Atherosclerosis 283 (2019) 92–99

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

A disintegrin and metalloprotease 22 accelerates neointima formation by activating ERK signaling

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HIGHLIGHTS

- ADAM22, a novel regulator of VSMCs, is dramatically upregulated during the development of neointima formation.
- ADAM22 enhances neointima formation by promoting VSMC proliferation, migration and phenotypic switching.
- The exacerbating function of ADAM22 in vascular remodeling is largely dependent on activating ERK signaling.

ARTICLE INFO

Keywords:
ADAM22
Neointima formation
Vascular smooth muscle cell
Proliferation
Migration
Phenotypic switching

ABSTRACT

Background and aims: Despite the advantage of arterial expansion for life-threatening vascular pathologies, the occurrence of neointima formation remains a prominent complication, with the underlying mechanisms largely unknown. A disintegrin and metalloprotease 22 (ADAM22) belongs to the family of ADAMs that possesses various biological capacities regulating vascular physiopathology. However, little is known about ADAM22 in vascular smooth muscle cell (VSMC)-mediated neointima formation. Here, we aimed to evaluate the potential functional regulation of ADAM22 in neointima formation and to further explore the underlying mechanisms.
Methods: In our study, platelet-derived growth factor-BB (PDGF-BB)-induced VSMC proliferation was examined using a 5-bromo-2′-deoxyuridine (BrdU) incorporation assay and a cell counting kit-8 (CCK8) assay, while VSMC migration was detected using a modified Boyden chamber method and a scratch-wound assay. The functional role of ADAM22 in neointima formation was evaluated based on a left carotid artery wire injury model in mice at 14 and 28 days.
Results: ADAM22 was significantly up-regulated in both PDGF-BB-challenged VSMCs and restenotic arteries of mice. When ADAM22 was overexpressed in VSMCs, cell proliferation, migration and phenotypic switching were simultaneously aggravated, whereas the opposite was observed when ADAM22 was knocked down *in vitro*. In ADAM22 heterozygote mice, wire-injury induced neointima formation was significantly ameliorated compared to wild-type control mice. Mechanistically, significantly up-regulated ERK phosphorylation is closely involved in the regulatory effects of ADAM22 in neointima formation. Interestingly, an ERK inhibitor largely reversed the aggravated VSMCs migration, proliferation and phenotypic switching induced by ADAM22 overexpression.
Conclusions: Our results indicate that ADAM22 accelerates neointima formation by enhancing VSMC migration, proliferation and phenotypic switching via promoting ERK phosphorylation. Suppressing ADAM22 expression may be an effective strategy for ameliorating neointima formation.

1. Introduction

As a common vascular disease, coronary atherosclerosis is a leading cause of mortality and morbidity in modern society [1]. Although stenting and bypass surgery have been widely used for the treatment of arteriosclerotic cardiovascular disease in the clinic, re-narrowing after surgery may appear due to the neointima formation [2]. Several processes, particularly vascular smooth muscle cell (VSMC) proliferation, migration and phenotypic switching, play pivotal roles in the development of neointima formation [2]. Under physiological conditions,

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https://doi.org/10.1016/j.atherosclerosis.2019.02.002
Received 21 September 2018; Received in revised form 30 December 2018; Accepted 1 February 2019
Available online 11 February 2019
0021-9150/

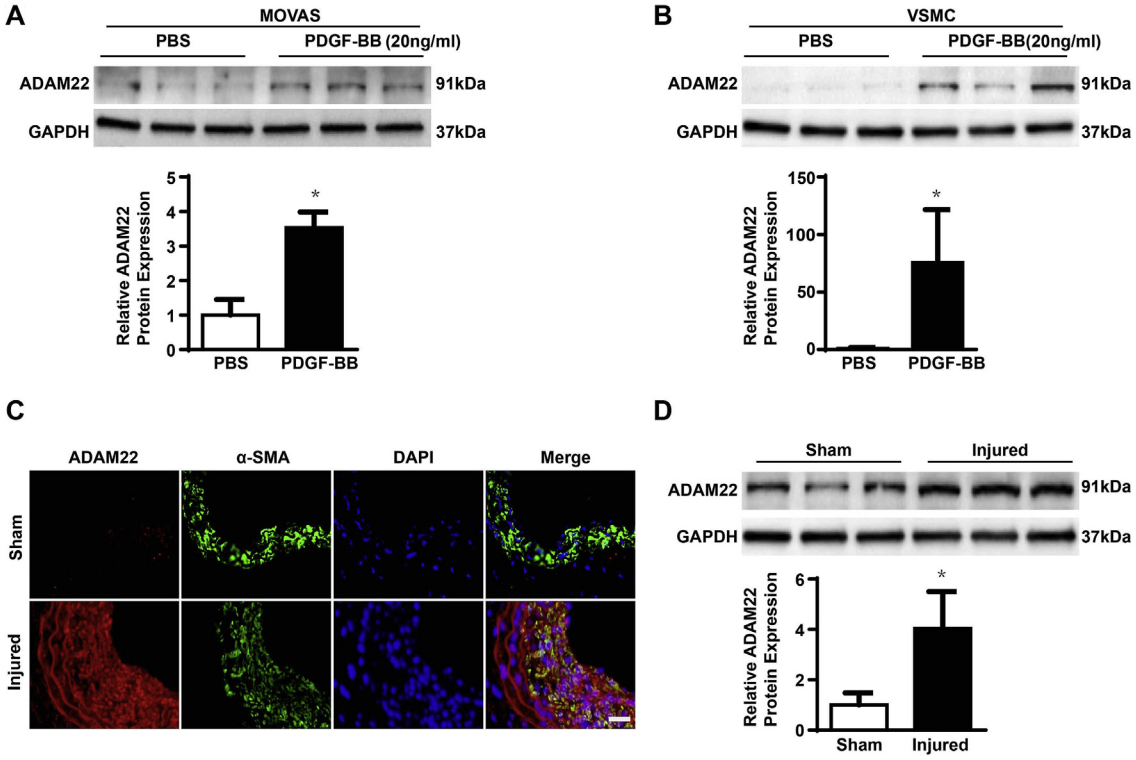

Fig. 1. Vascular injury alters ADAM22 expression.
(A and B) Representative Western blots of ADAM22 protein levels in MOVASs (A) and VSMCs (B) stimulated with PDGF-BB (20 ng/mL) for 48 h. (C) Immunofluorescence staining of ADAM22 (red) and α-SMA (green) in the carotid artery sections of WT mice. The arterial smooth muscle cells are indicated by α-SMA staining (n = 3 in each group). Scale bar, 50 μm. (D) The levels of ADAM22 proteins in the left carotid arteries of WT mice were determined by Western blotting. The arteries were harvested from sham-operated mice and injured mice at 28 days post-injury. In panels A, B, and D, the protein levels were quantified, GAPDH served as the loading control and blots are representative of three independent experiments. All values are presented as means ± SD, and the statistical significance is indicated. (A and B) *$p < 0.05$ *versus* the PBS control. (D) *$p < 0.05$ *versus* the sham-operated group.

VSMCs generate and maintain vascular tone by replicating at a slow rate, and inactive VSMCs maintain a contractile phenotype [3]. However, when the environmental conditions change, such as suffering from mechanical or stretch injury, the VSMCs can be activated and change from a contractile phenotype to a non-contractile phenotype [4]. Then, the activated VSMCs highly proliferate and migrate towards the stimulation point, leading to neointima formation [5]. It has been demonstrated that growth factors, such as platelet-derived growth factor (PDGF), secreted by inflammatory or endothelial cells, may accelerate the change of VSMCs from a contractile phenotype to a non-contractile phenotype [6]. Cell migration mainly depends on the combined action between the extracellular matrix and cytoskeleton [7]. Activated VSMCs alter extracellular matrix adhesion receptors and modulate cytoskeletal reorganization, both of which are essential for VSMCs' survival, contractility, migration and proliferation [5,8]. Unfortunately, the therapy for inhibiting neointima formation is limited, and in-depth investigations on the underlying mechanisms of neointima formation are, therefore, urgently required.

Disintegrin and metalloprotease (ADAM) family members are transmembrane proteins that belong to the zinc proteases superfamily, which are characterized by the existence of metalloproteases with integrin receptor-binding activities and the binding sites of several signal transducing proteins [9]. Previous studies have demonstrated the critical role of ADAMs in several physiological and pathological processes, including cell fate determination, muscle development, inflammation response and cancer [9]. In terms of cardiovascular diseases, ADAM15 was found to participate in promoting neovascularization in retinopathy of prematurity [10], while embryonic death with malformed vessels in yolk sacs was found in *ADAM10*$^{-/-}$ mice [11]. *ADAM17*$^{-/-}$ and *ADAM19*$^{-/-}$ mice exhibited similar imperfections in heart development [12,13]. Importantly, ADAMs family proteins have been reported to be directly implicated in cell proliferation and migration by regulating the shedding of epidermal growth factor receptors (EGFRs) ligands and the subsequent activation of G protein-coupled receptors (GPCRs) [9,14]. All of these studies strongly indicated the effects of ADAMs family proteins on vascular diseases.

A disintegrin and metalloprotease 22 (ADAM22) is a member of the ADAM family that is mainly implicated in cell adhesion [15]. Up to now, most studies have focused on the effects of ADAM22 on synapse, encephalopathy, cancer and heart pathology [16–19], whereas little is known about ADAM22 in vascular diseases. Based on prior reports about ADAMs family proteins, we hypothesize that ADAM22 plays a critical role in VSMC-mediated neointima formation. To test our hypothesis, mouse aortic smooth muscle cells (MOVASs) and ADAM22 heterozygote (ADAM22$^{+/-}$) mice were employed to investigate the effects of ADAM22 on VSMC proliferation, migration and phenotypic switching in response to vascular injury. Meanwhile, signaling assays indicated that ADAM22 facilitated VSMC-mediated neointima formation, at least in part, through enhanced ERK signaling pathway. Our results demonstrated that ADAM22 plays a crucial role in accelerating vascular injury-induced neointima formation.

2. Materials and methods

All animal experiments were performed in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011) and were approved by the Animal Care and Use Committee of Capital Medical University, Beijing, China. ADAM22 heterozygote (*ADAM22*$^{+/-}$) mice were purchased from the RIKEN BioResource Research Centre

(RBRC04187). Male wild-type (WT) mice and *ADAM22*$^{+/-}$ mice (10–12 weeks old, weighing between 24 and 27 g) were maintained with free access to food and water in a temperature- and humidity-controlled room with a 12/12-h light/dark cycle. Wire injury of the mouse carotid artery was performed as described previously [20–22]. An expanded and detailed Materials and Methods is available in the Supplemental Data.

3. Results

3.1. Vascular injury increases ADAM22 expression in VSMCs

To investigate the role of ADAM22 in neointima formation, MOVASs and primary mouse aortic smooth muscle cells were stimulated with PDGF-BB, which is widely accepted as a stimulus for neointima formation by accelerating VSMC proliferation and migration [23,24]. Our results demonstrate that the protein level of ADAM22 was significantly elevated at 48 h after treatment with 20 ng/mL PDGF-BB (Fig. 1A and B). Then, immunofluorescence staining was used to investigate the localization of ADAM22. Accordingly, up-regulated ADAM22 was mainly expressed in VSMCs, as indicated by α-smooth muscle actin (α-SMA), a specific marker for VSMCs (Fig. 1C). Consistently, ADAM22 protein expression levels were also markedly enhanced in the carotid arteries of mice of wire-injury induced neointima formation model (Fig. 1D). However, no evidence of ADAM22 mRNA expression change was observed in sham-operated arteries and at day 28 post-injury (Supplementary Fig. 1). These data suggest the involvement of ADAM22 in vascular injury-induced neointima formation.

3.2. ADAM22 enhances PDGF-BB-induced VSMC proliferation in vitro

Given the possible role of ADAM22 in the pathogenesis of neointima formation, MOVASs were infected with lenti-ADAM22. Gene and protein expressions were measured and compared with the control, in which complementary DNA (cDNA) of PP6 was cloned into VSMCs. As shown in Fig. 2A and B, both gene and protein expressions were markedly up-regulated in the Flag-tagged ADAM22 VSMCs, which confirmed the successful overexpression of ADAM22 in MOVASs. Next, cell proliferation was tested using CCK8 and BrdU methods. We observed no significant difference between Flag-tagged ADAM22 and the control in cell proliferation under normal conditions. However, when VSMCs were stimulated with PDGF-BB, the Flag-tagged ADAM22 cells exhibited significantly higher proliferation levels compared with PDGF-BB-treated control cells (Fig. 2C and D). Additionally, ADAM22 overexpression suppressed mRNA expressions of cyclin-dependent kinase inhibitor 1C (P21) and cyclin-dependent kinase inhibitor 1B (P27) protein levels, both of which are negative regulators of cell proliferation. Conversely, we observed increased cell proliferation markers proliferating cell nuclear antigen (PCNA) and CyclinD1 expressions in mRNA and protein levels when cells were challenged with PDGF-BB (Fig. 2E and F). Next, two lines of ADAM22 knock down MOVASs were successfully generated. MOVASs from line 2 exhibited a obvious decrease in ADAM22 expression level, and were therefore used in the subsequent phenotypic evaluations (Supplementary Fig. 2A). MOVASs were infected with lenti-shADAM22 or treated with pLKO.1 vector containing scrambled shRNA target GFP as a negative control (shGFP). In line with these observations, PDGF-BB treatment enhanced VSMC proliferation, whereas this effect was largely inhibited by knocking down expression of ADAM22 using shRNA (Supplementary Figs. 2B and C). These data suggest that ADAM22 may contribute to the process of neointima formation, at least in part, through accelerating cell proliferation in VSMCs.

3.3. ADAM22 accelerates PDGF-BB-induced VSMC migration and phenotypic switching in vitro

In addition to cell proliferation, the role of ADAM22 in cell migration was also measured *in vitro*. Using a modified Boyden chamber migration assay, we showed that the migration of MOVASs in PP6 control groups was induced by PDGFBB (20 ng/mL) treatment, which were significantly aggravated by ADAM22 overexpression (Fig. 3A). Consistently, the healing and VSMCs migration rates increased with the challenge of PDGF-BB, which was more significant in the PDGF-BB-treated Flag-ADAM22 cells than in the PDGF-BB-treated PP6 cells (Fig. 3B). Additionally, given that phenotypic switching mainly refers to the process of transformation from contractile smooth muscle cells to

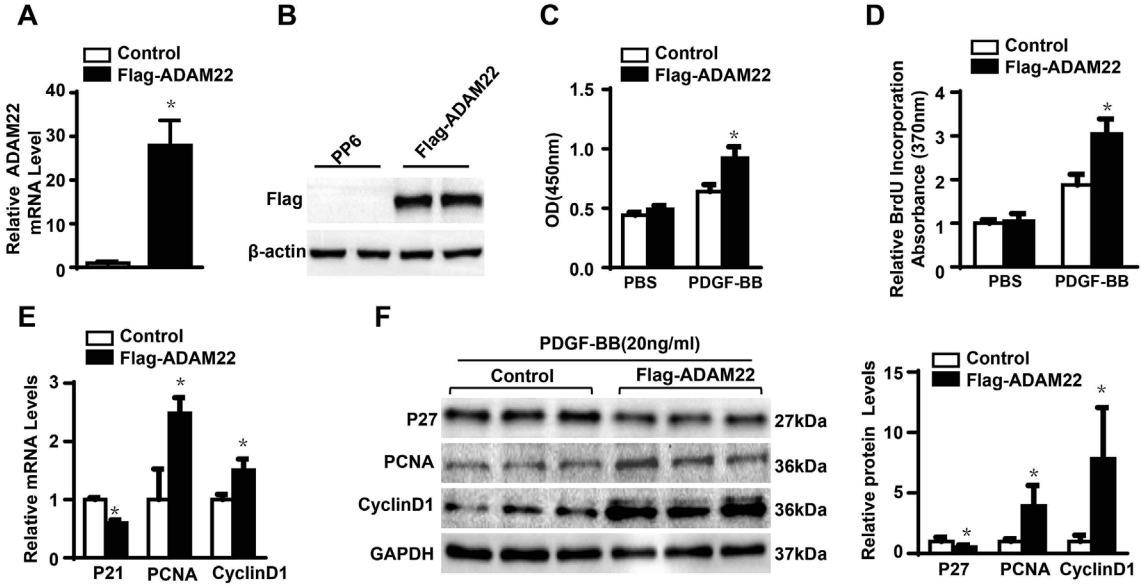

Fig. 2. ADAM22 increased PDGF-BB-induced VSMCs proliferation *in vitro*.
(A) Real-time PCR analysis of ADAM22 in MOVASs. (B) Immunoblot analysis of ADAM22 in MOVASs. (C–D) Cell proliferation was measured with the Cell Counting Kit-8 (CCK8) (C) and 5-Bromo-2′-Deoxyuridine (BrdU) (D) methods. (E) Real-time PCR analysis of p21, PCNA and CyclinD1. (F) Immunoblot analysis of p27, PCNA and CyclinD1. The protein levels were quantified. In panels B and F, β-actin (B) and GAPDH (F) served as the loading control. All values are presented as means ± SD for three independent experiments, and the statistical significance is indicated. (A–F) *$p < 0.05$ *versus* lenti-PP6 control group.

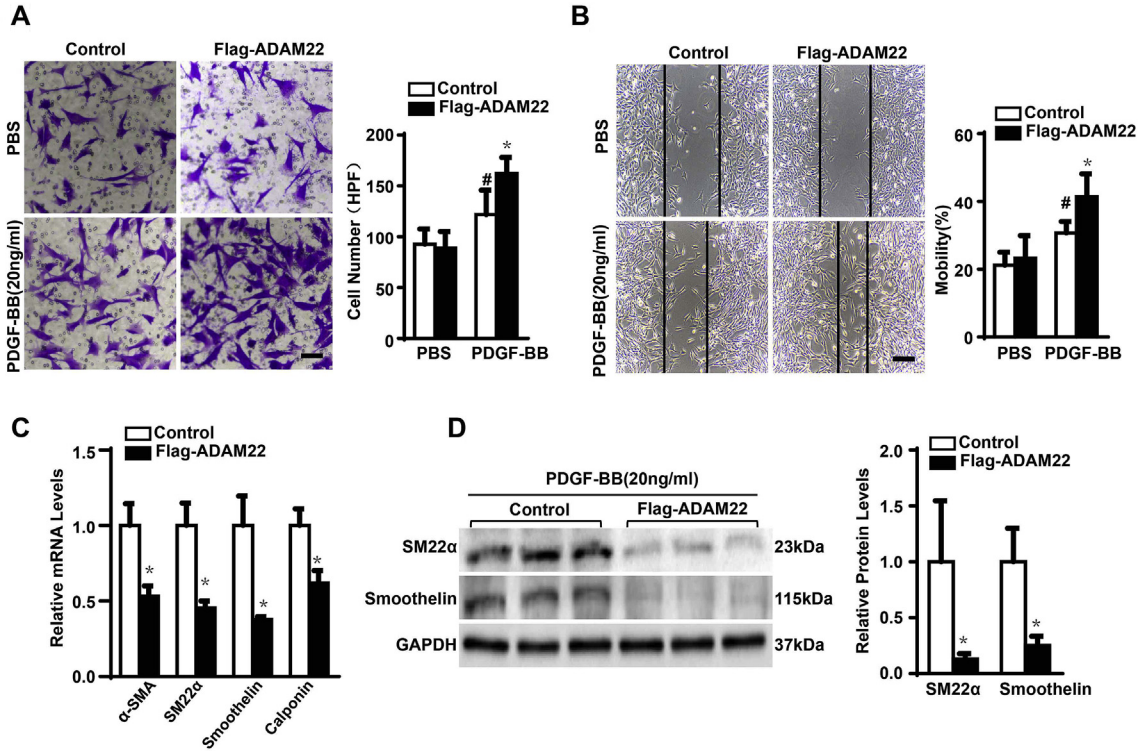

Fig. 3. ADAM22 enhanced PDGF-BB-induced VSMCs migration and phenotypic switching *in vitro*.
(A) Representative images of the transwell assays and the number of migrated VSMCs were counted in each group (n = 10 samples per group). Scale bar, 50 μm. (B) Representative images of cell scratch-wound assay and the statistical results on cell mobility (n = 6 samples per group). Scale bar, 50 μm. (C) Real-time PCR analysis of α-SMA, SM22α, smoothelin and calponin. (D) Immunoblot analysis of SM22α and smoothelin. GAPDH served as the loading control. In panels C and D are representative of three independent experiments. All values are presented as means ± SD, and the statistical significance is indicated. (A–B) #$p < 0.05$ *versus* PBS control group. (A–D) *$p < 0.05$ *versus* lenti-PP6 control.

synthetic smooth muscle cells, our results demonstrated that ADAM22 overexpression significantly decreased the gene expression of VSMC markers, such as α-SMA, SM22α, smoothelin and calponin. Meanwhile, ADAM22 overexpression suppressed the protein levels of SM22α and smoothelin (Fig. 3C and D). PDGF-BB promoted VSMC migration and suppressed the expression of smooth muscle genes in MOVASs. These effects were also inhibited by knocking down expression of ADAM22 using shRNA (Supplementary Figs. 2D and E). All these data demonstrated promotion effects of ADAM22 on VSMCs migration and phenotypic switching.

3.4. Heterozygous ADAM22 ablation inhibits neointima formation in mice

To further validate the pathophysiological role of ADAM22 *in vivo*, a wire-injury induced neointima formation model was established in both WT mice and *ADAM22*$^{+/-}$ mice, as *ADAM22*$^{-/-}$ mice died before weaning [25]. Histological assay demonstrated that the *ADAM22*$^{+/-}$ mice and WT mice led to comparable neointima in sham operation, while *ADAM22*$^{+/-}$ mice displayed markedly thinner neointima compared with WT mice at 14 and 28 days post-injury (Fig. 4A). No significant difference was observed in terms of the medial layer and external elastic lamina areas of the carotid arteries between WT and *ADAM22*$^{+/-}$ groups.

After confirming the pivotal regulation of ADAM22 in promoting neointima formation in mice, we further depicted its participation in VSMC proliferation and phenotypic switching *in vivo*. As shown in Fig. 4B, CyclinD1 and PCNA expressions were significantly decreased in *ADAM22*$^{+/-}$ mice at 14 and 28 days post-injury compared with WT controls (Fig. 4B). The Real-time PCR analysis showed that even though the P21 gene levels were obviously enhanced, CyclinD1 and PCNA gene expression was significantly suppressed in *ADAM22*$^{+/-}$ mice at 28 days post-injury (Fig. 4C). In line with these data, immunoblot analysis illustrated that vascular injury led to the up-regulation of P27 protein expression, as well as the down-regulation of CyclinD1 and PCNA protein expression (Fig. 4D).

The phenotypic switching of VSMCs was first measured by immunofluorescence staining. The results showed that *ADAM22*$^{+/-}$ mice exhibited markedly elevated α-SMA and SM22α following vascular injury, indicating the promotion effects of ADAM22 on VSMCs phenotypic switching (Fig. 4E). Additionally, the acceleration effects of ADAM22 on cell phenotypic switching were demonstrated by the enhanced gene expression of α-SMA, SM22α and calponin with an increase in the protein expression of SM22α and smoothelin in *ADAM22*$^{+/-}$ mice at 28 days post-injury (Fig. 4F and G). Therefore, these data indicate that ADAM22 plays a critical role in facilitating neointima formation *in vivo*.

3.5. ADAM22 activates ERK signaling to accelerate neointima formation

To further investigate the underlying mechanism of ADAM22 in neointima formation, we tested the AKT and MAPK signaling pathway, which is involved in neointima formation dependent on its potent capacity to mediate cell proliferation, migration and phenotypic switching [26–28]. As shown in Fig. 5A and Supplementary Fig. 3A, AKT, MAPK subunits, as well as ASK1 were largely activated by PDGF-BB treatment in VSMCs, as indicated by significantly increased phosphorylation. Intriguingly, ADAM22 overexpression in VSMCs only resulted in elevated phosphorylation levels of ERK and its upstream protein MEK when challenged with PDGF-BB. The regulatory role of ADAM22 was further evaluated in all the above signal molecules *in vivo*. According to the results, *ADAM22*$^{+/-}$ mice specifically blocked wire-injury induced phosphorylation of ERK, whereas the activation of AKT,

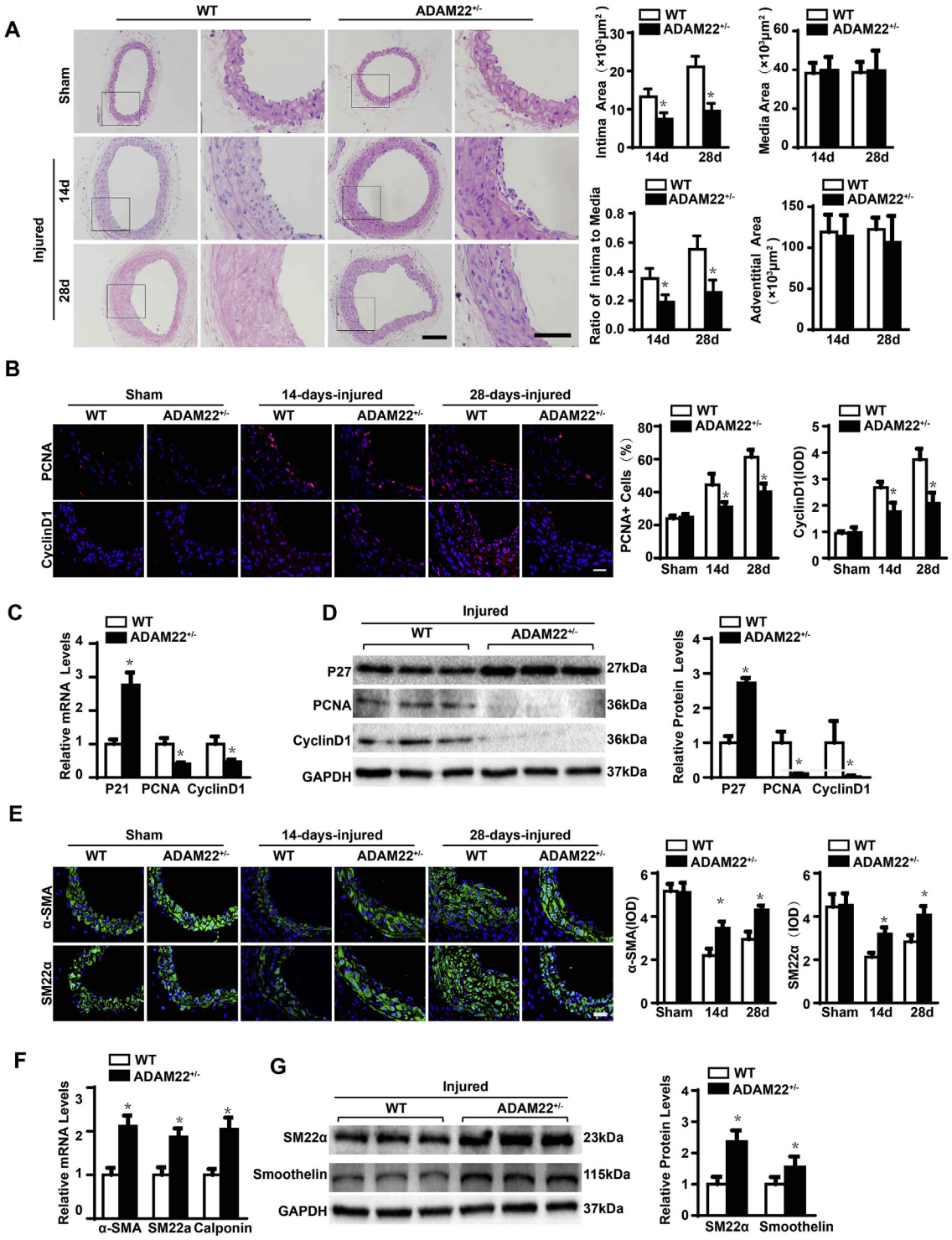

Fig. 4. ADAM22 promotes neointima formation *in vivo*.
(A) Haematoxylin and eosin staining was performed on sections derived from WT and *ADAM22*$^{+/-}$ mice at the indicated time points before and after injury. Quantitative analysis of the intima area, media area, ratio of intima to media, and external elastic lamina areas in the carotid artery sections of WT and *ADAM22*$^{+/-}$ mice (n = 5–8 per group at each time point). Scale bar = 50 μm. (B) Immunofluorescence staining of PCNA and CyclinD1 at the indicated time points before and after injury. The ratio of PCNA positive cells and the integral optical density (IOD) values for Cyclin D1 were calculated (n = 4–6 per group at each time point). Scale bar, 50 μm. (C) Real-time PCR analysis of p21, PCNA and Cyclin D1 at 28 days post-injury (n = 4–7 per group). (D) Immunoblot analysis of p27, PCNA and Cyclin D1 at 28 days post-injury. The blots represent three independent experiments. (E) Immunofluorescence staining of α-SMA and SM22α at the indicated time points before and after injury. The IOD values of α-SMA and SM22α were also provided (n = 4–7 per group at each time point). Scale bar, 50 μm. (F) Real-time PCR analysis of α-SMA, SM22α and calponin (n = 3 per group) and (G) immunoblot analysis of SM22α and smoothelin at 28 days post-injury. Blots are representative of three independent experiments. In panels D and G, GAPDH served as the loading control. All values are presented as means ± SD, and the statistical significance is indicated. (A–G) *$p < 0.05$ *versus* the WT group.

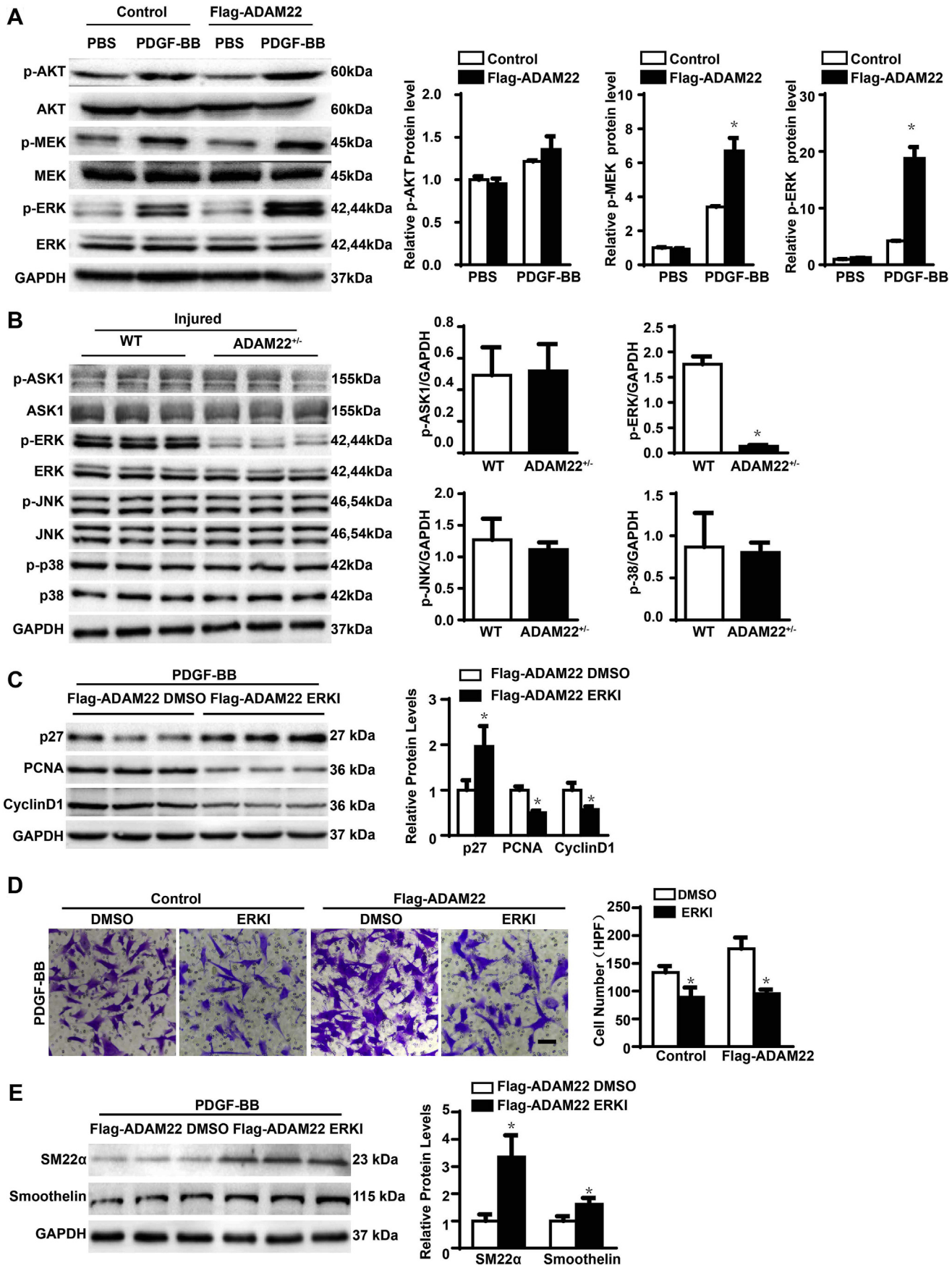

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Fig. 5. ADAM22 accelerated neointima formation via activation of ERK signaling.
(A) Immunoblot analysis of p-AKT, AKT, p-MEK, MEK, p-ERK and ERK in MOVASs treated with PDGF-BB. (B) Western blot analysis of p-ASK1, ASK1, p-ERK, ERK, p-JNK, JNK, p-P38 and P38 in WT and *ADAM22*$^{+/-}$ mice at 28 days post-injury. (C–E) Flag-tagged ADAM22 and PP6 controls were used to infect MOVASs before treated with ERKI or DMSO. Western blot analysis(C) of p27, PCNA and CyclinD1 and a transwell assay (D) was used to test the migration ability of VSMCs as well as immunoblot analysis (E) of SM22α and smoothelin. In panels A, B, C and E, GAPDH served as the loading control. These blots are representative of three independent experiments. In panels D, Scale bar, 50 μm; n = 6 samples per group. All values are presented as the means ± SD, and statistical significance is indicated. (A) $^{*}p < 0.05$ *versus* lenti-PP6 infected control group. (B) $^{*}p < 0.05$ *versus* the WT group. (C–E) $^{*}p < 0.05$ *versus* the DMSO-treated group.

ASK1, JNK and p38 were negligibly influenced in *ADAM22*$^{+/-}$ mice compared to the WT controls (Fig. 5B and Supplementary Fig. 3B). These data demonstrated that the accelerating effect of ADAM22 on neointima formation is independent of ASK1 and AKT signaling pathways, but at least in part, by activating the ERK signaling pathway.

Given that ADAM22 overexpression promoted ERK activation and exacerbated the process of VSMCs proliferation, migration and phenotypic switching, we evaluated whether inhibition of ERK activity could mitigate PDGF-BB-induced VSMC dysfunction. Flag-tagged ADAM22 and PP6 controls were used to infect MOVASs before treated with a suspension of ERK inhibitor (ERKI; MCE, U0126) or DMSO. Western blot analyses showed that ERKI greatly inhibited and largely abolished PDGF-BB-induced VSMC dysfunction, as evidenced by dramatic up-regulation of P27 protein expression and down-regulation of CyclinD1 and PCNA protein expression, as well as increased expression of SMC markers (Fig. 5C and E). More importantly, the VSMC migration assay indicated that ERKI treatment efficiently alleviated PDGF-BB-induced migration (Fig. 5D).

4. Discussion

VSMC proliferation, migration, and phenotypic switching have been recognized as key events in neointima formation. In this study, through both PDGF-BB *in vitro* and arterial injury *in vivo* experiments, we demonstrate that ADAM22 exerts a profound role in VSMC proliferation, migration and phenotypic switching, leading to accelerated VSMCs-mediated neointima formation via enhancing ERK phosphorylation. Based on data from the present study, we propose that inhibiting ADAM22 expression is a promising target for blocking or preventing neointima formation in clinic.

As a result of serious threat to patient's life expectancy, neointima formation has attracted increasing attention, and tremendous efforts have been made to search for an effective treatment approach in recent decades [20]. To well determine the role of ADAM22 in neointima formation, we first measured its expression levels in PDGF-BB-treated VSMCs and carotid artery wire injury model in mice, which implicate a potential role of ADAM22 in neointima formation. Interestingly, ADAM22 mRNA expressions showed no changed (Supplementary Fig. 1). Thus, the increased protein expression of ADAM22 upon vascular injury might be regulated by post-transcriptional modifications, which requires further investigation. While previous reports mainly focused on the role of ADAM22 in synaptic transmission, progressive encephalopathy and endocrine-resistant breast cancer [16,18,29], our present study is the first report on the involvement of ADAM22 in neointima formation. Regarding the cellular mechanism, it was found that ADAM22 might function in the development of the nervous system by regulating the maturation of postnatal synapses. Here, for the first time, we demonstrated that ADAM22 also acts as a critical player in cellular proliferation, migration and differentiation of VSMCs in the pathogenesis of neointima formation both *in vitro* and *in vivo*.

It is well known that PDGF is a key regulator for stimulating the proliferation and migration of VSMCs [30,31]. As a subtype of PDGF, PDGF-BB binds to its receptor and inducts the related pathways of Src homology 2 domain-containing signaling molecules, including phospholipase Cg and phosphatidylinositol 3-kinase [32,33], which subsequently activate downstream MAPK signaling and lead to corresponding cellular responses [34]. Several cellular physiological or pathological events, including cell proliferation and migration, have been demonstrated to be associated with MAPK activation [27,28]. Indeed, ERK and p38 have been well recognized as the main protein molecules that are responsible for initiating the cascade reaction in regulating VSMC proliferation and migration when cells are challenged with PDGF [30,31]. Bessard et al. reported that ERK knocked down had distinct functions in suppressing the growth of tumour cells both *in vitro* and *in vivo* [35]. Additionally, Jung et al. demonstrated that the ERK1 signaling pathway was involved in the inhibition of cordycepin-induced VSMC proliferation inhibition [36]. Consistent with previous studies, we showed significantly enhanced ERK activation after the cells were challenged with PDGF-BB. Interestingly, we observed a specific down-regulation of ERK phosphorylation, instead of JNK or p38, in *ADAM22*$^{+/-}$ mice as compared to their WT littermates. More importantly, inhibition of ERK activity also attenuated PDGF-BB-induced VSMCs proliferation, migration and phenotypic switching. Although the direct mechanisms and molecular linkers between ADAM22 and ERK remain to be further demonstrated, our present study indicate that the function of ADAM22 in regulating VSMCs proliferation and migration is mainly dependent on the ERK signaling pathway.

ADAM22 lacks a zinc-binding motif in metalloproteinase-like domain, which suggests of the absence of metalloproteinase activity and that ADAM22 functions as an integrin ligand [37,38]. Previous studies have demonstrated that some integrins, including integrin β1, integrin β3, integrin α6, integrin α9, integrin α1β8 and integrin αvβ3 were identified to interact with ADAM22 [39]. Since integrins play a critical role in the pathogenic progress of neointima hyperplasia [5,40,41], it is reasonable to hypothesize that the involvement of ADAM22 in the pathogenic process of neointima formation is mainly through the disintegrin activity.

In conclusion, this study provided the first evidence that ADAM22 accelerates PDGF-BB-induced proliferation, migration and phenotypic switching in VSMCs and strengthens the carotid artery wire-injury induced neointima formation in mice. ADAM22 significantly up-regulates the ERK signaling pathway, which may contribute to the acceleration effects of neointima formation following vascular injury. Therefore, our findings provide new insight and broaden our knowledge on the effects of ADAM22 in neointima formation, suggesting that ADAM22 may be a potent therapeutic target for the treatment of neointima formation following vascular injury.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

In this study, Shu-Min Zhang designed and performed the experiments, analyzed the data and wrote the manuscript; Le Jiang and Jian-Feng Liu and Xin Zhao performed the experiments; Bin Liang and Chang Liu analyzed the data; Chang-sheng Ma and Nian Liu provide useful advices and helping the revision of manuscript.

Financial support

This study was supported by grants from the National Natural

Science Foundation of China NO.81500384; NO.81600262; NO.81530016; NO.81670291; and NO.81770318), National Key Research and Development Program of China (2016YFC0900901), Beijing Postdoctoral Science Foundation (2017-ZZ-125), and China Postdoctoral Science Foundation (2016M601069).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.02.002.

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