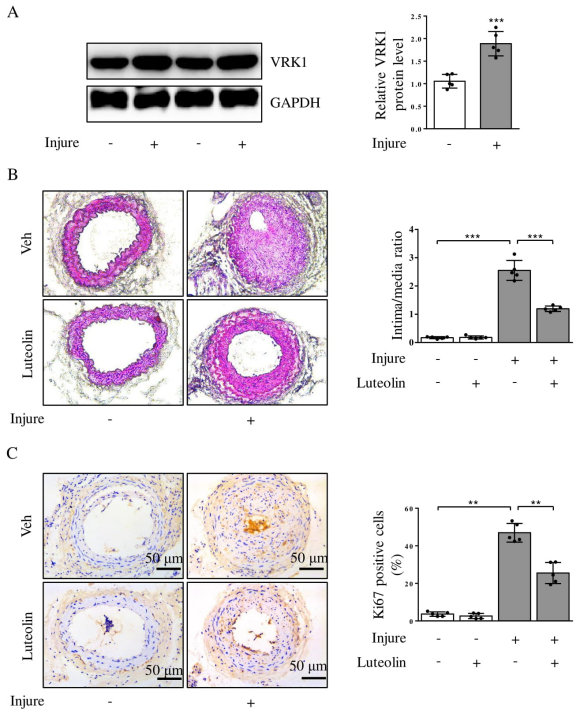

Fig. 4. Inactivation of VRK1 ameliorates neointima hyperplasia after vascular injury. (A) Relative vascular expression of VRK1 at day 28 after vascular injury (n = 5) was determined by immunoblotting. (B) Representative hematoxylin and eosin (H&E) staining results of carotid arteries of mice at day 28 after sham operation or vascular injury (left) and corresponding quantification for ratio of intima/media (right) are shown (n = 5). Magnification at 200×. (C) Immunohistochemistry staining of Ki-67 (brown) in sections of carotid arteries of mice at day 28 after sham operation or vascular injury (left) and corresponding quantification for Ki-67-positive cells within neointima (right) are shown (n = 5). Magnification at 200×. Data are shown as mean ± S.D. **P < 0.01 and ***P < 0.001 denote statistical comparison between the two marked groups.

increased ratio of intima/media. Meanwhile, the neointima hyperplasia after injury was significantly alleviated by VRK1 inactivation (Fig. 4B). Abnormal VSMC proliferation is a critical cause of vascular restenosis (17). Thus, we performed Ki-67 immunostaining to assess the proportion of proliferating cells. The number of Ki-67-positive cells within neointima was significantly increased in the injured artery than in the sham artery, which was markedly decreased after VRK inactivation by luteolin (Fig. 4C).

We next investigated whether β-catenin was also involved in VRK1-mediated regulation of vascular neointima hyperplasia *in vivo*. We found that vascular expression of β-catenin was obviously increased after injury, which was then reduced by luteolin (Supplementary Fig. S4A). Besides, SKL2001 *in vivo* abolished the protective effect of VRK1 inhibition on neointima hyperplasia (Supplementary Fig. S4B). These data indicate that VRK1 inhibition plays a critical role in attenuating intima hyperplasia in response to vascular injury by reducing β-catenin expression.

DISCUSSION

Abnormal proliferation and migration of VSMCs play an important role in the development of vascular neointima hyperplasia and restenosis after percutaneous vascular interventions (17). Therefore, searching for an effective strategy to block proliferation or migration of VSMCs might be essential for treating mechanical injury-associated vascular restenosis. In the present study, we found that silencing VRK1 attenuated β-catenin expression and decreased the proliferation and migration of VSMCs. These effects were abolished by restoration of β-catenin expression. We also revealed that VRK1 inhibition resulted in decreased mTORC1 activity in VSMCs. Re-activation of mTORC1 abolished the inhibitory effect of silencing VRK1 on phenotypic transition of VSMCs, whereas mTORC1 re-activation failed to recover the attenuated proliferation and migration of VSMCs after silencing β-catenin. Finally, we demonstrated that VRK1 inhibition ameliorated vascular neointima hyperplasia after injury. Our current findings suggest that VRK1 is a potential target for treating vascular restenosis.

Identified as a chromatin remodeling enzyme, VRK1 is known to regulate epigenetic modification and affect gene expression (18). VRK1 is considered as a regulator of cell cycle and proliferation of fibroblasts and various cancer cells (18). Additionally, VRK1 can promote the migration of several types of cells (12, 19). In correspondence with roles of VRK1 in proliferation and migration of above-mentioned cell lines, our current study revealed that VRK1 was highly expressed in proliferating VSMC and injured artery. VRK1 inhibition reduced the total cell number, suppressed the proliferation and migration of VSMCs, and ameliorated injury-induced vascular neointima hyperplasia. These results demonstrate that VRK1 is a positive regulator for phenotypic transition of VSMC during vascular restenosis after injury. However, the precise mechanism for VRK1-mediated regulation of VSMC is currently unclear.

A previous study has demonstrated that the regulatory effect of VRK1 on cell proliferation and migration depends on β-catenin activation (12). Whether β-catenin is involved in VRK1-mediated regulation of VSMC remains unknown. β-catenin is originally recognized for its role in adherens junctions and stabilizing cell-cell contacts (20). As a critical component of Wnt signaling pathway, β-catenin plays a crucial role in controlling the proliferation of cells in several cancers (20). In addition, β-catenin signaling is associated with accelerated proliferation, migration of VSMCs, and vascular intima thickening (20, 21). Consistent with aforementioned findings, we found that β-catenin expression was positively regulated by VRK1. More importantly, restoration of β-catenin expression by SKL2001 reversed VRK1 inhibition-induced decrease in proliferation and migration of VSMCs and neointima hyperplasia. β-catenin is also associated with vascular inflammation (22), which is plays an important role in vascular restenosis (2). Whether inflammation is also involved in VRK1/β-catenin-mediated regulation of vascular restenosis needs further exploration. These data reveal a close

connection between β-catenin signaling and VSMC phenotypic transition at the downstream of VRK1.

mTOR is a highly conserved serine/threonine kinase. It serves as an important regulator of cellular growth and metabolism (23). mTOR has two distinct multi-protein complexes, mTORC1 and mTORC2. mTORC1 activation is involved in cell proliferation via its regulation of cellular processes such as transcription, translation, and ribosome biogenesis (23). mTORC1 also plays a pivotal role in VSMC proliferation (17). A previous study has demonstrated that mTORC1 is essential for β-catenin translation and accumulation during tumorigenesis (14). In the current study, mTORC1 activity was positively regulated by VRK1. More importantly, decreased β-catenin expression, proliferation, and migration of VSMC after silencing VRK1 were all rescued by restoration of mTORC1 activity. Furthermore, once β-catenin expression was silenced, mTORC1 re-activation failed to retrieve the impaired proliferation and migration of VSMCs after VRK1 inhibition. These observations suggest that VRK1-dependent regulation of β-catenin can promote the proliferation and migration of VSMCs in a mTORC1-dependent manner.

Taken together, our results demonstrate that VRK1 inhibition can suppress the proliferation and migration of VSMCs and neointima hyperplasia in response to vascular injury by reducing β-catenin expression. Furthermore, VRK1 inhibition can cause downregulation of β-catenin by suppressing mTORC1 activity. This research suggests that VRK1 is a novel candidate target for treating vascular restenosis-associated diseases.

MATERIALS AND METHODS

Cell culture and treatment

VSMC was isolated from thoracic aortas of 8- to 10-week-old C57BL/6J mice by using enzyme digestion method. Briefly, isolated VSMCs were cultured in complete medium (HyClone, Carlsbad, CA) with 10% fetal calf serum (Invitrogen, Carlsbad, CA) and 1% streptomycin/penicillin (ThermoFisher Scientific, Waltham, MA). VSMCs were incubated at 37°C in 5% CO_2 atmosphere. Recombined human PDGF-BB (R&D Systems, Minneapolis, MI) was used to treat cells in different doses and time courses. SKL2001 (MCE, Shanghai) was used to pre-treat VSMCs with a dose of 40 μmol/L for 6 hours (24).

Western blotting

Western blotting assay was conducted as previously described (17). Extractions of VSMCs and artery tissues were lysed with RIPA buffer (Beyotime Institute of Biotechnology, Shanghai). Protein samples were segregated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA). Primary antibodies against VRK1, β-catenin, phospho (p)-mTOR^{Ser2448}, mTOR, p-S6$^{Ser235/236}$, S6, p-4EBP1$^{Thr37/46}$, 4EBP1, Caspase 3, Caspase 9 and GAPDH were purchased from Cell Signaling Technology (Danvers, MA). Quantitative analysis of protein band was performed by using Image J software (Bethesda, MA).

Immunofluorescence

Immunofluorescent (IF) staining was performed as described before (17). Briefly, VSMCs were washed by PBS, fixed by 4% paraformaldehyde for 20 minutes, and then blocked in 1% blocking solution at room temperature. VSMCs were then incubated with a primary antibody against Ki-67 (1:1000; Cell Signaling Technology) overnight at 4°C. After washed with PBS, VSMCs were then incubated with an Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (1:2500; Molecular Probes Inc., Eugene, OR) for 1 hour in dark. Finally, VSMCs were stained with DAPI (5 mg/ml; VECTOR Labs, Burlingame, CA) in room temperature for 5 seconds. Images were captured using an immunofluorescent microscopy (Leica MPS 60; Wetzlar, HD). The fluorescence intensity was measured via Image J software (Bethesda, MA).

Animal experiments

C57BL/6J mice (Dashuo Animal Science and Technology, Chengdu, Sichuan) were raised as following described: room temperature, 12-hour light/dark cycle, periodic air changes and free access to food and water. Establishing vascular injury model was performed as described before (10). Briefly, mice were firstly anesthetized and received a small midline incision in the neck area. The left proximal common carotid artery and left internal carotid artery were temporarily clamped. The left external carotid artery was permanently ligated. A guidewire was put into the left common carotid artery and passed backward and forward to denude the endothelium. The clamps were then removed to restore the blood flow. Luteolin (100 mg/kg) was intraperitoneally injected for three times per week when suffering the injury. SKL2001 (6 mg/kg) was intraperitoneally injected for 7 days when suffering the injury. Four weeks later, mice were deeply anesthetized with pentobarbital (100 mg/kg) and then received decapitation (16). All experiments in our current study were approved by the Institutional Animal Care and Use Committee and the Ethic Committee of The General Hospital of Western Theater Command.

Immunohistochemistry

Morphometric analyses of carotid arteries were performed by hematoxylin and eosin (HE) and Ki-67 staining as previously described (10). For H&E staining, the artery sections (4 μm) were stained with hematoxylin and eosin. For Ki-67 staining, the artery sections were incubated overnight with a primary antibody against Ki-67 (Cell Signaling Technology) at 4°C after being blocked. After washed by PBS, sections were followed by incubation with a secondary antibody and finally counterstained with mayer hematoxylin to visualize nuclei. Images were obtained by using Image-Pro Plus software.

Statistical analysis

Unpaired Student' t-test was utilized to compare two independent groups. One-way analysis of variance (ANOVA) was performed for multi-comparisons with appropriate post hoc tests. Data are presented as mean ± S.D. All tests were two-tailed. $P <$

0.05 was considered to be statistically significant.

The precise protocols are available in Supplementary information.

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AUTHOR CONTRIBUTION

X.S.S and W.W.Z contributed equally to this study. X.S.S and W.W.Z conceived the project. X.S.S designed the study. X.S.S, Y.J.Y and D.C.Y supervised the entire research. W.W.Z and Q.W performed most of the experimental work and conducted data analysis. J.Q.Z provided some technical supports. X.S.S and W.W.Z contributed to figure preparation. X.S.S, Y.J.Y and D.C.Y discussed the study. X.S.S organized the data and wrote the manuscript. All authors reviewed the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Thygesen K, Alpert JS, Jaffe AS et al (2018) Fourth universal definition of myocardial infarction. Circulation 138, e618-e651
2. Byrne RA, Joner M and Kastrati A (2015) Stent thrombosis and restenosis: what have we learned and where are we going? The Andreas Gruntzig Lecture ESC 2014. Eur Heart J 36, 3320-3331
3. Nicolais C, Lakhter V, Virk HUH et al (2018) Therapeutic options for in-stent restenosis. Curr Cardiol Rep 20, 7
4. Lacolley P, Regnault V, Segers P and Laurent S (2017) Vascular smooth muscle cells and arterial stiffening: relevance in development, Aging, and Disease. Physiol Rev 97, 1555-1617
5. Owens GK, Kumar MS and Wamhoff BR (2004) Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 84, 767-801
6. Pendyala LK, Yin X, Li J et al (2009) The first-generation drug-eluting stents and coronary endothelial dysfunction. JACC Cardiovasc Interv 2, 1169-1177
7. Campillo-Marcos I, Garcia-Gonzalez R, Navarro-Carrasco E and Lazo PA (2021) The human VRK1 chromatin kinase in cancer biology. Cancer Lett 503, 117-128
8. Valbuena A, Sanz-Garcia M, Lopez-Sanchez I, Vega FM and Lazo PA (2011) Roles of VRK1 as a new player in the control of biological processes required for cell division. Cell Signal 23, 1267-1272
9. Vinograd-Byk H, Sapir T and Cantarero L et al (2015) The spinal muscular atrophy with pontocerebellar hypoplasia gene VRK1 regulates neuronal migration through an amyloid-beta precursor protein-dependent mechanism. J Neurosci 35, 936-942
10. Sun X, Li S, Gan X, Chen K, Yang D and Yang Y (2020) NF2 deficiency accelerates neointima hyperplasia following vascular injury via promoting YAP-TEAD1 interaction in vascular smooth muscle cells. Aging 12, 9726-9744
11. An W, Luong LA and Bowden NP et al (2021) Cezanne is a critical regulator of pathological arterial remodelling by targeting beta-catenin signalling. Cardiovasc Res [Online ahead of print]
12. Wang L, Zhai R, Shen H, Song G, Wan F and Li Q (2021) VRK1 promotes proliferation, migration, and invasion of gastric carcinoma cells by activating beta-catenin. Neoplasma 68, 1005-1014
13. Ha JM, Yun SJ and Kim YW et al (2015) Platelet-derived growth factor regulates vascular smooth muscle phenotype via mammalian target of rapamycin complex 1. Biochem Biophys Res Commun 464, 57-62
14. Fu Y, Huang B and Shi Z et al (2013) SRSF1 and SRSF9 RNA binding proteins promote Wnt signalling-mediated tumorigenesis by enhancing beta-catenin biosynthesis. EMBO Mol Med 5, 737-750
15. Houssaini A, Abid S and Derumeaux G et al (2016) Selective tuberous sclerosis complex 1 gene deletion in smooth muscle activates mammalian target of rapamycin signaling and induces pulmonary hypertension. Am J Respir Cell Mol Biol 55, 352-367
16. Lin D, Kuang G, Wan J, Zhang X, Li H and Gong X (2017) Luteolin suppresses the metastasis of triple-negative breast cancer by reversing epithelial-to-mesenchymal transition via downregulation of beta-catenin expression. Oncol Rep 37, 895-902
17. Sun X, Li S and Gan X et al (2019) Wild-type p53-induced phosphatase 1 promotes vascular smooth muscle cell proliferation and neointima hyperplasia after vascular injury via p-adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin complex 1 pathway. J Hypertens 37, 2256-2268
18. Colmenero-Repiso A, Gomez-Munoz MA and Rodriguez-Prieto I et al (2020) Identification of VRK1 as a new neuroblastoma tumor progression marker regulating cell proliferation. Cancers 12, 3465
19. Ren Z, Geng J and Xiong C et al (2020) Downregulation of VRK1 reduces the expression of BANF1 and suppresses the proliferative and migratory activity of esophageal cancer cells. Oncol Lett 20, 1163-1170
20. Tsaousi A, Williams H and Lyon CA et al (2011) Wnt4/beta-catenin signaling induces VSMC proliferation and is associated with intimal thickening. Circ Res 108, 427-436
21. Hulin-Curtis S, Williams H, Wadey KS, Sala-Newby GB and George SJ (2017) Targeting Wnt/beta-catenin activated cells with dominant-negative N-cadherin to reduce neointima formation. Mol Ther Methods Clin Dev 5, 191-199
22. Gao Z, Xu X and Li Y et al (2021) Mechanistic Insight into PPARgamma and Tregs in atherosclerotic immune inflammation. Front Pharmacol 12, 750078
23. Hall MN (2008) mTOR-what does it do? Transplant Proc 40, S5-S8
24. Zhou P, Zhang X and Guo M et al (2019) Ginsenoside Rb1 ameliorates CKD-associated vascular calcification by inhibiting the Wnt/beta-catenin pathway. J Cell Mol Med 23, 7088-7098