Biochemical and Biophysical Research Communications 439 (2013) 235–240

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Apelin-13-induced proliferation and migration induced of rat vascular smooth muscle cells is mediated by the upregulation of Egr-1

Qi-Feng Liu [a], Hong-Wei Yu [b], Lu You [a], Ming-Xin Liu [a], Ke-Yan Li [a,b], Gui-Zhou Tao [a,*]

[a] Department of Cardiology, The First Affiliated Hospital of Liaoning Medical University, Jinzhou 121001, China
[b] Department of Cardiology, Jinzhou Central Hospital, Jinzhou 121001, China

ARTICLE INFO

Article history:
Received 4 August 2013
Available online 22 August 2013

Keywords:
Apelin-13
Vascular smooth muscle cells
Early growth response factor-1
Migration
Proliferation
Osteopontin

ABSTRACT

Apelin-13 plays an important role in the migration and proliferation of vascular smooth muscle cells (VSMCs); however, the underlying mechanisms are still unclear. Egr-1 is a nuclear transcription factor, which is considered to be the critical initiating factor of the processes of VSMC proliferation and migration. Egr-1 is known to regulate the expression of osteopontin (OPN), which is a marker of the phenotypic modulation that is a necessary condition of VSMC proliferation and migration. We hypothesized that the role of Apelin-13 is mediated via upregulation of Egr-1. To test this hypothesis, we analyzed the effects of Apelin-13 treatment on Egr-1 mRNA and protein expression in A10 rat aortic VSMCs by RT-PCR and Western blotting, respectively. Results showed that, Apelin-13 upregulated the expression of Egr-1. Furthermore, treatment with the extracellular-regulated protein kinase (ERK) inhibitor, PD98059, inhibited the upregulation of Egr-1 by Apelin-13. In addition, this upregulation was inhibited by treatment of VSMCs with the Egr-1 specific deoxyribozyme ED5 (DNAenzyme/10-23 DRz). Furthermore, ED5 treatment was found to significantly inhibit Apelin-13-induced migration and proliferation of VSMCs using transwell and MTT assays, respectively. The evaluation of *OPN* mRNA and protein expression levels by RT-PCR and Western blot analyses revealed that ED5 treatment also inhibited Apelin-13-induced OPN upregulation. The results of this study indicated that Apelin-13 upregulates Egr-1 via ERK. Furthermore, Apelin-13 induced the proliferation and migration of VSMCs as well as the upregulation of OPN via the upregulation of Egr-1. These results will provide an important theoretical and experimental basis for the control of inappropriate remodeling of vessel walls, and will hopefully lead to the prevention and treatment of vascular remodeling diseases.

1. Introduction

Vascular remodeling diseases, including atherosclerosis, restenosis (RS) following reconstructive vascular surgery and hypertension, have become an increasingly serious threat to human health. After much investigation, a common pathophysiological process has been implicated in the pathogenesis of vascular disease: vascular remodeling. To date, studies have demonstrated that migration and proliferation of the vascular smooth muscle cells (VSMCs) in the vascular tunica media constitute at least part of the pathological basis of vascular remodeling [1–3].

The G protein-coupled receptor endogenous ligand Apelin plays an important role in the cardiovascular system [4,5]. Many Apelin subtypes have been identified, of which Apelin-13 and Apelin-36 are the major forms. Apelin-13 has been shown to mediate the migration and proliferation of VSMCs, although its mechanism is still not clear.

Early growth response factor-1 (Egr-1) is an immediate-early gene and zinc finger transcription factor that regulates the expression of multiple proliferation-associated genes [6]. The expression level of Egr-1 correlates closely with cell proliferation [7,8].

Although Apelin-13 and Egr-1 both play significant roles in the migration and proliferation of VSMCs, relatively little is known about the relationship between these two factors. VSMCs are divided into contraction and synthetic subtypes and transformation from the contraction phenotype to the synthetic phenotypic is a necessary condition of VSMC proliferation and migration. The secreted glycoprotein osteopontin (OPN) is a marker of VSMC phenotypic modulation. Synthetic OPN expression is high in smooth muscle cells. Apelin-13 has been reported to promote synthetic transformation of VSMC resulting in upregulation of OPN expression. Our previous experiments showed that Egr-1 regulates OPN expression directly via binding to the OPN promoter and also indirectly through upregulation of TGF-β expression.

* Corresponding author. Fax: +86 244170831.
E-mail address: guizhoutao-@hotmail.com (G.-Z. Tao).

0006-291X/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbrc.2013.08.051

Therefore, we speculated that Apelin-13-induced migration and proliferation of VSMCs is mediated by upregulation of Egr-1. To test this hypothesis, we analyzed the effects of Apelin-13 treatment on Egr-1 expression in A10 rat aortic VSMCs. Furthermore, treatment with the extracellular-regulated protein kinase (ERK) inhibitor, PD98059, inhibited the upregulation of Egr-1 by Apelin-13. In addition, Apelin-13-induced migration and proliferation of VSMC as well as OPN expression were monitored following treatment of VSMCs with the Egr-1 specific deoxyribozyme (DNAenzyme/10-23 DRz, ED5). It is hoped that elucidation of the mechanism underlying Apelin-13-induced VSMC proliferation and migration and the role of Egr-1 in these processes will lead to the prevention and treatment of vascular remodeling diseases.

2. Materials and methods

2.1. Cell culture, vectors, and transfection

Rat A10 aortic VSMCs were purchased from the ATCC (Rockville, MD, USA). VSMCs were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS).

The DNA enzyme, ED5, was synthesized by TaKaRa Biotechnology Co. (Japan). The sequence was as follows: 5′-CCGCTGCCA GGCTAGCTACAA CGACCGGACGT-3′. The catalytic domain is underlined. The 5′ and 3′ termini of the oligonucleotides were protected from exonuclease activity by a phosphorothioate linkage.

The VSMCs were cultured until they reached 80% confluence before treatment with 0.1 μmol/L ED5 for 2 h. VSMCs were then cultured in the presence of 2 μmol/L Apelin-13 for 4 h. Egr-1 and OPN expression was analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot.

2.2. RT-PCR

Total RNA was extracted from VSMCs using TRIzol Reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. RT-PCR analysis was performed using a RNA PCR Kit (AMV) Ver.3.0 (TaKaRa Dalian, China) according to the manufacturer's instructions. The following PCR primers were used:

Egr-1, sense: 5′-CAGTCGTAGTGACCACCTTACCA-3′; antisense: 5′-AGGTTGCTGTCATGTCTGAAAGAC-3′.

Opn, sense: 5′-GCTGAAGCCTGACCCATCT-3′; antisense: 5′-TCCC GTTGCTGTCCTGAT-3′.

GAPDH, sense: 5′-GACTGACAGCCCCAGAGTGT-3′; antisense: 5′-GACCAGCTTCCCATTCTCAG-3′.

PCR conditions were as follows: 94 °C for 4 min; 94 °C for 1 min, annealing at 57 °C (*Egr-1*), 54 °C (*Opn*), or 60 °C (GAPDH) for 30 s, 72 °C for 30 s, 35 cycles; 72 °C for 10 min. Products were resolved by 1% agarose gel electrophoresis, and bands were visualized by ethidium bromide staining. Densitometric analysis of bands was performed using BioImaging Systems (UVP, CA, USA).

2.3. Western blot analysis

Protein lysates (80 μg) from cells were resolved by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to PVDF membranes (Sigma, Aldrich-Inc. MO, USA). Membranes were blocked with TBST buffer containing 5% skimmed milk and then incubated with primary antibodies (anti-Egr-1 (1:300), anti-OPN (1:500), anti-ERK (1:200), anti-PERK (1:200), or α-tubulin (1:500), Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4 °C. Samples were then incubated with a HRP-IgG secondary antibody, and enhanced chemiluminescence (ECL) was used to visualize the bands (Thermo Fisher Scientific Inc., Fremont, CA, USA). Band quantification was performed using Quantity One (Bio-Rad, Hercules, CA, USA). When required, blots were stripped using an antibody stripping solution (KeyGen, Nanjing, China) and reprobed with additional antibodies. Experiments were performed in triplicate.

2.4. MTT assay

VSMC proliferation was assayed using a colorimetric (MTT) assay kit (Amersco, Solon, OH, USA). Approximately 5000 cells/well were plated into a 96-well plate and incubated with Apelin-13 (2 μM) or ED5 (0.1 μM) for 24 h. At 4 h before the end of the incubation, 100 μg of MTT was added to each well. MTT was removed and the cells were solubilized with 150 μl dimethyl sulfoxide (DMSO). The OD at 490 nm (OD_{490}) was determined for each well using a microculture plate reader (Bio-Rad, CA, USA). MTT assays were performed in triplicate.

2.5. Transwell assay

Transwell inserts with an 8.0 μm pore size (Corning Inc., NY, USA) were used for cell invasion assays. Briefly, 100 μl of a cell suspension (5×10^5 cells/ml) was added to the upper chamber, and 600 μl medium containing 10% FBS was added to the lower chamber as the chemoattractant. After incubation for 24 h, the filters were fixed with 100% methanol for 15 min and subjected to hematoxylin staining. Quantification of the number of migrated cells was counted in 10 random high-power fields (at 200× magnification) using an inverted microscope. The experiment was repeated three times, and the data are shown as the mean ± standard deviation (SD).

2.6. Statistical analyses

All values are expressed as mean ± SD. SPSS11.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The data were analyzed using one-way analysis of variance (ANOVA) followed by a least significant difference test (LSD) for multiple comparisons. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Apelin-13 upregulates Egr-1 in VSMCs

Egr-1 expression was analyzed in rat A10 aortic VSMCs cultured in the presence of Apelin-13 (2 μmol/L for 4 h) to investigate the hypothesis that Apelin-13-induced migration and proliferation of VSMCs is mediated by upregulation of Egr-1. Our results showed that Egr-1 was significantly upregulated in VSMCs cultured in the presence of Apelin-13 at both the mRNA and protein levels (Fig. 1; $^{**}P < 0.01$ vs. control group).

3.2. Apelin-13 upregulates Egr-1 expression via the ERK pathway

Reports have shown that Apelin-13 promotes the proliferation of VSMC principally through the PKC–ERK signaling pathway [9] and Erg-1 is activated by ERK protein kinase. Therefore, we hypothesized that Apelin-13 upregulates Egr-1 in VSMCs. PD98059 was added to VSMCs in order to clarify the mechanism of how Apelin-13-mediated influences Egr-1 expression (Fig. 2). The results showed that Egr-1 expression was reduced in PD98059-treated cells compared to untreated cells ($P < 0.01$). This demonstrates that PD98059 can effectively block the Apelin-13-mediated upregulation of Egr-1 expression and that Apelin-13 acts through the ERK pathway to upregulate Egr-1.

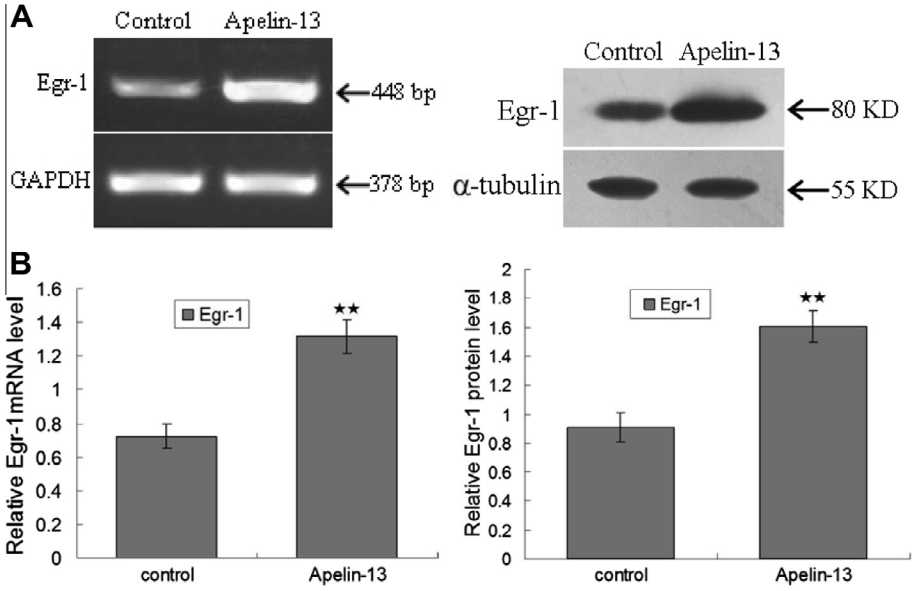

Fig. 1. Effects of Apelin-13 on Egr-1 expression in VSMCs. (A) Under control conditions, low Egr-1 mRNA and Egr-1 protein expression was detected in VSMCs by RT-PCR and Western blot analyses, respectively. (B) Quantification of the Egr-1 mRNA and protein expression was upregulated in VSMCs treated with Apelin-13 compared to control cells. The experiments were repeated at least three times. The results are represented as mean ± SD of three independent experiments. $^{\star\star}P < 0.01$ vs. control group.

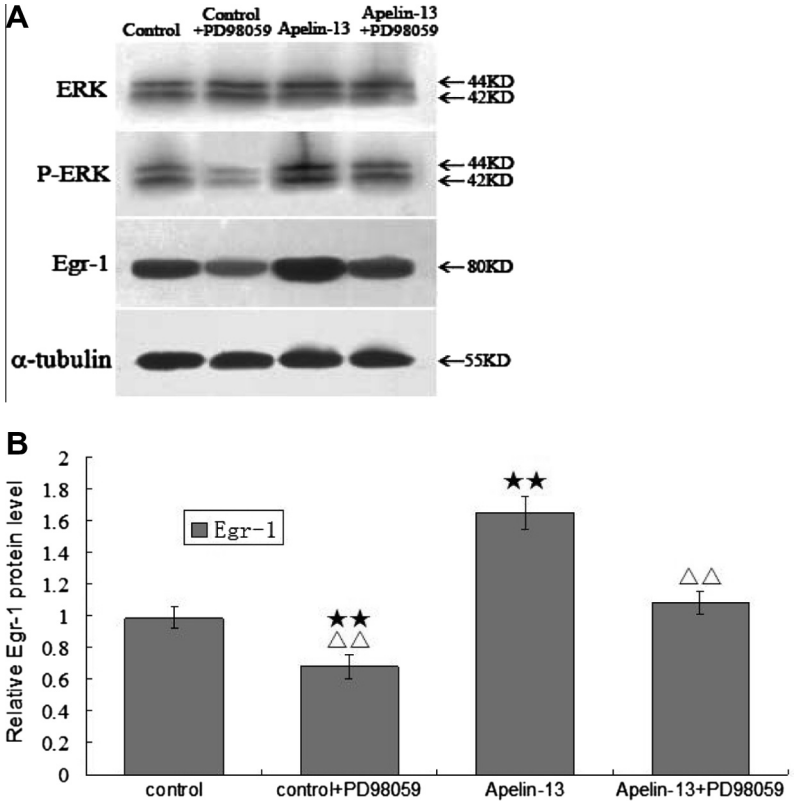

Fig. 2. The effects of PD98059 on Egr-1 expression in Apelin-13-treated VSMCs. (A) Western blot analysis to determine the effect of PD98059 on Egr-1 expression in Apelin-13-treated VSMCs. α-Tubulin served as the loading control. (B) Quantification of the results indicated that the Egr-1 levels were decreased in Apelin-13-treated and control VSMCs treated with PD98059. These experiments were repeated at least three times. The results are represented as the mean ± SD of three independent experiments. $^{\star\star}P < 0.01$ vs. control group; $^{\Delta\Delta}P < 0.01$ vs. Apelin-13-treated group.

3.3. Effects of Egr-1 on Apelin-13-induced VSMC proliferation

The effects of Egr-1 on Apelin-13-induced VSMC proliferation were analyzed in MTT assays (Table 1). Apelin-13-treated VSMCs exhibited significantly higher levels of cell proliferation relative to control VSMCs ($P < 0.01$). Cell proliferation was inhibited significantly in both Apelin-13-treated and control VSMCs treated with ED5 ($P < 0.01$), thus indicating that Apelin-13-induced VSMC proliferation is mediated via Egr-1 upregulation.

3.4. Effects of ED5 on Apelin-13-induced VSMC migration

Transwell assays were carried out to determine the role of Egr-1 on Apelin-13-induced VSMC migration (Fig. 3). Apelin-13-treated

Table 1
Effect of Egr-1 on VSMCs proliferation mediated by Apelin-13 in VSMCs determined in MTT assays.

	Control	Apelin-13	Control + ED5	Apelin-13 + ED5
A_{490}	0.225 ± 0.049	0.447 ± 0.063[a]	0.142 ± 0.040[a,b]	0.258 ± 0.045[b]

[a] $P < 0.01$ vs. control group.
[b] $P < 0.01$ vs. Apelin-13-treated group.

cells exhibited a significantly higher migration than control cells ($P < 0.01$). Furthermore, both Apelin-13-treated and control VSMCs treated with ED5 exhibited a significant decrease in migration relative to untreated cells ($P < 0.01$), thus indicating that Apelin-13-induced VSMC migration is mediated through Egr-1.

3.5. Apelin-13 upregulated OPN expression through Egr-1

Our previous experiments demonstrated that Egr-1 regulates OPN expression directly via binding to the OPN promoter and also indirectly through up regulation of TGF-β expression. Therefore, the role of OPN in the effects of Apelin-13 on VSMCs observed in this study was investigated by RT-PCR and Western blot analysis of OPN mRNA and protein expression, respectively (Fig. 4). Expression of OPN was significantly higher in Apelin-13-treated VSMC compared with control cells at both the mRNA and protein levels ($P < 0.01$), thus indicating that Apelin-13 upregulated OPN expression. Furthermore, a significant decrease in OPN expression was observed at both the mRNA and protein levels in both Apelin-13-treated and control VSMCs following treatment with ED5 compared with untreated cells ($P < 0.01$).

4. Discussion

With an overall increase in social and economic development, aging populations, and widespread dietary changes, vascular disease has become an increasingly serious threat to human health. After much study, vascular remodeling has been identified as a common pathophysiological process implicated in vascular disease. Currently, many studies have demonstrated that migration and proliferation of VSMCs in the vascular tunica media form the pathological basis for vascular remodeling.

Apelin is the endogenous ligand for the G-protein-coupled APJ receptor and plays an important role in regulating the cardiovascular system. Generally, Apelin, which is released from endothelial cells, regulates vascular tone and myocardial contractility in VSMC and cardiomyocytes. The function of Apelin is mediated via its interaction with the APJ receptor to perform a critical role in lowering blood pressure, protecting myocardial injury, improving heart function, promoting the formation of new blood vessels and regulating water and salt metabolism [10–13]. Multiple isoforms of Apelin have been identified including Apelin-36 and Apelin-13, which is the major isoform. It has recently been reported that Apelin-13 promotes rat VSMC proliferation and migration via the PKC–ERK and PI3 K/Akt signaling pathways [14,15], although the identity of the transcription factor that is activated in this pathway is not yet clear.

The Egr-1 transcription factor is expressed at very low levels, if at all, in normal unperturbed endothelium. However, Egr-1 expression is rapidly upregulated following mechanical injury to induce VSMC division, proliferation and intimal hyperplasia. Studies have shown that Egr-1 mediates VSMC migration and proliferation. In a previous study, we synthesized a specific DRz targeting rat Egr-1 mRNA (ED5). Deoxyribozymes (DRz), or DNA enzymes, are small single-stranded DNA fragments with enzymatic activity that cleave specific RNA strands, thereby regulating protein expression. Transfection of ED5 into the rat carotid artery following balloon injury resulted in the inhibition of VMSC migration and proliferation. Furthermore, significant inhibition of Egr-1 RNA and protein, as well as a significant reduction in neointimal hyperplasia was observed. It was speculated that these effects are mediated by

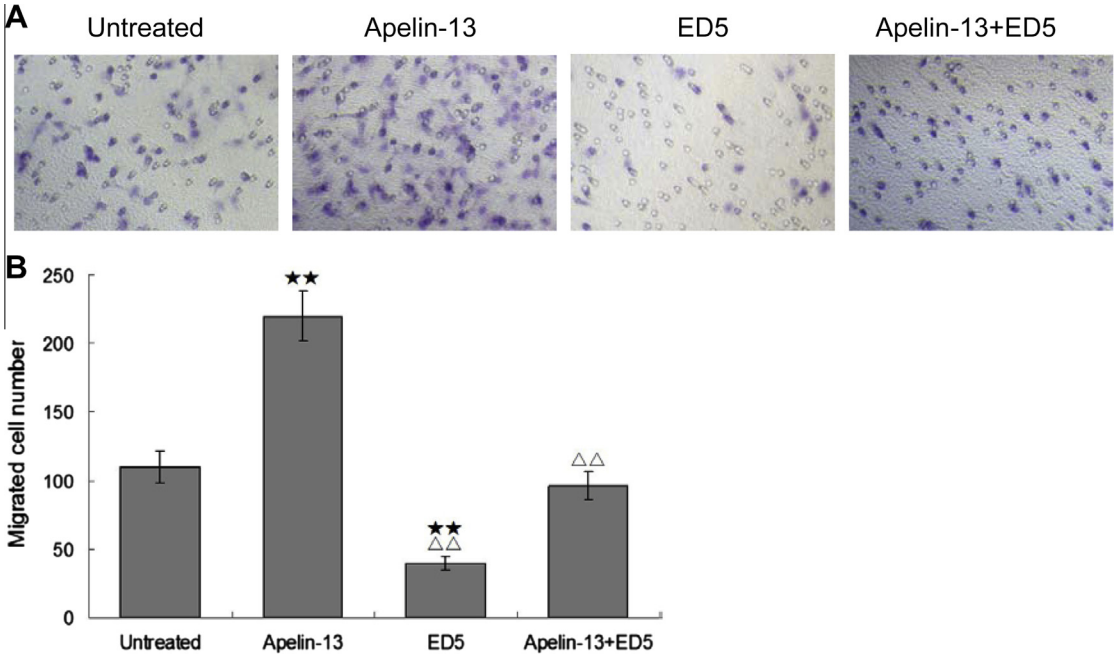

Fig. 3. Effects of ED5 on the migration of Apelin-13-induced VSMCs. (A) Representative hematoxylin-stained cell images from transwell assays to determine the effects of ED5 on migration of and Apelin-13-treated and control VSMCs. (B) Quantification of the number of migrated cells showed that Apelin-13-treated VSMCs exhibited significantly greater migration potential than control VSMCs. The migration potential of Apelin-13-treated and control cells decreased significantly following ED5 treatment. These experiments were repeated at least three times. The results are represented as the mean ± SD of three independent experiments. ★★$P < 0.01$ vs. control group; $^{\Delta\Delta}P < 0.01$ vs. Apelin-13-treated group.

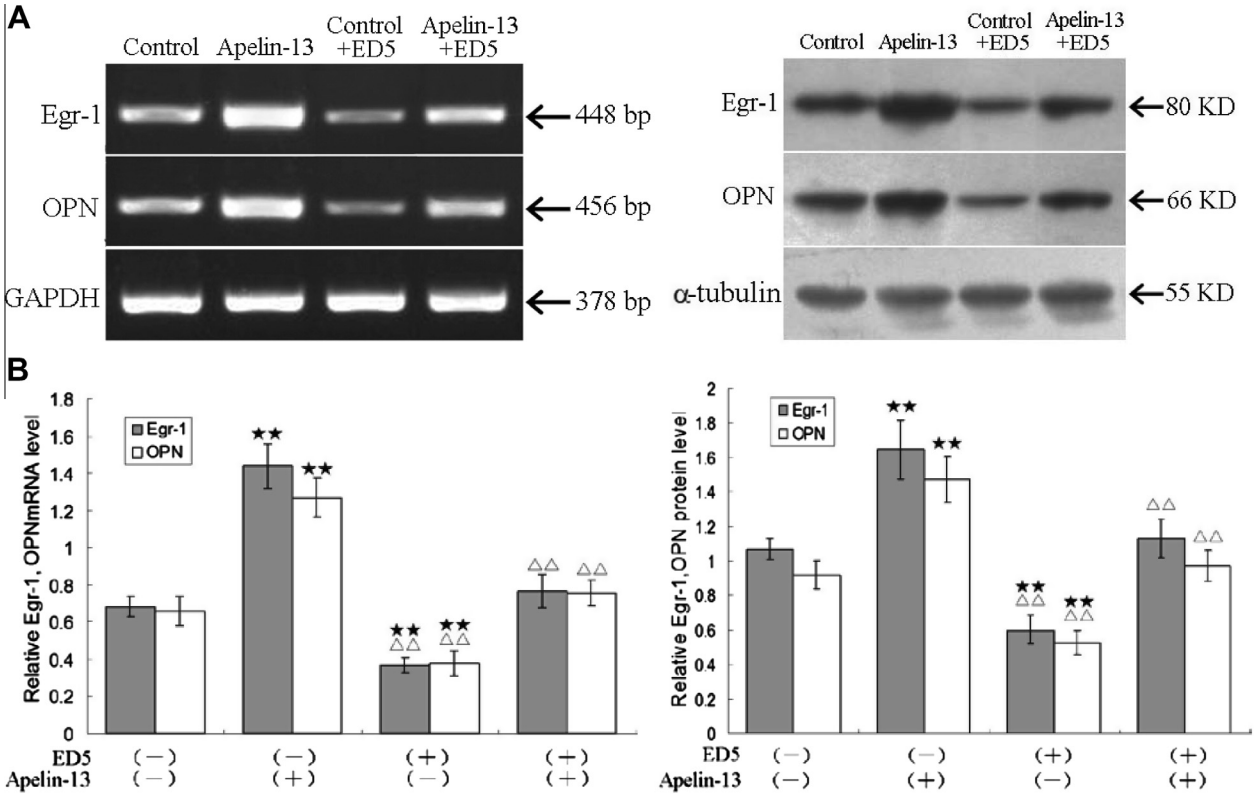

Fig. 4. Effects of ED5 on OPN expression in Apelin-13-treated VSMCs. (A) Western blot analysis to determine the effect of ED5 on OPN expression in Apelin-13-treated VSMCs. α-tubulin served as a loading control. (B) Quantification of the results indicated that the levels of Egr-1 were decreased in Apelin-13-treated and control VSMCs treated with ED5. Furthermore, OPN expression was reduced in both Apelin-13-treated and control VSMCs following ED5 treatment. These experiments were repeated at least three times. The results are represented as the mean ± SD of three independent experiments. $^{\star\star}P < 0.01$ vs. control group; $^{\Delta\Delta}P < 0.01$ vs. Apelin-13-treated group.

downregulated expression of transforming growth factor-β1 (TGF-β1), and inhibition of the phenotypic change of VSMCs, thus improving vascular endothelial function [16,17].

Although Apelin-13 and Egr-1 both play significant roles in mediating the processes of VSMC migration and proliferation, relatively little is known about the relationship between these two factors. Our own and previous studies indicate that Apelin-13 upregulates-Egr-1.

In this study, Egr-1 expression was analyzed in rat A10 aortic VSMCs cultured in the presence of Apelin-13 to investigate whether Apelin-13 upregulates the expression of Egr-1. We demonstrated that both Egr-1 mRNA and protein were more highly expressed in VSMCs that were cultured with Apelin-13 compared to untreated control cells, indicating that Apelin-13 upregulates the expression of Egr-1.

Based on our above results, we plan to clarify the mechanism of Apelin-13-mediated changes in Egr-1 expression. Apelin-13 promotes the proliferation and migration of rat VSMCs, although the mechanism of receptor signaling and intracellular secondary messenger induction has not yet been elucidated. Reports have shown that Apelin-13 promotes VSMC proliferation mainly through the PKC–ERK signaling pathway and Erg-1 is activated by ERK protein kinase. Therefore, we hypothesized that Apelin-13 upregulates Egr-1 via the ERK pathway in VSMCs. D98059 was added to VSMCs in order to clarify the mechanism by which Apelin-13 mediates changes in Egr-1 expression. The results showed that Egr-1 expression was reduced in PD98059-treated cells compared to untreated cells. This demonstrates that PD98059 can effectively block the Apelin-13-mediated upregulation of Egr-1 expression and Apelin-13 acts via the ERK pathway to upregulate Egr-1.

Apelin-13 promotes the proliferation and migration of rat VSMC; therefore, we hypothesized that this effect is mediated via upregulation of Egr-1. We carried out MTT assays to measure VSMC proliferation in the presence of Egr-1 inhibitors and found that Apelin-13-treated VSMCs exhibited higher levels of cell proliferation relative to control VSMCs. Furthermore, ED-5 treatment significantly inhibited cell proliferation both in Apelin-13-treated and control VSMCs. At the same time, transwell assays were carried out to determine the role of Egr-1 on VSMC migration mediated by Apelin-13. The results of these experiments showed that Apelin-13-treated cells exhibited a significantly higher migration potential than control cells. Furthermore, a significant decrease in migration relative to untreated cells was observed in both Apelin-13-treated and control VSMCs treated with ED5. The results of these experiments suggested that Apelin-13-induces proliferation and migration of VSMCs through upregulation of Egr-1.

Phenotypic modulation of VSMCs from the contraction phenotype to the synthetic phenotypic is a necessary condition of vascular smooth muscle cell proliferation and migration. OPN is a marker of this transformation and synthetic OPN expression is high in smooth muscle cells. According to reports in the literature, Apelin-13 promotes the transformation of VSMC to the synthetic phenotype and is likely to upregulate OPN expression. Our previous experiments showed that Egr-1 regulates OPN expression directly via binding to the OPN promoter and also indirectly through upregulation of TGF-β expression [18,19]. Therefore, we hypothesized that Apelin-13 upregulates OPN expression through Egr-1. Our experiments demonstrated that OPN expression was significantly greater at both the mRNA and protein levels in Apelin-13 treated VSMCs compared to control cells, thus indicating that

Apelin-13 upregulates OPN expression. Furthermore, a significant decrease in OPN expression was observed at both the mRNA and protein levels in Apelin-13-treated and control VSMCs following ED5 treatment. The results of these experiments suggest that Apelin-13 upregulates OPN expression through Egr-1.

In summary, our results indicate that the Apelin-13-induced proliferation and migration induced of rat vascular smooth muscle cells is mediated by the upregulation of Egr-1. Furthermore, we note that the effects of Apelin-13 on rat VSMCs were not completely abrogated by ED5. This implies that other signaling molecules/pathways are involved in this process, which future studies will investigate. We hope that an increased understanding of the molecular mechanisms of the action of Apelin-13 on rat VSMCs will provide important insights into the inappropriate remodeling of vessel walls. This may in turn lead to new approaches in the prevention and treatment of vascular remodeling diseases.

Acknowledgments

We would like to thank the native English speaking scientists of Elixigen Company for editing our manuscript. This work was supported by a grant from Chinese National Natural Science Foundation (No. 30871074).

References

[1] G. Li, S.J. Chen, S. Oparil, Y.F. Chen, J.A. Thompson, Direct in vivo evidence demonstrating neointimal migration of adventitial fibroblasts after balloon injury of rat carotid arteries, Circulation 101 (2000) 1362–1365.
[2] R.S. Schwartz, T.D. Henry, Pathophysiology of coronary artery restenosis, Rev. Cardiovasc. Med. 3 (2002) 4–9.
[3] Y. Xu, H. Arai, T. Murayama, T. Kita, M. Yokode, Hypercholesterolemia contributes to the development of atherosclerosis and vascular remodeling by recruiting bone marrow-derived cells in cuff-induced vascular injury, Biochem. Biophys. Res. Commun. 363 (2007) 782–787.
[4] G. Barnes, A.G. Japp, D.E. Newby, Translational promise of the apelin-APJ system, Heart 10 (2010) 11–16.
[5] H. Fukushima, N. Kobayashi, H. Takeshima, W. Koguchi, T. Ishimitsu, Effects of olmesartan on Apelin/APJ and Akt/endothelial nitric oxide synthase pathway in Dahl rats with end-stage heart failure, J. Cardiovasc. Pharmacol. 55 (2010) 83–88.
[6] R.H. Luan, G.L. Jia, W. Li, Z.S. Jia, J.Q. Lian, Inhibition of vascular smooth muscle cell proliferation by specific c-myc mRNA cleaving hammerhead ribozyme, J. Fourth Mil. Med. Univ. 24 (2003) 517–521.
[7] G. Thiel, G. Cibelli, Regulation of life and death by the zinc finger transcription factor Egr-1, J. Cell Physiol. 193 (2002) 287–292.
[8] L.M. Khachigian, Early growth response-1 in cardiovascular pathobiology, Circ. Res. 98 (2006) 186–191.
[9] X.H. Mao, L.F. Li, J. Gao, L. Yang, W. Liu, X.P. Qin, L.X. Chen, Apelin-13 promotes proliferation of vascular smooth muscle cells by downregulating caveolae expression, J. Clin. Rehabil. Tissue Eng. Res. 15 (2011) 8603–8608.
[10] M. Eyries, G. Siegfried, M. Ciumas, K. Montagne, M. Agrapart, F. Lebrin, F. Soubrier, Hypoxia-induced apelin expression regulates endothelial cell proliferation and regenerative angiogenesis, Circ. Res. 103 (2008) 432–440.
[11] T. Zhao, Q. Lu, Y. Tao, X.Y. Liang, K. Wang, Y.R. Jiang, Effects of apelin and vascular endothelial growth factor on central retinal vein occlusion in monkey eyes intravitreally injected with bevacizumab: a preliminary study, Mol. Vis. 17 (2011) 1044–1055.
[12] S. Sakimoto, H. Kidoya, H. Naito, M. Kamei, H. Sakaguchi, N. Goda, A. Fukamizu, K. Nishida, N. Takakura, A role for endothelial cells in promoting the maturation of astrocytes through the apelin/APJ system in mice, Development 139 (2012) 1327–1335.
[13] Q. Lu, Y.R. Jiang, J. Qian, Y. Tao, Apelin-13 regulates proliferation, migration and survival of retinal Müller cells under hypoxia, Diabetes Res. Clin. Pract. 99 (2013) 158–167.
[14] P.F. Shan, Y. Lu, R.R. Cui, Y. Jiang, L.Q. Yuan, E.Y. Liao, Apelin attenuates the osteoblastic differentiation of vascular smooth muscle cells, PLoS One 6 (2011) e17938.
[15] C. Liu, T. Su, F. Li, L. Li, X. Qin, W. Pan, F. Feng, F. Chen, D. Liao, L. Chen, PI3K/Akt signaling transduction pathway is involved in rat vascular smooth muscle cell proliferation induced by apelin-13, Acta Biochim. Biophys. Sin. 42 (2010) 396–402.
[16] G.N. Liu, Y.X. Teng, W. Yan, Transfected synthetic DNA enzyme gene specifically inhibits Egr-1 gene expression and reduces neointimal hyperplasia following balloon injury in rats, Int. J. Cardiol. 129 (2008) 118–124.
[17] Y. Wu, W. Han, G.N. Liu, A DNA enzyme targeting Egr-1 inhibits rat vascular smooth muscle cell proliferation by down-regulation of cyclin D1 and TGF-beta1, Braz. J. Med. Biol. Res. 43 (2010) 17–24.
[18] H.W. Yu, Q.F. Liu, G.N. Liu, Positive regulation of the Egr-1/ osteopontin positive feedback loop in rat vascular smooth muscle cells by TGF-β, ERK, JNK, and p38 MAPK signaling, Biochem. Biophys. Res. Commun. 396 (2010) 451–456.
[19] H.W. Yu, Q.F. Liu, G.N. Liu, Egr-1 upregulates OPN through direct binding to its promoter and OPN upregulates Egr-1 via the ERK pathway, Mol. Cell. Biochem. 332 (2009) 77–84.