MALT1 accelerates proatherogenic vascular smooth muscle cell growth, invasion and synthetic phenotype switching via nuclear factor-κB signaling-dependent way

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Abstract. Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) modulates T helper cell differentiation and nuclear factor-κB (NF-κB) pathway-mediated inflammation and potentially regulates lipid metabolism, which are all critical factors involved in atherosclerosis. The present study aimed to investigate the effect of MALT1 on the cellular functions of proatherogenic vascular smooth muscle cells (VSMCs). Therefore, to establish a human proatherogenic VSMC model, VSMCs were treated with different doses of oxidized low-density lipoprotein (oxLDL). Subsequently, the effect of MALT1 overexpression or knockdown in proatherogenic VSMCs treated with or without NF-κB activator was also explored. The results showed that treatment of proatherogenic VSMCs with oxLDL significantly elevated the mRNA and protein expression levels of MALT1 in a dose-dependent manner. Furthermore, MALT1 overexpression enhanced cell viability, invasion and phenotype switching and reduced apoptosis in proatherogenic VSMCs. However, MALT1 knockdown exerted the opposite effect on the above cellular functions. Additionally, the results revealed that MALT1 could positively regulate the NF-κB pathway in proatherogenic VSMCs. Moreover, treatment of proatherogenic VSMCs with NF-κB activator not only exacerbated the dysregulation of cellular functions, but also hampered the effect of MALT1 knockdown on attenuating cell growth, invasion and synthetic phenotype switching, thus suggesting that NF-κB was essential for the regulation of MALT1-triggered functions in proatherogenic VSMCs. In conclusion, the current study suggested that MALT1 could exacerbate cell viability, mobility and synthetic phenotype switching of proatherogenic VSMCs in a NF-κB signaling-dependent manner. Therefore, MALT1 could be considered as a potential therapeutic target for atherosclerosis.

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Introduction

Atherosclerosis is a critical pathological process that may result in the stenosis of the artery, eventually leading to cerebral-cardiovascular diseases, such as coronary artery disease and ischemic stroke, two notorious diseases associated with high mortality rate worldwide (1-3). Currently, the treatment approaches for the above cerebral-cardiovascular diseases, such as thrombolysis, thrombectomy and percutaneous coronary intervention, have greatly improved the clinical outcomes of patients (4-7). However, fundamental management strategies to prevent or even reverse the process of atherosclerosis are still lacking. Therefore, exploring potential treatment targets for atherosclerosis is of great importance.

It has been reported that the dysregulation of vascular smooth muscle cells (VSMCs) is critically involved in the pathogenesis and progression of atherosclerosis (8). It is generally considered that the abnormal proliferation or invasion of VSMCs can promote the formation of atherosclerotic lesions, as well as elevate lipid accumulation, another key event involved in the progression of atherosclerosis (9). A previous study also demonstrated that the switching of VSMCs from a contractile phenotype towards a synthetic phenotype could enhance inflammation, thus also contributing to atherosclerosis (10). Additionally, the apoptosis of VSMCs at the late stage of atherosclerosis could facilitate the rupture of atherosclerotic lesion (11). Therefore, inhibiting the abnormal cellular functions of VSMCs could be a potential strategy for managing atherosclerosis.

Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is part of the caspase recruitment domain recruited membrane associated protein 3/B-cell lymphoma 10/MALT1 (CBM) signaling complex that regulates the activation of the nuclear factor-κB (NF-κB) signaling pathway involved in several diseases and more particularly in allergy and cancer (12-14). However, it has become gradually accepted that MALT1 may be involved in other pathological

processes. Therefore, a previous study showed that MALT1 could activate the NF-κB signaling pathway to elevate inflammation in the vasculature (14). In addition, the aforementioned study also revealed that CBM complex-deficient mice could not develop atherosclerosis following stimulation with angiotensin, thus supporting that MALT1 could be involved in atherosclerosis (14). However, whether MALT1 could modulate the cellular functions of VSMCs to regulate atherosclerosis remains unknown.

Therefore, the current study aimed to evaluate the effect of MALT1 on proatherogenic VSMC proliferation, apoptosis, invasion and phenotype switching, as well as its potential underlying mechanism of action.

Materials and methods

Cell culture. Human primary VSMCs were purchased from Bluefcell Bio and maintained in DMEM supplemented with 10% FBS (both from Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin solution (Sangon Biotech Co. Ltd.) at 37˚C and 5% CO_2. The use of VSMCs was approved by the Ethics Committee of Affiliated Hospital of Inner Mongolia Medical University with approval number KY (2020015).

VSMC activation with oxidized low-density lipoprotein (oxLDL). VSMCs at a density of $2x10^4$ cells/well were seeded into a 6- or 96-well plate. Following incubation for 24 h at 37˚C, VSMCs were stimulated with 0, 25, 50, 100 or 200 μg/ml oxLDL (Beijing Solarbio Science & Technology Co., Ltd.) (15). At 24 h after stimulation at 37˚C, further experiments, including reverse transcription-quantitative (RT-q) PCR, western blot analysis and cell viability, cell apoptosis and cell invasion assays were performed.

MALT1 regulation experiment. The lentivirus overexpressing MALT1 (Lv-MALT1) or knocking down MALT1 (Lv-anti-MALT1) and the empty lentivirus (lentivirus containing empty vector, Vector) were obtained from Shanghai GenePharma Co., Ltd. The frame of vectors overexpressing MALT1 or knocking down MALT1 are shown in Fig. S1. Briefly, VSMCs were seeded into culture plates and were then transfected with the above lentiviruses using 6 μg/ml polybrene (cat. no. 40804ES76; Shanghai Yeasen Biotechnology Co., Ltd.). Untransfected VSMCs served as the control group. Following incubation for 72 h at 37˚C (16), VSMCs were activated with 100 μg/ml oxLDL for 24 h and were then collected for RT-qPCR, western blotting, cell viability, cell apoptosis and cell invasion assays.

VSMC treatment with phorbol 12-myristate 13-acetate (PMA). PMA (1 μM; MedChemExpress) (17), a NF-κB activator, was adopted to evaluate the MALT1-mediated regulation of the NF-κB signaling pathway. Briefly, VSMCs were transfected with Lv-anti-MALT1 or empty lentivirus for 72 h, as previously described. Subsequently, VSMCs were divided into the following four groups: Vector group, where cells were transfected with empty lentivirus and were not treated with PMA; Lv-anti-MALT1 group, where cells were transfected with MALT1 knockdown lentivirus and were not treated with PMA; PMA group, where VSMCs were transfected with empty lentivirus and treated with PMA; and Lv-anti-MALT1 + PMA group, where cells were transfected with Lv-anti-MALT1, followed by treatment with PMA. Untransfected and untreated VSMCs served as the control group. VSMCs in all groups were cultured in medium supplemented with 100 μg/ml oxLDL. Following treatment for 24 h at 37˚C, cells were harvested for RT-qPCR, western blot, cell viability, cell apoptosis and cell invasion assays.

RT-qPCR. The mRNA expression levels of MALT1 in VSMCs were assessed using RT-qPCR. Briefly, total RNA was extracted from $1x10^6$ VSMCs using a Trizol (Beyotime Institute of Biotechnology) and RT-PCR and qPCR amplification were performed using the RT reagent kit (Takara Bio, Inc.) and qPCR mix kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocols, respectively. For qPCR, the following thermocycling conditions were performed: One cycle of 95˚C for 5 min, followed by 40 cycles at 95˚C for 2 min and 61˚C for 20 sec. qPCR was performed in triplicate. The relative expression levels of MALT1 were analyzed using the $2^{-\Delta\Delta Cq}$ method (18). The primer sequences used were as follows: For MALT1, forward, 5'-TCTTGGCTG GACAGTTTGTGA-3' and reverse, 5'-GCTCTCTGGGAT GTCGCAA-3'; and for GAPDH, forward, 5'-GAGTCCACT GGCGTCTTCAC-3' and reverse, 5'-ATCTTGAGGCTGTTG TCATACTTCT-3'.

Western blot analysis. Total proteins were extracted from VSMCs using a RIPA reagent (Shanghai Yeasen Biotechnology Co., Ltd.) and quantified with a BCA kit (Mlbio). Subsequently, the 20 μg protein extracts were separated by SDS-PAGE on 4-20% precast gels (Beyotime Institute of Biotechnology) and were then transferred onto nitrocellulose membranes (MilliporeSigma). The membranes were then blocked with 5% BSA (Beyotime Institute of Biotechnology) for 1 h at 37˚C, followed first by incubation with primary antibodies at 4˚C overnight and then with the corresponding secondary antibody at 37˚C for 1 h. The protein bands were visualized using an ECL reagent (UNIV). Densitometry was performed using Image J (version 1.8.0; National Institutes of Health). The antibodies used for western blot analysis were all purchased from Affinity Biosciences and were as follows: Anti-MALT1 (dilution, 1:1,000; cat. no. DF6867), anti-α-smooth muscle actin (SMA; dilution, 1:1,000; cat. no. AF1032), anti-osteopontin (OPN; dilution, 1:1,000; cat. no. AF0227), anti-phosphorylated (p)-IκBα (dilution, 1:500; cat. no. AF2002), anti-IκBα (dilution, 1:500; cat. no. af5002), anti-p-p65 (dilution, 1:500; cat. no. AF2006), anti-p65 (dilution, 1:500; cat. no. AF5006), anti-GAPDH (dilution, 1:5,000; cat. no. AF7021) and HRP conjugated goat-anti rabbit secondary antibody (dilution, 1:10,000; cat. no. S0001).

Cell viability assay. The viability of VSMCs was assessed using a Cell Counting Kit-8 (CCK-8; MilliporeSigma). Briefly, VSMCs were seeded into 96-well culture plates (Wuxi NEST Biotechnology Co., Ltd.) and were then treated for 24 h as previously described. Subsequently, cells were supplemented with CCK-8 reagent for 2 h and the optical density was measured using a microplate reader (BioTek Instruments, Inc.).

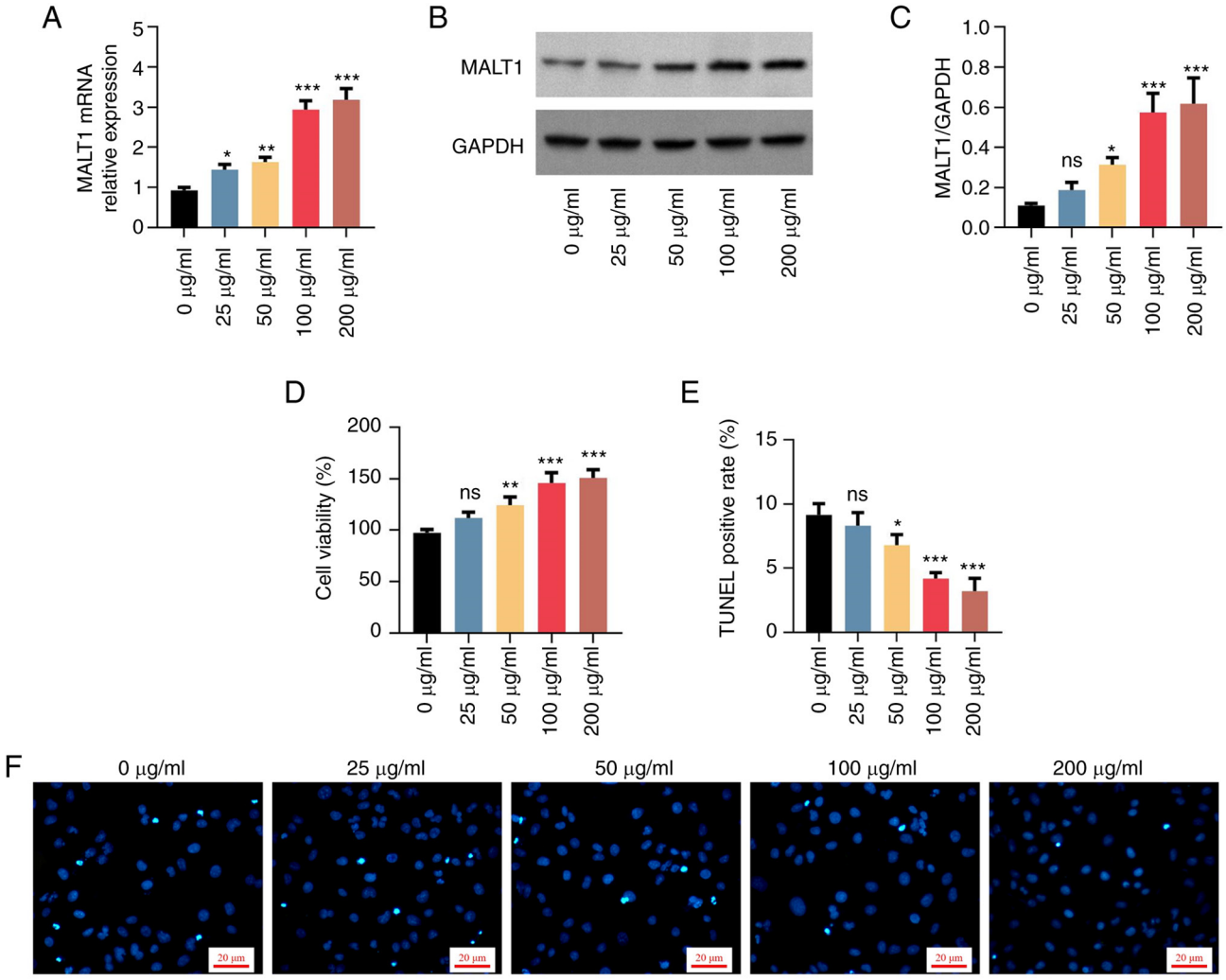

Figure 1. Assessment of MALT1 expression, viability and apoptosis of vascular smooth muscle cells treated with different doses of oxLDL for 24 h. (A) Comparison of the MALT1 mRNA expression levels among different groups. (B) Detection of the protein expression levels of MALT1 in different groups. Comparison of (C) the protein expression levels of MALT1, (D) cell viability and (E) cell apoptosis among different groups is presented. (F) Detection of cell apoptosis via TUNEL assay in different groups. *P<0.05, **P<0.01 and ***P<0.001, vs. cells treated with 0 μg/ml oxLDL. ns, non-significant; oxLDL, oxidized low-density lipoprotein; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1.

Cell apoptosis assay. The apoptosis of VSMCs was assessed using a TUNEL apoptosis kit (Beyotime Institute of Biotechnology). Briefly, following treatment, VSMCs were fixed with 4% paraformaldehyde (Beyotime Institute of Biotechnology) for 10 min at room temperature, followed by incubation with TUNEL reagent for 1 h at 37˚C. Finally, VSMCs were stained with DAPI (5 mg/l, Sangon Biotech Co. Ltd.) for 10 min at room temperature.

Cell invasion assay. The invasion ability of VSMCs was evaluated using Transwell assays. Briefly, treated VSMCs were seeded into the upper chamber, which was precoated in Matrigel at 37˚C for 1 h (Corning, Inc.), while the lower chamber was supplemented with complete medium. Following incubation for 24 h at 37˚C, cells were stained with crystal violet (0.1%, Sangon Biotech Co. Ltd.) at room temperature for 20 min.

Statistical analysis. The differences among multiple groups were compared with one-way ANOVA followed by Dunnett's or Tukey's multiple comparisons test using GraphPad Prism 8.0 (Dotmatics). P<0.05 was considered to indicate a statistically significant difference.

Results

OxLDL upregulates MALT1, enhances cell viability and inhibits apoptosis in VSMCs. The mRNA expression levels of MALT1 were notably elevated in a dose-dependent manner following VSMC treatment with 25-200 μg/ml oxLDL (all P<0.05; Fig. 1A). Consistently, the protein expression levels of MALT1 were also significantly increased in a dose-dependent manner in VSMCs treated with 50-200 μg/ml oxLDL (all P<0.05; Fig. 1B and C). However, no statistical significance was observed in the 25 μg/ml oxLDL treatment group. In addition, oxLDL enhanced cell proliferation (Fig. 1D) suppressed cell apoptosis (Fig. 1E and F). It enhanced invasion (Fig. 2A and B), downregulated α-SMA and upregulated OPN (Fig. 2C-E) in a dose-dependent manner. However, again, no statistical significance was observed in the 25 μg/ml oxLDL treatment group. The effect of oxLDL on cell proliferation, apoptosis, invasion

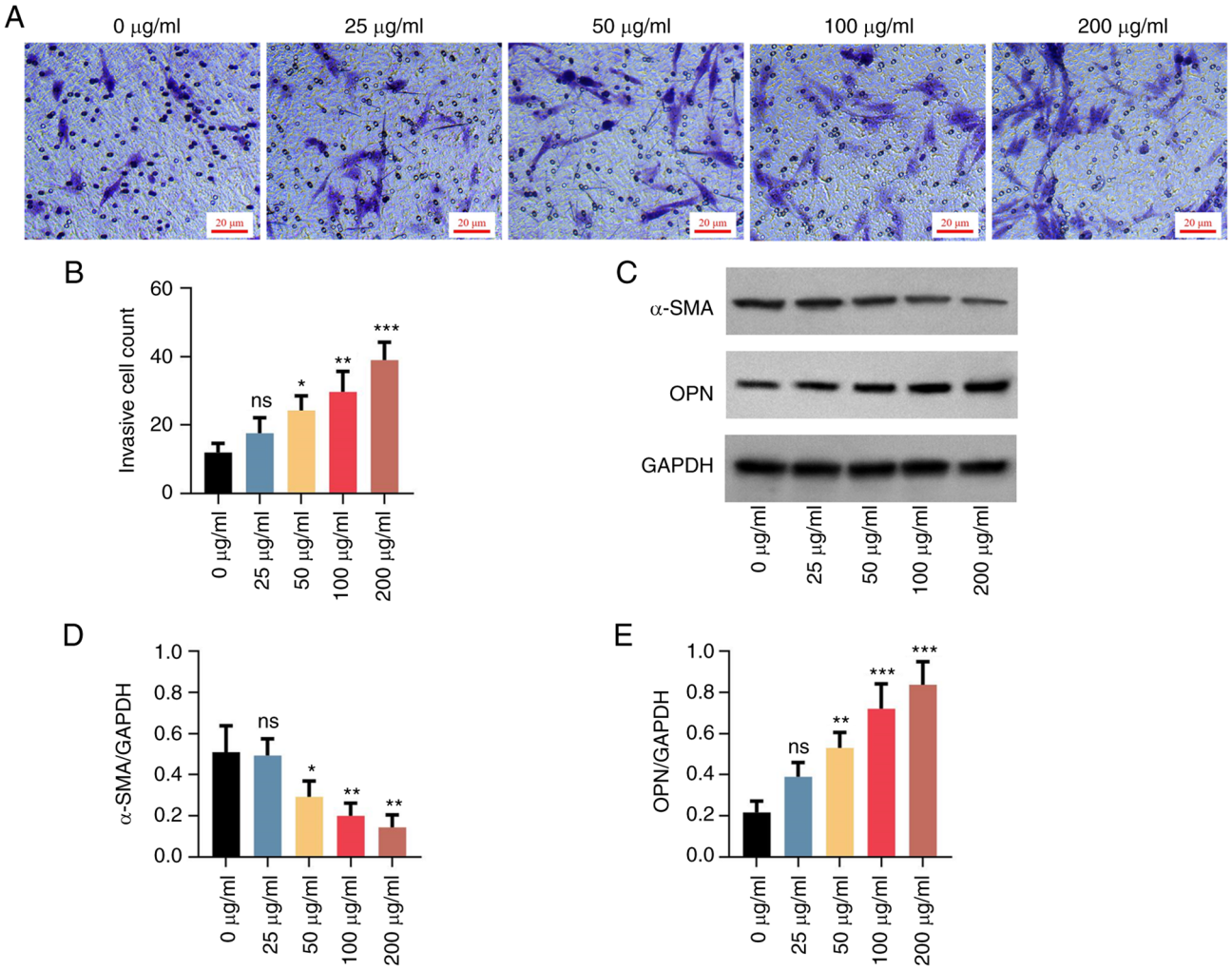

Figure 2. Assessment of the invasion ability and phenotype switching of VSMCs treated with different doses of oxLDL for 24 h. (A) Evaluation of cell invasion among different groups using crystal violet staining. (B) Comparison of cell invasion among different groups is presented. (C) Protein expression levels of α-SMA and OPN in different groups. Comparison of the (D) α-SMA and (E) OPN protein expression levels among different groups. *P<0.05, **P<0.01 and ***P<0.001 vs. cells treated with 0 μg/ml oxLDL. ns, non-significant; VSMCs, vascular smooth muscle cells; oxLDL, oxidized low-density lipoprotein; α-SMA, α-smooth muscle actin; OPN, osteopontin.

and varying α-SMA and OPN levels showed a dose-dependent manner between 0 and 100 μg/ml, but it reached a plateau between 100 and 200 μg/ml. Therefore, a lower dose at the plateau was chosen for the following experiment (which is common practice). The above data supported the successful establishment of the proatherogenic VSMC model.

MALT1 positively regulates cell viability, invasion and phenotype switching and negatively regulates apoptosis in proatherogenic VSMCs. Subsequently, to evaluate the effect of MALT1 on the cellular functions of proatherogenic VSMCs, the expression of MALT1 was modulated in VSMCs transfected with the corresponding lentivirus. The results showed that the mRNA (Fig. 3A) and protein (Fig. 3B and C) expression levels of MALT1 were increased in VSMCs transfected with Lv-MALT1 (both P<0.001) compared with the Vector group. By contrast, MALT1 was downregulated in cells transfected with Lv-anti-MALT1 (both P<0.05) compared with the Vector group, thus suggesting that the transduction of VSMCs with lentiviral particles was successful. Furthermore, compared with the Vector group, the viability of proatherogenic VSMCs was elevated and reduced by MALT1 overexpression and knockdown, respectively (both P<0.01; Fig. 3D). Additionally, apoptosis assessment by TUNEL assay revealed that the number of apoptotic cells was decreased in the Lv-MALT1 group and increased in the Lv-anti-MALT1 group compared with the Vector group (Fig. 3E). Consistently, semi-quantified analysis confirmed that the changes in the number of apoptotic VSMCs were statistically significant compared with the Vector group (P<0.05, for Lv-MALT1; P<0.001, for Lv-anti-MALT1; Fig. 3F).

Transwell assay and crystal violet staining showed that the invasion ability of proatherogenic VSMCs was enhanced by MALT1 overexpression and reduced by MALT1 knockdown compared with the Vector group (Fig. 4A). Semi-quantified analysis verified the aforementioned effects (both P<0.01; Fig. 4B). Furthermore, the protein expression levels of VSMC phenotype markers were evaluated by western blot analysis (Fig. 4C). Therefore, the results demonstrated that the expression of the contractile phenotype marker, α-SMA, was inhibited by MALT1 overexpression and elevated by MALT1 knockdown (both P<0.05; Fig. 4D). However, the opposite

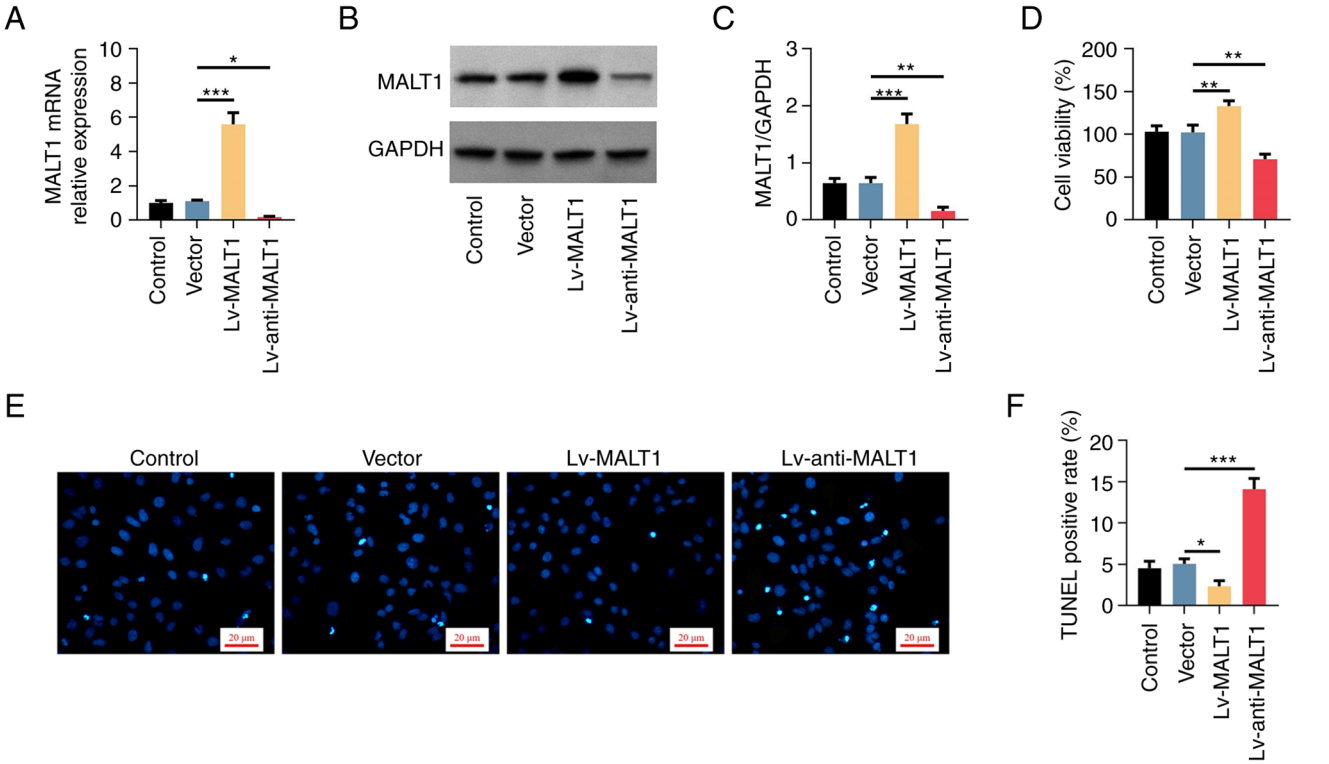

Figure 3. Effect of MALT1 on the viability and apoptosis of proatherogenic VSMCs. VSMCs were first transfected with MALT1 overexpression or knockdown lentiviral plasmids and were then treated with 100 μg/ml oxidized low-density lipoprotein for 24 h. (A) Comparison of the MALT1 mRNA expression levels among different groups in transfected VSMCs. (B) The protein expression levels of MALT1 among different groups were detected following VSMC transfection with lentiviral plasmids. (C) Protein expression levels of MALT1 and (D) cell viability were compared among different groups of transfected VSMCs. (E) Cell apoptosis rate of transfected VSMCs was compared via TUNEL assay. (F) Comparison of cell apoptosis among different groups of transfected VSMCs. *P<0.05, **P<0.01 and ***P<0.001. MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; VSMCs, vascular smooth muscle cells.

effects were observed in the protein expression levels of the synthetic phenotype marker OPN (both P<0.01; Fig. 4E), thus indicating that the phenotype of proatherogenic VSMCs was regulated by the expression of MALT1.

MALT1 activates the NF-κB signaling pathway in proatherogenic VSMCs. Subsequently, the activation status of the NF-κB signaling pathway, a potential downstream pathway of MALT1, was detected in proatherogenic VSMCs using western blot analysis (Fig. 5A). The data revealed that compared with the Vector group, p-IκBα was upregulated by MALT1 overexpression and downregulated by MALT1 knockdown (both P<0.05; Fig. 5B). Additionally, the protein expression levels of p-p65 were increased (P<0.001) and reduced (P<0.05) by MALT1 overexpression and knockdown, respectively (Fig. 5C).

MALT1 regulates the cellular functions of proatherogenic VSMCs via the NF-κB signaling pathway. To reveal the association between MALT1 and NF-κB signaling in the cellular functions of proatherogenic VSMCs, MALT1-depleted VSMCs and MALT1-containg VSMCs were treated with PMA, a NF-κB pathway activator. Western blot analysis showed that cell treatment with PMA upregulated both p-IκBα and p-p65, compared with the Vector group (both P<0.001; Fig. 6A). Furthermore, treatment of MALT1-depleted proatherogenic VSMCs with PMA increased the levels of p-IκBα and p-p65, which were reduced by MALT1 knockdown (both P<0.001; Fig. 6B and C). Regarding the cellular functions of proatherogenic VSMCs, PMA promoted cell viability (P<0.01; Fig. 6D), suppressed cell apoptosis (P<0.05; Fig. 6E and F), increased cell invasion (P<0.01; Fig. 7A and B), downregulated α-SMA and upregulated OPN (both P<0.05; Fig. 7C-E) compared with the Vector treatment group. Furthermore, additional treatment with PMA further reduced the viability of VSMCs, that had been elevated by MALT1 knockdown (P<0.01; Fig. 6D). In terms of apoptosis, additional PMA treatment suppressed the MALT1 knockdown-mediated enhanced cell apoptosis (P<0.001; Fig. 6E and F). Furthermore, additional treatment of VSMCs with PMA promoted the cell invasion, that was inhibited by MALT1 knockdown (both P<0.01; Fig. 7A and B). Finally, additional treatment with PMA restored the MALT1 knockdown-mediated high levels of α-SMA and low levels of OPN in proatherogenic VSMCs (both P<0.01; Fig. 7C-E). The aforementioned findings suggested that the NF-κB signaling pathway was essential for regulating the MALT1-triggered cellular functions of proatherogenic VSMCs.

Discussion

Currently, several factors have been identified to be closely associated with the risk of atherosclerosis, including hyperlipidemia, diabetes mellitus, smoking, increasing age and biological sex (19,20). Among the mentioned risk

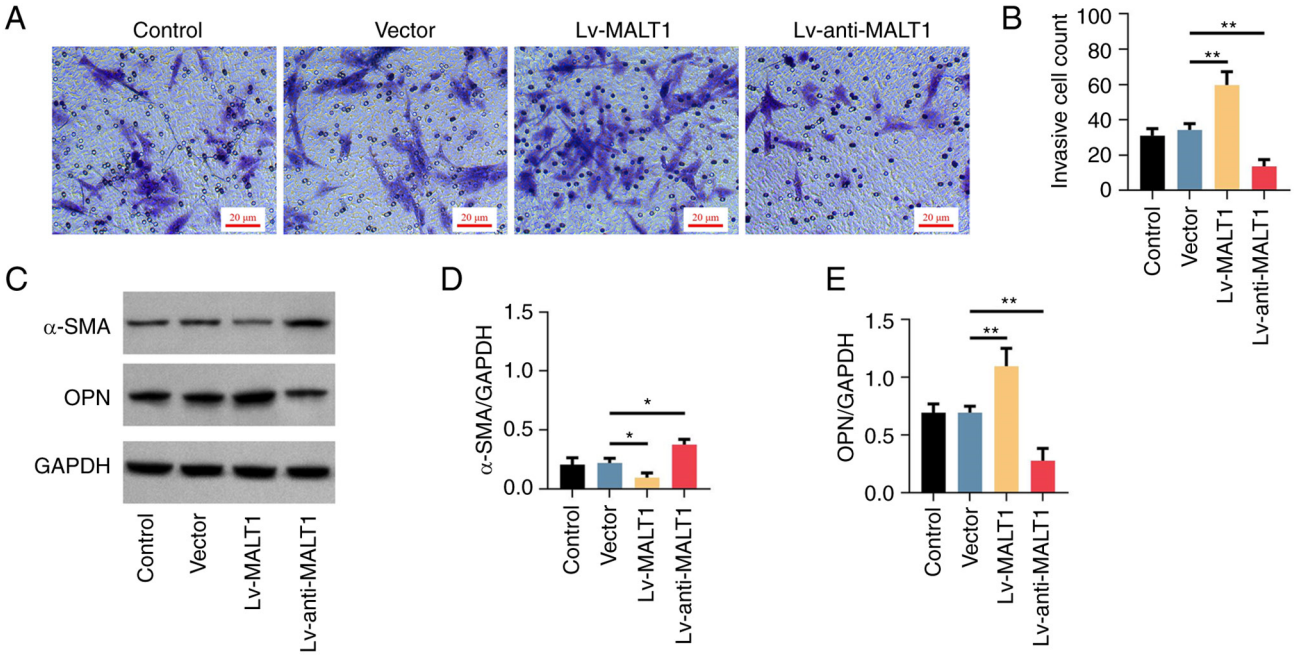

Figure 4. Effect of MALT1 on the invasion and phenotype switching of proatherogenic VSMCs. VSMCs were first transfected with MALT1 overexpression or knockdown lentiviral plasmids and were then treated with 100 μg/ml oxidized low-density lipoprotein for 24 h. (A) The cell invasion ability of transfected VSCMs from different groups was evaluated using crystal violet staining. (B) Cell invasion ability was compared among different groups. (C) Protein expression levels of α-SMA and OPN were compared among different groups. The levels of (D) α-SMA and (E) OPN in transfected VSMCs in different groups are shown. *P<0.05 and **P<0.01. MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; VSMCs, vascular smooth muscle cells; α-SMA, α-smooth muscle actin; OPN, osteopontin.

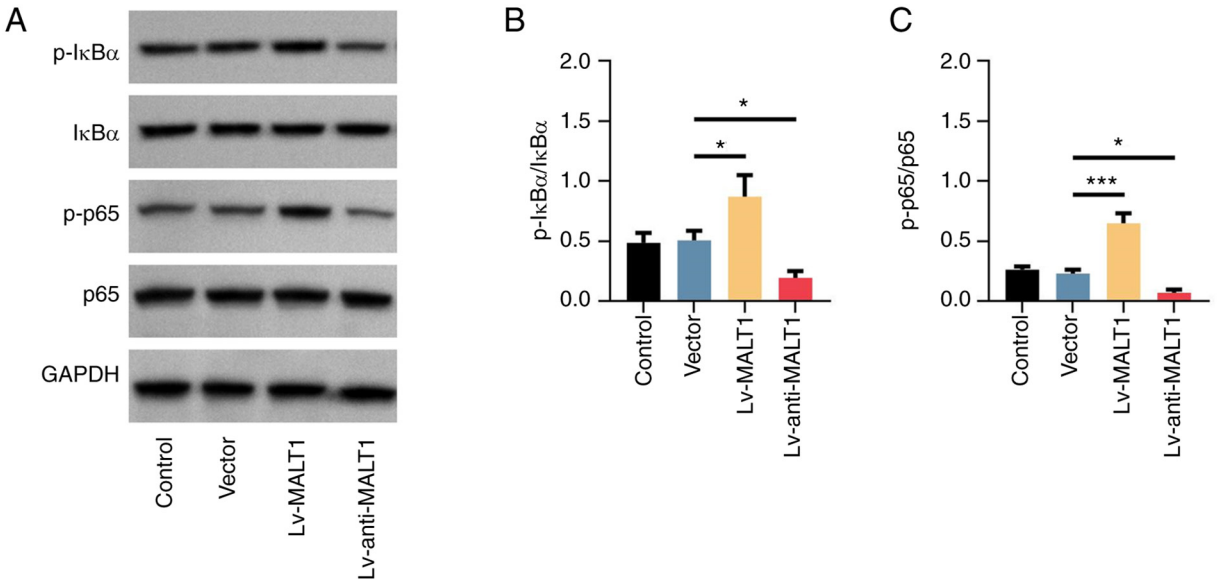

Figure 5. Effect of MALT1 mucosa-associated lymphoid tissue lymphoma translocation protein 1 on nuclear factor-κB signaling in proatherogenic VSMCs. VSMCs were first transfected with MALT1 overexpression or knockdown lentiviral plasmids and were then treated with 100 μg/ml oxidized low-density lipoprotein for 24 h. (A) The protein expression levels of p-IκBα and p-p65 in different groups of transfected VSMCs. The expression levels of (B) p-IκBα and (C) p-p65 were compared among different groups. *P<0.05 and ***P<0.001. MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; p-, phosphorylated.

factors, hyperlipidemia is considered to be the most critical one (21). It has been reported that oxLDL, one of the major members of lipidemia, is a main culprit of atherosclerosis and is involved in the formation, progression and rupture of atherosclerotic lesions (22). In addition, oxLDL is widely used in preclinical studies to mimic atherosclerotic conditions (23-25). In the current study, oxLDL was also used to establish a proatherogenic VSMC model. Treatment of VSMCs with oxLDL promoted cell viability, cell invasion and synthetic phenotype and suppressed cell apoptosis in a dose-dependent manner. Additionally, in the current study, treatment of proatherogenic VSMCs with oxLDL increased

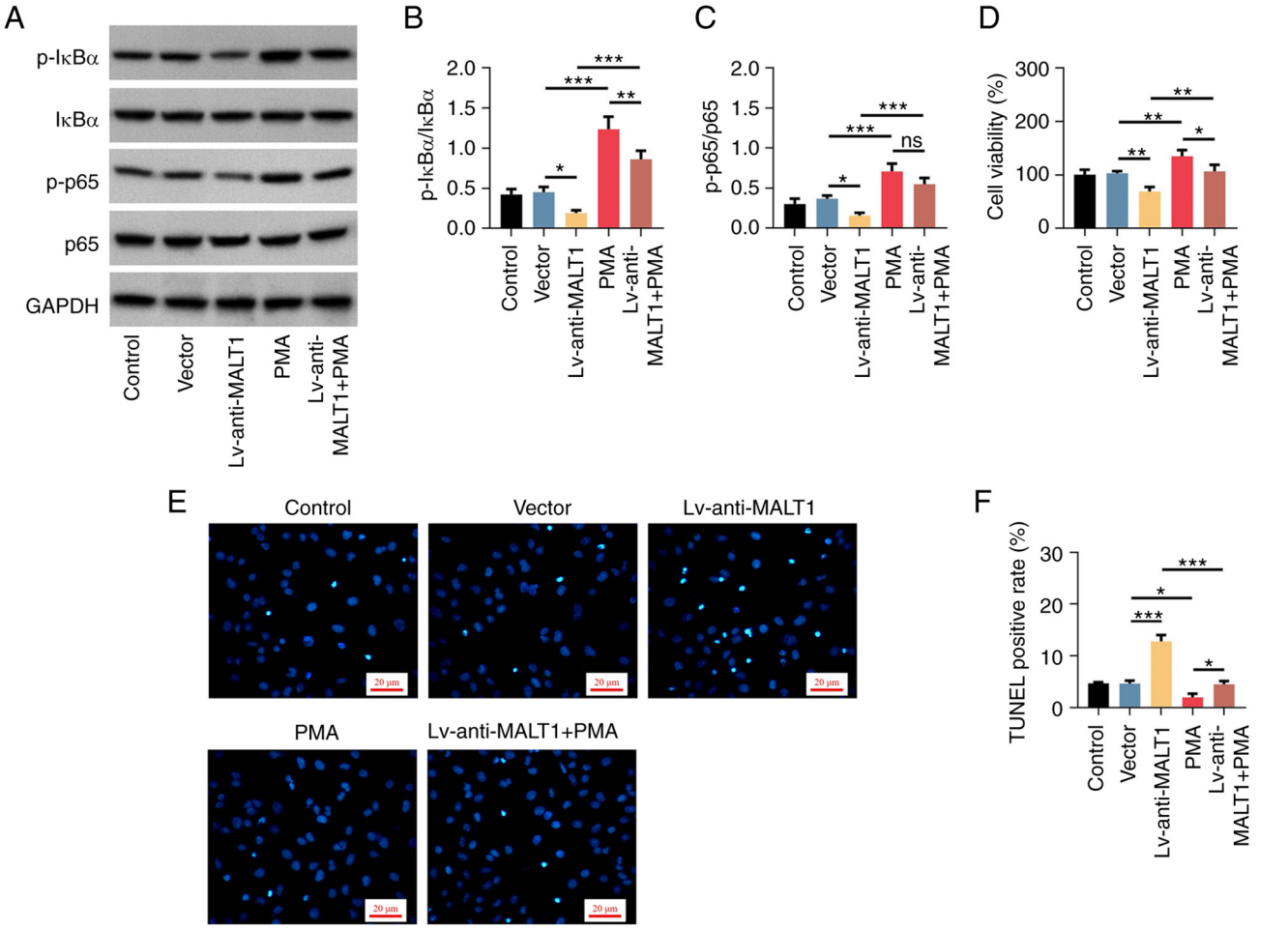

Figure 6. Effect of nuclear factor-κB activator on the MALT1 knockdown-mediated regulation of proatherogenic VSMC viability and apoptosis. VSMCs were first transfected with MALT1 knockdown lentiviral plasmids and were then treated with 100 μg/ml oxidized low-density lipoprotein for 24 h. (A) Protein expression levels of p-IκBα and p-p65 were detected in MALT1-depleted cells treated or not with PMA. The expression of (B) p-IκBα and (C) p-p65 and (D) cell viability was compared among different groups of MALT1-depleted VSMCs treated with or without PMA. (E) The apoptosis of MALT1-depleted cells treated with or without PMA was assessed using TUNEL assay. (F) The comparison of apoptosis among different groups of MALT1-depleted VSMCs treated with or without PMA. *P<0.05, **P<0.01 and ***P<0.001. ns, non-significant; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; VSMCs, vascular smooth muscle cells; p-, phosphorylated; PMA, phorbol 12-myristate 13-acetate.

the mRNA and protein expression levels of MALT1 in a dose-dependent manner. A previous study showed that MALT1 was upregulated in patients with acute ischemic stroke compared with healthy subjects (26). The above finding was partly in line with the results of the present study. Together with the previous study, these data suggested that MALT1 could be associated with atherosclerosis. This may be due to the fact that oxLDL could interact with the members of the CRAMA protein family, thus upregulating MALT1 (27).

It has been suggested that MALT1 is a potential regulator of atherosclerosis. For example, previous studies demonstrated that MALT1 could positively regulate the differentiation of T helper 17 (Th17) cells, a vital class of immune cells involved in promoting atherosclerosis progression (28,29). Additionally, MALT1 could also activate NF-κB signaling, which in turn induced inflammation to positively regulate atherosclerosis (13,30). Furthermore, another study revealed that angiotensin could not promote the development of atherosclerosis in mice deficient in CBM complex (14). However, whether MALT1 can directly regulate the dysregulation of proatherogenic VSMCs remains to be elucidated. The results of the current study showed that MALT1 overexpression enhanced the dysregulation of proatherogenic VSMCs, as supported by the increased cell proliferation, invasion and synthetic phenotype and reduced cell apoptosis. However, MALT1 knockdown exerted the opposite effects. The aforementioned findings could be due to: i) MALT1 could activate downstream signaling pathways, such as the NF-κB and Janus kinase pathways to modulate the cellular functions of proatherogenic VSMCs (13,31); ii) MALT1 could promote the pathogenesis of atherosclerosis via activating the G protein-coupled type 1 receptor for angiotensin II via the CBM complex (14); and iii) MALT1 could promote the differentiation of Th17 cells, thus inducing the secretion of interleukin-17, which in turn could further promote the pathogenesis of atherosclerosis (28,32).

The NF-κB pathway is a vital signaling pathway involved in the regulation of multiple cellular functions, such as cell survival, immune response and inflammation, thus participating in the onset of several diseases, including cancer, autoimmune diseases and center nervous

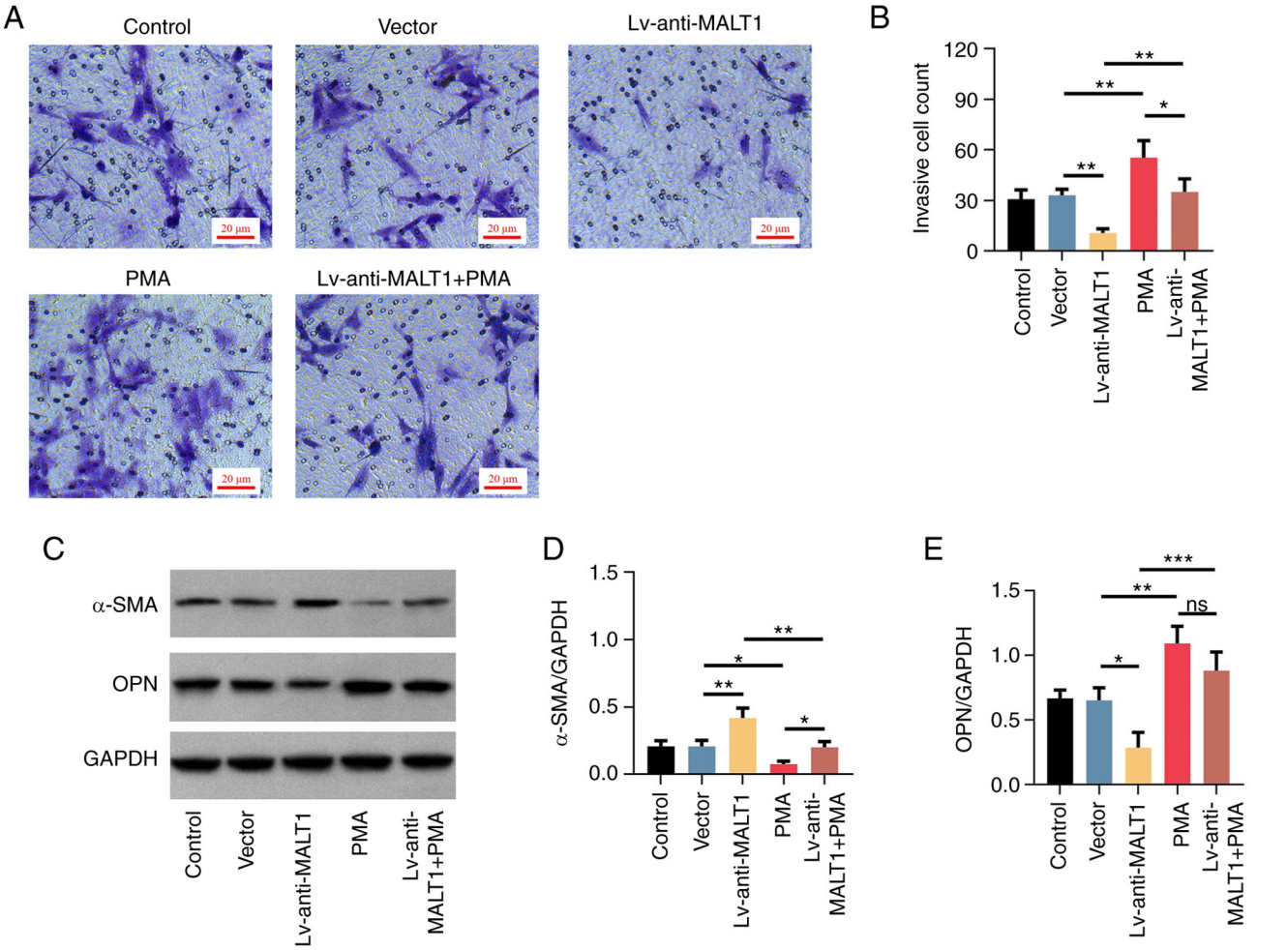

Figure 7. Effect of nuclear factor-κB activator on the MALT1 knockdown-mediated regulation of proatherogenic VSMC invasion and phenotype switching. VSMCs were first transfected with MALT1 knockdown lentiviral plasmids and were then treated with 100 μg/ml oxidized low-density lipoprotein for 24 h. (A) The cell invasion ability of MALT1-depleted cells treated with or without PMA was evaluated using crystal violet staining. (B) The comparison of cell invasion among different groups of MALT1-depleted VSMCs treated with or without PMA treatment. (C) The protein expression levels of α-SMA and OPN in different groups of MALT1-depleted cells treated with or without PMA. The comparison in the expression levels of (D) α-SMA and (E) OPN among different groups of MALT1-depleted VSMCs treated with or without PMA. *P<0.05, **P<0.01 and ***P<0.001. ns, not significant; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; VSMCs, vascular smooth muscle cells; PMA, phorbol 12-myristate 13-acetate; α-SMA, α-smooth muscle actin; OPN, osteopontin.

system diseases (33,34). Notably, it has been reported that the NF-κB pathway is critically involved in atherosclerosis (35-37) and it is the primary downstream target of MALT1 (13,14). Therefore, the current study further investigated whether the NF-κB signaling pathway was essential for the MALT1-mediated modulation of proatherogenic VSMC dysregulation. First, the results revealed that MALT1 could positively regulate the NF-κB pathway in proatherogenic VSMCs, which was in agreement with a previous study (14). Second, the data suggested that the activation of NF-κB signaling could hamper the effect of MALT1 knockdown on attenuating the dysregulation of proatherogenic VSMCs. Taken together, the above results indicated that MALT1 could exaggerate the dysregulation of proatherogenic VSMCs via activation of the NF-κB signaling pathway. It was therefore hypothesized that the high levels of MALT1 could activate the CBM complex, thus promoting the activation of the NF-κB pathway (38). Furthermore, the NF-κB pathway was involved in the functional alteration of VSMCs (39). However, the above findings should be further verified *in vivo*. Additionally, whether MALT1 could facilitate atherosclerosis via other processes, such as lipid accumulation, inflammation and foam cell formation, should be further evaluated. Cell images at a lower magnification could provide an alternative view on cell apoptosis and invasion.

Collectively, the results of the present study suggested that MALT1 could increase cell growth, invasion and synthetic phenotype switching via activating NF-κB signaling in proatherogenic VSMCs. The aforementioned findings could provide the basis for the development of MALT1-based treatment approaches for atherosclerosis. However, further validation experiments are needed.

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Availability of data and materials

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

Authors' contributions

LB contributed to the conception and design of the study. HZ contributed to data acquisition, analysis and interpretation of data. LB and HZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The use of VSMCs was approved by the Ethics Committee of Affiliated Hospital of Inner Mongolia Medical University [approval number KY (2020015)].

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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