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Localization and function of KLF4 in cytoplasm of vascular smooth muscle cell

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ABSTRACT

The Krüppel-like factor 4 is a DNA-binding transcriptional regulator that regulates a diverse array of cellular processes, including development, differentiation, proliferation, and apoptosis. The previous studies about KLF4 functions mainly focused on its role as a transcription factor, its functions in the cytoplasm are still unknown. In this study, we found that PDGF-BB could prompt the translocation of KLF4 to the cytoplasm through CRM1-mediated nuclear export pathway in vascular smooth muscle cells (VSMCs) and increased the interaction of KLF4 with actin in the cytoplasm. Further study showed that both KLF4 phosphorylation and SUMOylation induced by PDGF-BB participates in regulation of cytoskeletal organization by stabilizing the actin cytoskeleton in VSMCs. In conclusion, these results identify that KLF4 participates in the cytoskeletal organization by stabilizing cytoskeleton in the cytoplasm of VSMCs.

1. Introduction

The phenotypic modulation of vascular smooth muscle cells (VSMCs) is known to be critical in blood vessel formation during embryogenesis and in pathological states such as atherogenesis, restenosis, and hypertension [1]. Platelet-derived growth factor (PDGF) is a kind of chemokine and mitogenic agents to stimulate VSMC phenotype modulation (transforming from contractile to synthetic phenotype) [2]. It is well known that VSMC phenotype modulation accompanied by reorganization of cytoskeleton, wherein cytoskeletal reorganization is mainly reflected in the actin filament depolymerization and polymerization [3,4]. Recent researches reported that many transcriptional factors, such as SRF and MRTFA/B could modulate the cytoskeletal organization and participate in VSMC phenotype remodeling [5–7].

The Krüppel-like factors (KLFs) are DNA-binding transcriptional regulators that regulate a diverse array of cellular processes, including development, differentiation, proliferation, and apoptosis [8,9]. As one of the KLFs, KLF4 mainly distributes in the nucleus and induces VSMC differentiation by activating VSMC contraction-associated genes, including SM α-actin and SM22α [10,11]. We and others have demonstrated that PDGF-BB affects VSMC phenotype by inducing the expression of KLF4 [12–14]. However, the influence of PDGF-BB on VSMC cytoskeletal organization as well as molecular mechanisms whereby KLF4 regulates cytoskeletal organization is still unclear at present. In addition, the previous studies about KLF4 functions mainly focused on its role as a transcription factor, its functions in the cytoplasm are also unknown. In this study, we observed the distribution of KLF4 in the cytoplasm and investigated its role in regulating the cytoskeleton organization.

2. Methods

The present study was approved by the Institutional Animal Care and Use Committee of Hebei Medical University (Shijiazhuang, China). The study protocol conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication number 85-23, revised 1996).

2.1. Cell culture and treatment

VSMCs were isolated from the thoracic aorta of male Sprague–Dawley rats (90–100 g) as described previously [15]. VSMCs were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) with 10% fetal bovine serum (FBS) and maintained in 5% CO_2 at 37 °C in a humidified atmosphere. For PDGF-BB treatment, VSMCs were growth-arrested by incubation in serum-free DMEM for 24 h and then treated with different concentration of PDGF-BB (R&D Systems) for the stated periods.

2.2. Western blotting

Proteins were isolated from VSMCs as described previously [16], then separated on sodium dodecyl sulfate–polyacrylamide

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gel electrophoresis (SDS–PAGE), and transferred onto polyvinylidene difluoride membrane (Millipore). Membranes were blocked with 5% milk in Tris–HCl Tween 20 buffer for 2 h at 37 °C and incubated overnight with specific antibodies. After incubation with appropriate secondary antibody, the membranes were developed with the Chemiluminescence Plus Western blot analysis kit (Santa Cruz Biotechnology).

2.3. Fluorescence staining

Fluorescence staining was performed as described previously [17]. The cells were stained for 20 min with TRITC/phalloidin (1 μg/mL) in blocking solution (1% BSA and 0.1% Triton X-100 in NaCl/Pi) in the dark at room temperature to localize F-actin.

2.4. GST pull-down assay

In order to produce GST fusion proteins, full-length and transcriptional activation domain-related regions of KLF4 were generated in a pGEX-3X vector inframe with the N-terminal GST tag. All new constructs were confirmed by restriction digestion followed by sequencing. Protein expression was induced by reaction with 0.2 mM isopropyl thio-β-D-galactoside at 30 °C for 3 h. Bacterial lysates were purified over glutathione-agarose. For the pull-down assay, actin was incubated with GST Sepharose beads or with KLF4/GST Sepharose beads. After centrifugation, the pellets were washed, and the interacting proteins were separated by SDS /PAGE and identified by western blotting with an anti-actin Ig [16,18].

2.5. Luciferase assay

293A cells were purchased from ATCC (Manassas, VA, USA) and maintained in high-glucose DMEM supplemented with 10% FBS. Briefly, cells were seeded in a 24-well plate and grown for 24 h before transfection with pEGFP-KLF4 and pEGFP-KLF4mut plasmids. Cells were transfected using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Cells were then harvested and luciferase activities were measured using a Dual Luciferase Assay Kit (Promega). SM22α promoter activity was expressed as the relative activity ratio of firefly luciferase to Renilla luciferase [19]. All promoter constructs were evaluated in a minimum of three separate wells per experiment.

3. Results

3.1. PDGF-BB treatment results in translocation of KLF4 to the cytoplasm through CRM1-mediated nuclear export pathway in VSMCs

We first observed the subcellular localization of KLF4 and the effect of PDGF-BB on its subcellular localization in VSMCs by immunostaining for KLF4. As shown in Fig. 1A, KLF4 was predominantly localized to the nucleus of VSMCs in the absence of serum and translocated to the cytoplasm in response to PDGF-BB stimulation in a time-dependent manner. Further VSMCs treated by PDGF-BB for different times were fractionated into nuclear and cytoplasmic proteins, and KLF4 in each fraction was detected by western blotting. Results showed that KLF4 in the cytoplasm gradually increased within 2 h after PDGF-BB treatment, whereas, in contrast, nuclear KLF4 decreased relatively during this time course (Fig. 1B).

To determine the involvement of the nuclear export factor CRM1 in the nuclear export of KLF4 induced by PDGF-BB in VSMCs, the effect of leptomycin B (LMB), an inhibitor of the CRM1 pathway, on the nuclear export of KLF4 was investigated. As shown in Fig. 1C, LMB treatment significantly inhibited the translocation of KLF4 to the cytoplasm induced by PDGF-BB. Consistent with localization of KLF4 by immunofluorescence, Western blot analyses revealed that LMB treatment markedly reduced cytoplasmic KLF4 exported in response to PDGF-BB (Fig. 1D, lane 2 versus lane 4). By contrast, in VSMCs treated with LMB, KLF4 in the nuclear extracts relatively increased. These results suggest that PDGF-BB promotes KLF4 translocation from the nucleus to the cytoplasm, and that CRM1 pathway mediates nuclear export of KLF4 induced by PDGF-BB.

3.2. PDGF-BB stimulates the interaction of KLF4 with actin in the cytoplasm

To examine whether KLF4 can interact directly with actin, double-label indirect immunofluorescence assay was performed to compare the subcellular distributions of KLF4 (green) and actin (red) in VSMCs treated with or without PDGF-BB (Fig. 2A). By this approach, we found that KLF4 and actin were extensively colocalized in cells along the actin stress fibers (Fig. 2A) after treatment with PDGF-BB. To further examine the interaction between KLF4 and actin, GST pull-down assay was used. A KLF4 construct containing the full-length KLF4 was expressed as a GST fusion protein. Equivalent amounts of the GST-KLF4 fusion protein or GST protein bound to glutathione matrices were incubated with lysates from VSMCs treated with or without PDGF-BB. The matrix-bound GST fusion proteins were examined by Western blotting for their ability to bind actin in these lysates. As shown in Fig. 2B, the GST-KLF4 could pull down the endogenous actin, whereas GST alone was unable to interact with actin, and PDGF-BB prompted this interaction (data not shown). These morphological and biochemical results strongly identified that PDGF-BB induces the nuclear export of KLF4 and enhances the interaction of KLF4 with actin in the cytoplasm. In order to detect the structural basis of KLF4 interaction with actin, different truncated KLF4 expression plasmids pGEX-4T-KLF4-2 (1–117aa), pGEX-4T-KLF4-3 (1–181aa) and pGEX-4T-KLF4-4 (1–371aa) were constructed. GST pull down assay showed that there was a interaction between GST-KLF4-3 and actin, suggesting that 117–181 amino acid residues of KLF4 were the structural domain of the interaction between actin and KLF4 (Fig. 2C).

3.3. KLF4 participates in the organization of actin cytoskeleton

Because we have found that KLF4 interacted with actin, we sought to determine whether KLF4 interaction with actin could affect the organization of actin cytoskeleton. To test this, we transfected VSMCs with pEGFP or pEGFP-KLF4 and analyzed the formation of stress fibers by immunofluorescence analysis. As shown in Fig. 3A, overexpression of KLF4 induced the clustering of the myofilaments, the actin filaments were recruited into thick and long actin bundles in KLF4-overexpressed VSMCs, suggesting that KLF4 promotes the stress fiber formation. PDGF-BB treatment decreased the formation of stress fibers (Suppl. Fig. 1A). To further evaluate the role of KLF4 in actin cytoskeleton remodeling, the micromyogenic event was studied in the presence of cytochalasin B (CB), an actin filament depolymerizing agent, or jasplakinolide (JPK), an actin filament stabilizing agent. As shown in Suppl. Fig. 1B, after treatment of VSMCs with cytochalasin B for 60 min, actin bundling and stress fibers have almost disappeared, indicating that actin cytoskeleton is depolymerized. Overexpression of KLF4 partly abolished the effect of cytochalasin B on the cytoskeleton. In contrast, after the application of JPK, the micromyogenic event was augmented. However, overexpression of KLF4 did not further increase the formation of stress fibers (Suppl. Fig. 1C). These results suggest that KLF4 stabilizes the actin cytoskeleton by interacting with actin in VSMCs.

Because KLF4 stabilizes the cytoskeleton, we wanted to detect whether the cytoskeleton remodeling affects the subcellular

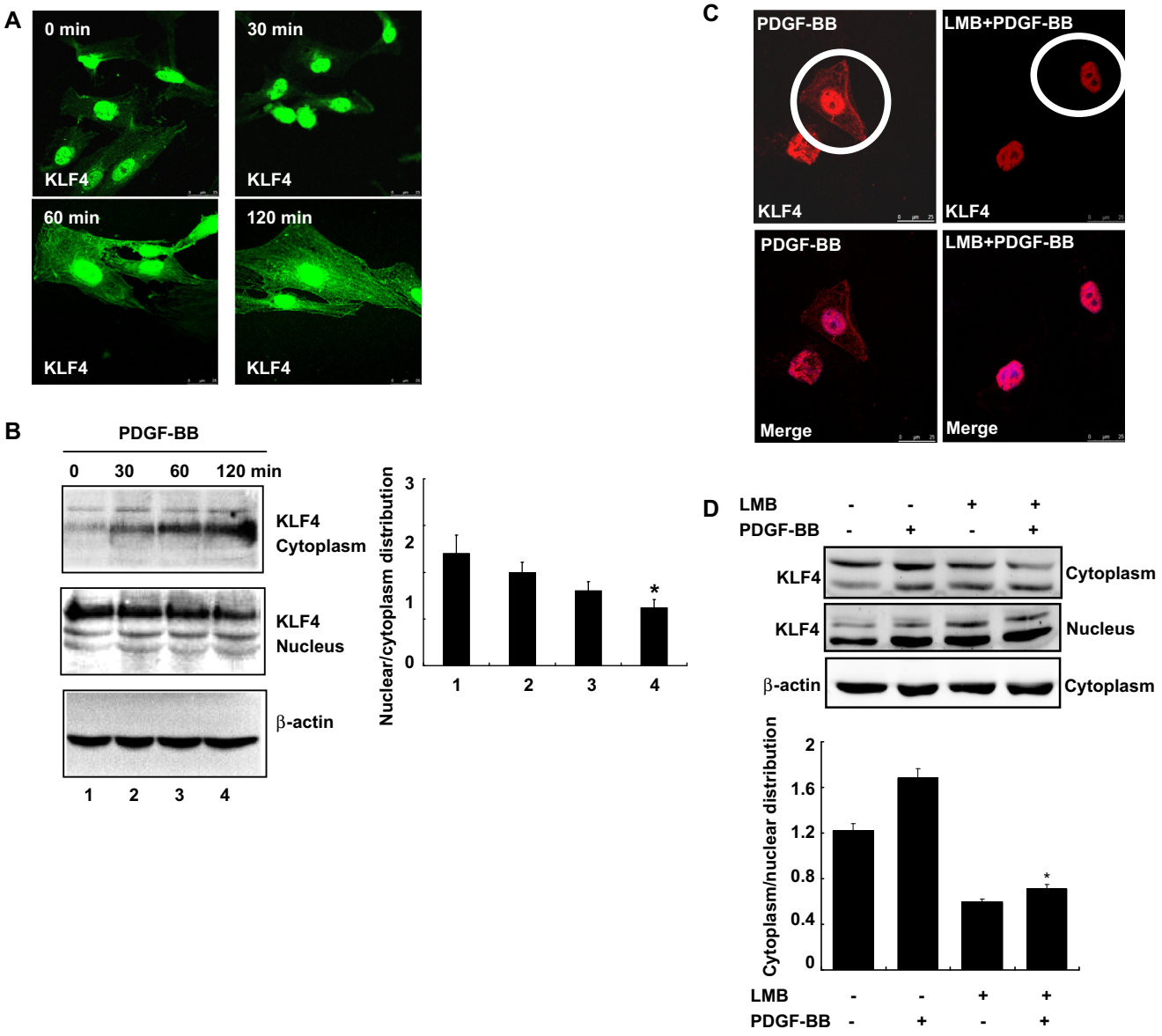

Fig. 1. PDGF-BB promotes the nuclear export of KLF4 in a CRM1-dependent manner. (A) VSMCs were treated with PDGF-BB (20 ng/ml) for the indicated times. Cells were then fixed, and direct fluorescence labeling with an anti-KLF4 antibody was carried out. Scale bar, 50 μm. (B) VSMCs were treated with PDGF-BB (20 ng/ml) for the indicated times. The cytoplasmic and nuclear extracts were collected, respectively. Equal amount of proteins were separated by SDS–PAGE and analyzed by Western blot analysis to examine the subcellular distribution of KLF4. A representative result from three independent experiments was shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus the control group (0 min). (C) VSMCs were pretreated with LMB for 6 h and then treated with PDGF-BB for 2 h. The cells were fixed and stained by immunofluorescence. The distribution of KLF4 and the co-localization of KLF4 and actin were observed by laser scanning confocal microscope. (D) The proteins in cytoplasm and nucleus were collected, respectively. Equal amount of proteins were separated by SDS–PAGE and analyzed by Western blot analysis to examine the subcellular distribution of KLF4. A representative result from three independent experiments was shown (upper panel), whereas band intensities that were measured are shown on the lower panel. $^{*}p < 0.05$ versus the control group.

distribution of KLF4. To this aim, KLF4 distribution in the cytoplasm and the nucleus was detected by Western blotting after VSMCs were treated with cytochalasin B or JPK. As shown in Fig. 3A, actin polymerization induced by JPK increased the cytoplasmic of KLF4 2-fold over that seen with the control group, whereas cytochalasin B treatment reduced the distribution of KLF4 in the cytoplasm compared with JPK-treated cells. By contrast, cytoskeleton depolymerization induced by cytochalasin B increased the distribution of KLF4 in the nucleus. Immunofluorescence analysis showed that cytochalasin B treatment led to cytoskeleton depolymerization, myofilament dispersion, and increase of KLF4 level in the nucleus, and there was no significant fluorescence overlap between TRITC labeled-KLF4 and FITC labeled-actin; JPK treatment increased formation of stress fibers and actin bundles, and prompted co-localization of KLF4 and actin (Fig. 3B). These results indicated that KLF4 participates in organization of actin cytoskeleton by interacting with actin in the cytoplasm.

3.4. Both KLF4 phosphorylation and SUMOylation induced by PDGF-BB are responsible for organization of actin cytoskeleton

Our previous study has shown that KLF4 could be SUMOylated and phosphorylated by PDGF-BB [20]. In order to determine the relationship between KLF4 modification and actin cytoskeleton organization, phosphorylation-deficient mutant expression vectors for KLF4, pEGFP-KLF4 S251A and pEGFP-KLF4 S255A, and SUMOylation-deficient mutant expression vector pEGFP-KLF4 K225/229R

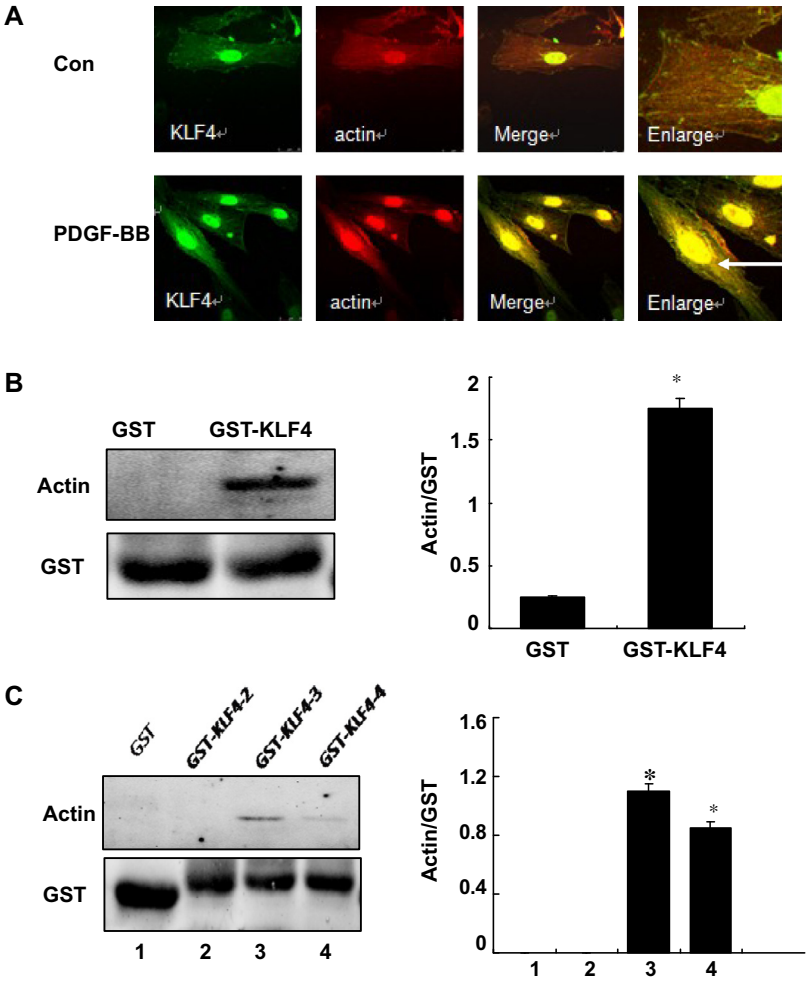

Fig. 2. PDGF-BB increases the interaction between KLF4 and actin in the cytoplasm. (A) VSMCs were treated with PDGF-BB (20 ng/ml) for 2 h. Cells were then fixed and stained with anti-KLF4 antibody or anti-actin antibody and DAPI for nuclei. Subcellular distribution of endogenous KLF4 (green), actin (red) was shown in the panels. Arrows indicate co-localization of KLF4 with actin fibers (yellow staining in the enlarged panels). Scale bar, 50 μm. (B) Purified recombinant GST or GST-KLF4 fusion protein coupled to glutathione-Sepharose were analyzed by SDS–PAGE and immunoblotted using anti-actin (upper) or anti-GST (lower) antibody. A representative result from three independent experiments was shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus the GST group. (C) Purified recombinant GST, GST-KLF4-2, GST-KLF4-3, or GST-KLF4-4 fusion protein coupled to glutathione-Sepharose were incubated with muscle actin. After extensive washing, Sepharose beads were analyzed by SDS–PAGE and immunoblotted using anti-actin (upper) or anti-GST (lower) antibody. A representative result from three independent experiments was shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus the GST group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were used to transfect VSMCs, followed by PDGF-BB stimulation, to observe the impact of KLF4 phosphorylation and SUMOylation on cytoskeleton organization. Immunofluoresence staining showed that the cytoskeleton of VSMCs transfected with S251A or S255A mutant became stronger and thicker, aggregated towards the cell edge, and that cellular morphology was altered by these phosphorylation-deficient mutants (Suppl. Fig. 2). Upon PDGF-BB stimulation, the cells became smaller, and the stress fiber bundles decreased. Accordingly, in VSMCs transfected with SUMOylation-deficient mutant, although the stress fibers also became stronger and thicker, it arranged turbidly and lost its bunchy arrangement (Suppl. Fig. 2). These results indicated that both the KLF4 phosphorylation and SUMOylation induced by PDGF-BB are responsible for the organization of actin cytoskeleton.

3.5. KLF4 plays a role in cytoskeletal organization through promoting expression of contraction-associated genes

As we all know, KLF4 plays a role in inhibiting cell proliferation and inducing cell differentiation through stimulating expression of contraction-associated genes (such as SM α-actin and SM 22α). KLF4 contains three domains: DNA binding domain, transcriptional activation domain and nuclear localization sequence, wherein a transcriptional activation domain exists in the 91–117 amino acid residues on the amino terminal end of KLF4. Whether KLF4-induced cytoskeletal organization is related with its activation on contraction-associated gene expression? We constructed deletion mutant of KLF4 transcriptional activation domain and observed its influence on SM22α promoter activity through reporter gene analysis. First we validated that the KLF4 mutant lost its ability to activate SM22α promoter activity (Fig. 4A). To further confirm whether the KLF4 mutant still plays a role in cytoskeletal organization, actin filaments were detected by phalloidin staining. The results showed that the cytoskeletal myofilament became diffuse after VSMCs were transfected with KLF4 deletion mutant, indicating that KLF4 plays a role in cytoskeletal organization through promoting expression of contraction-associated genes (Fig. 4B).

4. Discussion

KLF4, as a multifunctional transcription factor, is mainly distributed in the nucleus[12,21,22]. Our studies found that PDGF-BB can

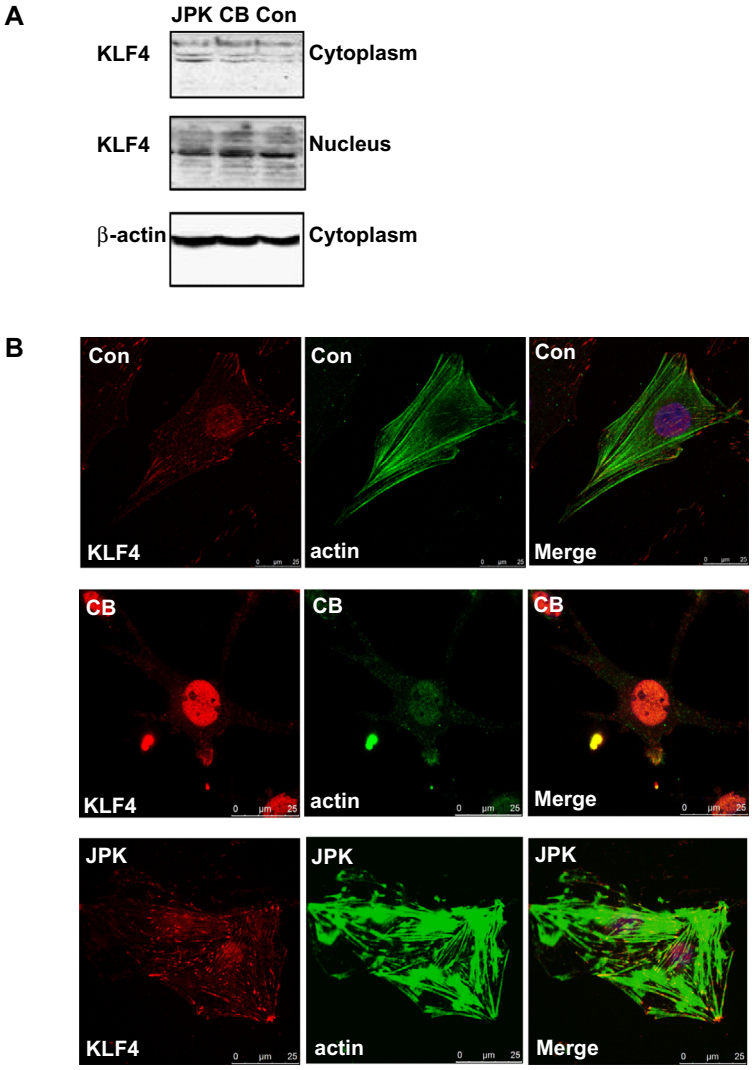

Fig. 3. Cytoskeletal reorganization affects KLF4 interaction with actin in the cytoplasm. (A) VSMCs were treated with JPK (50 nm/L) or CB (50 nm/L) and lysed, and the proteins of cytoplasm and nucleus were separated and analyzed by Western blot analysis to examine the subcellular distribution of KLF4 using anti-KLF4 antiboby. (B) VSMCs were treated with CB and JPK for 2 h,and then the cells were fixed and stained by immunofluorescence.The distribution of KLF4 and the co-localization of KLF4 and actin were observed by laser scanning confocal microscope. Scale bar, 50 μm.

promote KLF4 nuclear export, which is distributed in the cytoplasm (Fig. 1A and B). Whether and how KLF4 functions within the cytoplasm is unknown. Pandya et al. found that although KLF4 could exist in the cytoplasm, localization of KLF4 in the nucleus of breast cancer cells is a prognostic factor [23]. Wamhoff et al. also found that KLF4 localized to both cytoplasmic and nuclear compartments of SMCs undergoing phenotypic modulation [24]. But the function of KLF4 in the cytoplasm is still unclear.

CRM1 pathway is an important way of nuclear export of transcription factor [25], we used LMB, an inhibitor of CRM1, to pretreat VSMCs to verify whether translocates to the cytoplasm through CRM1 pathway. Western blotting and immunofluorescence results showed that PDGF-BB promoted KLF4 translocation from the nucleus to cytoplasm, and that CRM1 pathway mediated nuclear export of KLF4 induced PDGF-BB (Fig. 1C). To further examine the function of KLF4 in the cytoplasm, GST pull-down assays and confocal assay were used. These morphological and biochemical results strongly identified that PDGF-BB induced the nuclear export of KLF4 increased the interaction of KLF4 with actin in the cytoplasm (Fig. 2A–C). In order to detect the structural basis of KLF4 interaction with actin, different truncated KLF4 expression plasmids were constructed and identified that 117–181 amino acid residues of KLF4 were the structural domain of the interaction between actin and KLF4 (Fig. 2C).

The actin cytoskeleton, long thought to be a static scaffold for the maintenance of cell shape, polarity, and mechanical support, undergoes dynamic remodeling involving scores of proteins that regulate the cytoskeleton [26]. It is well known that except for cytoskeleton factors, many transcriptional factors participate in the regulation of the cytoskeleton. Miano et al. found that SRF is a dispensable transcription factor for cellular growth but an absolutely essential orchestrator of actin cytoskeleton and contractile homeostasis [27,28]. Ly et al. also showed that myocardin-related transcription factor (MRTF), an actin-regulated coactivator of serum response factor, is a major link between the actin skeleton and transcriptional control [29]. Morita et al. reported that MRTF-A/B act as pivotal mediators of stress fiber and focal adhesion formation via the transcriptional regulation of a subset of cytoskeletal/focal adhesion genes [30,31]. We therefore asked whether KLF4 exhibits localization to the cytoplasm in synthetic VSMCs participates in cytoskeleton remodeling during VSMC phenotype modulation. As shown in Suppl. Fig. 1 and Fig. 3, PDGF–BB could promote KLF4 localization to the cytoskeleton and participation in cytoskeleton organization by stabilizating F-actin stress fibers.

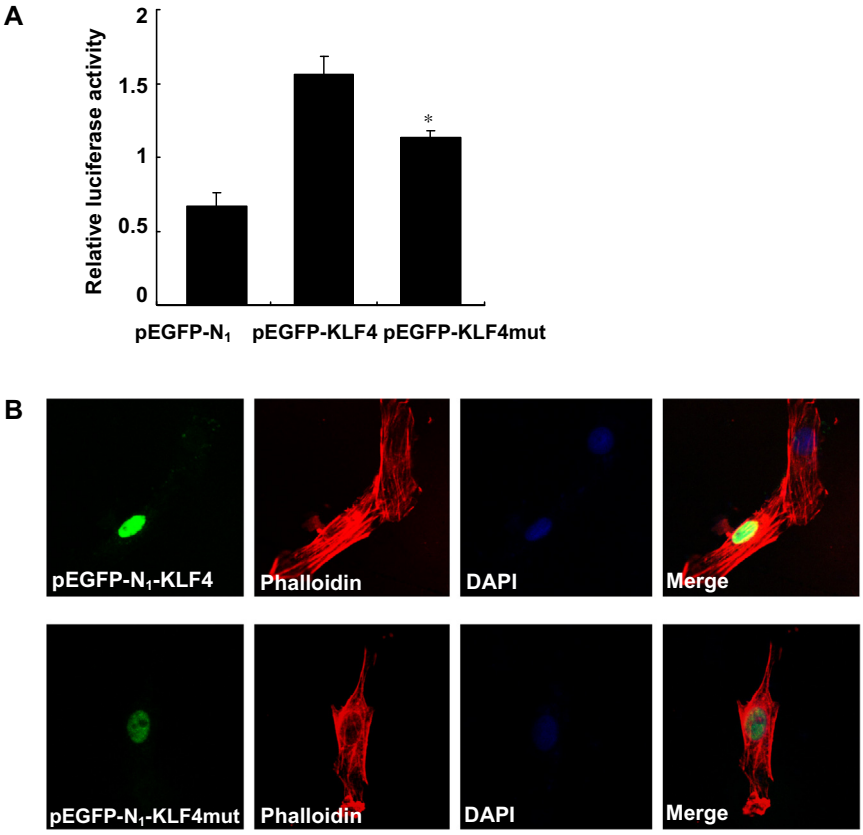

Fig. 4. VSMC contraction-associated genes activated by KLF4 play an important role in cytoskeletal organization. (A) A293 cells were transiently cotransfected with the SM22α luciferase reporter plasmid together with either pEGFP empty vector, pEGFP-KLF4 or pEGFP-KLF4mut. 48 h later, the cells were harvested, and the activity of firefly luciferase was measured and normalized to that of Renilla luciferase. The bars represent the means ± S.E. from three independent experiments. $^{*}p < 0.05$ versus pEGFP-KLF4 group. (B) VSMCs were transfected with pEGFP-KLF4, pEGFP-KLF4mut. F-actin stress fibers were stained with rhodamine-phalloidin. The myofilament changes in the cytoskeleton were detected by laser scanning confocal microscope. Scale bar, 50 μm.

Many researchers reported that KLF4 can be modified, such as phosphorylation [12], acetylation [32–34], SUMOylation [35,36], and ubiquitylation [36,37] by different treatment. Our research found that Sp1 is dissociated from the KLF4-Sp1 complex through PKC-δ-mediated KLF4 phosphorylation at Thr401 by TGF-β treatment [15]. Kim et al. identified that ERK1 and ERK2 regulate embryonic stem cell self-renewal through phosphorylation of KLF4 [38]. Kawai-Kowase et al. found that PIAS1 induces SUMOylation of KLF4 followed by ubiquitin-dependent degradation [36]. We identified that all-trans retinoic acid increases KLF4 acetylation by inducing histone de-acetylase 2 (HDAC2) phosphorylation [32]. Salmon et al. demonstrated that pELK-1–KLF4 complex could recruit HDACs to SM marker gene and mediate epigenetic silencing [33]. All these data showed that different modifications of KLF4 had different functions. In order to determine the relationship between KLF4 modification and cytoskeleton remodeling, phosphorylation- and SUMOylation-deficient mutant expression vectors for KLF4 were used to transfect VSMCs to observe the impact of KLF4 phosphorylation and SUMOylation on cytoskeleton remodeling. Both the KLF4 phosphorylation and SUMOylation induced by PDGF-BB are responsible for the regulation of cytoskeleton remodeling (Suppl. Fig. 2), suggesting that the modification of KLF4 participates in the regulation of cytoskeleton organization in VSMCs.

KLF4 plays an important role in inhibiting cell proliferation and inducing differentiation through stimulating expression of contraction-associated genes (such as SM α-actin and SM 22α) [39,40]. KLF4 mainly contains three domains: DNA binding domain, transcriptional activation domain and nuclear localization sequence, wherein a transcriptional activation domain with high degeneration exists in the 91–117 amino acid residues of KLF4 amino terminal end, it is the main structural features that make KLF4 play transcriptional activation role [10,41]. To identify whether KLF4-induced cytoskeletal changes have relationship with its activation on contraction-associated gene expression or not, we constructed deletion mutants of KLF4 transcriptional activation domain and observed its influence on cell cytoskeleton. First, we validated that KLF4 mutants deleted for transcriptional activation domain lost the ability activate SM22α promoter activity by reporter gene assay. Second, we found that the myofilament of cytoskeleton became diffuse after VSMCs were transfected with the KLF4 deletion mutants, thereby losing its function in promoting filament bundles compared with wild-type KLF4, which indicates that KLF4 plays a role in cytoskeleton remodeling through promoting expression of contraction-associated genes (Fig. 4B).

This is the first report that PDGF-BB can promote interaction between KLF4 and actin in the cytoplasm. Our results confirm that KLF4 plays an important role in cytoskeleton remodeling through promoting the expression of contraction-associated genes, such as SM22α. This finding is beneficial for deeply understanding the role of KLF4 in cardiovascular disease, and provides a new study basis for seeking the treatment methods of vascular remodeling.

Author contributions

Liu Y, Zheng B, Zhang XH, Nie CQ, Li YH carried out the experiments., Wen JK and Zheng B reviewed the data and helped in the design and preparation of the manuscript.

Disclosures

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.05.067.

References

[1] R. Hutcheson, R. Terry, J. Chaplin, E. Smith, A. Musiyenko, J.C. Russell, T. Lincoln, P. Rocic, MicroRNA-145 restores contractile vascular smooth muscle phenotype and coronary collateral growth in the metabolic syndrome, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 727–736.
[2] E.A. Zemskov, I. Mikhailenko, E.P. Smith, A.M. Belkin, Tissue transglutaminase promotes PDGF/PDGFR-mediated signaling and responses in vascular smooth muscle cells, J. Cell. Physiol. 227 (2012) 2089–2096.
[3] U. Hedin, J. Thyberg, J. Roy, A. Dumitrescu, P.K. Tran, Role of tyrosine kinases in extracellular matrix-mediated modulation of arterial smooth muscle cell phenotype, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 1977–1984.
[4] M. Han, L.H. Dong, B. Zheng, J.H. Shi, J.K. Wen, Y. Cheng, Smooth muscle 22 alpha maintains the differentiated phenotype of vascular smooth muscle cells by inducing filamentous actin bundling, Life Sci. 84 (2009) 394–401.
[5] H. Doi, T. Iso, M. Yamazaki, H. Akiyama, H. Kanai, H. Sato, K. Kawai-Kowase, T. Tanaka, T. Maeno, E. Okamoto, M. Arai, L. Kedes, M. Kurabayashi, HERP1 inhibits myocardin-induced vascular smooth muscle cell differentiation by interfering with SRF binding to CArG box, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 2328–2334.
[6] G. Lagna, M.M. Ku, P.H. Nguyen, N.A. Neuman, B.N. Davis, A. Hata, Control of phenotypic plasticity of smooth muscle cells by bone morphogenetic protein signaling through the myocardin-related transcription factors, J .Biol. Chem. 282 (2007) 37244–37255.
[7] S. Nakamura, K. Hayashi, K. Iwasaki, T. Fujioka, H. Egusa, H. Yatani, K. Sobue, Nuclear import mechanism for myocardin family members and their correlation with vascular smooth muscle cell phenotype, J. Biol. Chem. 285 (2010) 37314–37323.
[8] Y. Yang, B.G. Goldstein, H.H. Chao, J.P. Katz, KLF4 and KLF5 regulate proliferation, apoptosis and invasion in esophageal cancer cells, Cancer Biol. Ther. 4 (2005) 1216–1221.
[9] Z. Wu, S. Wang, Role of kruppel-like transcription factors in adipogenesis, Dev. Biol. 373 (2013) 235–243.
[10] B. Zheng, M. Han, J.K. Wen, Role of Kruppel-like factor 4 in phenotypic switching and proliferation of vascular smooth muscle cells, IUBMB Life 62 (2010) 132–139.
[11] S.G. Sun, B. Zheng, M. Han, X.M. Fang, H.X. Li, S.B. Miao, M. Su, Y. Han, H.J. Shi, J.K. Wen, MiR-146a and Kruppel-like factor 4 form a feedback loop to participate in vascular smooth muscle cell proliferation, EMBO Rep. 12 (2011) 56–62.
[12] K. Yu, B. Zheng, M. Han, J.K. Wen, ATRA activates and PDGF-BB represses the SM22alpha promoter through KLF4 binding to, or dissociating from, its cis-DNA elements, Cardiovasc. Res. 90 (2011) 464–474.
[13] R.A. Deaton, Q. Gan, G.K. Owens, Sp1-dependent activation of KLF4 is required for PDGF-BB-induced phenotypic modulation of smooth muscle, Am. J. Physiol. Heart Circ. Physiol. 296 (2009) H1027–1037.
[14] B. Zheng, M. Han, M. Bernier, X.H. Zhang, F. Meng, S.B. Miao, M. He, X.M. Zhao, J.K. Wen, Kruppel-like factor 4 inhibits proliferation by platelet-derived growth factor receptor beta-mediated, not by retinoic acid receptor alpha-mediated, phosphatidylinositol 3-kinase and ERK signaling in vascular smooth muscle cells, J. Biol. Chem. 284 (2009) 22773–22785.
[15] X.H. Zhang, B. Zheng, C. Gu, J.R. Fu, J.K. Wen, TGF-beta1 downregulates AT1 receptor expression via PKC-delta-mediated Sp1 dissociation from KLF4 and Smad-mediated PPAR-gamma association with KLF4, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 1015–1023.
[16] B. Zheng, M. Han, Y.N. Shu, Y.J. Li, S.B. Miao, X.H. Zhang, H.J. Shi, T. Zhang, J.K. Wen, HDAC2 phosphorylation-dependent Klf5 deacetylation and RARalpha acetylation induced by RAR agonist switch the transcription regulatory programs of p21 in VSMCs, Cell Res. 21 (2011) 1487–1508.
[17] B. Zheng, J.K. Wen, M. Han, HhLIM is a novel F-actin binding protein involved in actin cytoskeleton remodeling, FEBS J. 275 (2008) 1568–1578.
[18] J.H. Shi, B. Zheng, S. Chen, G.Y. Ma, J.K. Wen, Retinoic acid receptor alpha mediates all-trans-retinoic acid-induced Klf4 gene expression by regulating Klf4 promoter activity in vascular smooth muscle cells, J. Biol. Chem. 287 (2012) 10799–10811.
[19] B. Zheng, M. Han, J.K. Wen, R. Zhang, Human heart LIM protein activates atrial-natriuretic-factor gene expression by interacting with the cardiac-restricted transcription factor Nkx2.5, Biochem. J. 409 (2008) 683–690.
[20] T. Yoshida, M. Yamashita, M. Hayashi, Kruppel-like factor 4 contributes to high phosphate-induced phenotypic switching of vascular smooth muscle cells into osteogenic cells, J. Biol. Chem. 287 (2012) 25706–25714.
[21] B.N. Davis-Dusenbery, M.C. Chan, K.E. Reno, A.S. Weisman, M.D. Layne, G. Lagna, A. Hata, Down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-beta and bone morphogenetic protein 4, J. Biol. Chem. 286 (2011) 28097–28110.
[22] H.J. Kee, J.S. Kwon, S. Shin, Y. Ahn, M.H. Jeong, H. Kook, Trichostatin A prevents neointimal hyperplasia via activation of Kruppel like factor 4, Vascul. Pharmacol. 55 (2011) 127–134.
[23] A.Y. Pandya, L.I. Talley, A.R. Frost, T.J. Fitzgerald, V. Trivedi, M. Chakravarthy, D.C. Chhieng, W.E. Grizzle, J.A. Engler, H. Krontiras, K.I. Bland, A.F. LoBuglio, S.M. Lobo-Ruppert, J.M. Ruppert, Nuclear localization of KLF4 is associated with an aggressive phenotype in early-stage breast cancer, Clin. Cancer Res. 10 (2004) 2709–2719.
[24] S.M. Garvey, D.S. Sinden, P.D. Schoppee Bortz, B.R. Wamhoff, Cyclosporine up-regulates Kruppel-like factor-4 (KLF4) in vascular smooth muscle cells and drives phenotypic modulation in vivo, J. Pharmacol. Exp. Ther. 333 (2010) 34–42.
[25] H. Inoue, M. Kauffman, S. Shacham, Y. Landesman, J. Yang, C.P. Evans, R.H. Weiss, CRM1 blockade by selective inhibitors of nuclear export attenuates kidney cancer growth, J. Urol. 189 (2012) 2317–2326.
[26] E.G. Stringham, N. Marcus-Gueret, L. Ramsay, K.L. Schmidt, Live cell imaging of the cytoskeleton, Methods Enzymol. 505 (2012) 203–217.
[27] J.M. Miano, X. Long, K. Fujiwara, Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus, Am. J. Physiol. Cell Physiol. 292 (2007) C70–81.
[28] J.M. Miano, Role of serum response factor in the pathogenesis of disease, Lab. Invest. 90 (2010) 1274–1284.
[29] D.L. Ly, F. Waheed, M. Lodyga, P. Speight, A. Masszi, H. Nakano, M. Hersom, S.F. Pedersen, K. Szaszi, A. Kapus, Hyperosmotic stress regulates the distribution and stability of myocardin-related transcription factor, a key modulator of the cytoskeleton, Am. J. Physiol. Cell Physiol. 304 (2013) C115–127.
[30] T. Morita, T. Mayanagi, K. Sobue, Reorganization of the actin cytoskeleton via transcriptional regulation of cytoskeletal/focal adhesion genes by myocardin-related transcription factors (MRTFs/MAL/MKLs), Exp. Cell Res. 313 (2007) 3432–3445.
[31] T. Morita, T. Mayanagi, K. Sobue, Dual roles of myocardin-related transcription factors in epithelial mesenchymal transition via slug induction and actin remodeling, J. Cell Biol. 179 (2007) 1027–1042.
[32] F. Meng, M. Han, B. Zheng, C. Wang, R. Zhang, X.H. Zhang, J.K. Wen, All-trans retinoic acid increases KLF4 acetylation by inducing HDAC2 phosphorylation and its dissociation from KLF4 in vascular smooth muscle cells, Biochem. Biophys. Res. Commun. 387 (2009) 13–18.
[33] M. Salmon, D. Gomez, E. Greene, L. Shankman, G.K. Owens, Cooperative binding of KLF4, pELK-1, and HDAC2 to a G/C repressor element in the SM22alpha promoter mediates transcriptional silencing during SMC phenotypic switching in vivo, Circ. Res. 111 (2012) 685–696.
[34] P.M. Evans, W. Zhang, X. Chen, J. Yang, K.K. Bhakat, C. Liu, Kruppel-like factor 4 is acetylated by p300 and regulates gene transcription via modulation of histone acetylation, J. Biol. Chem. 282 (2007) 33994–34002.
[35] S. Tahmasebi, M. Ghorbani, P. Savage, K. Yan, G. Gocevski, L. Xiao, L. You, X.J. Yang, Sumoylation of Kruppel-like factor 4 inhibits pluripotency induction but promotes adipocyte differentiation, J. Biol. Chem. 288 (2013) 12791–12804.
[36] K. Kawai-Kowase, T. Ohshima, H. Matsui, T. Tanaka, T. Shimizu, T. Iso, M. Arai, G.K. Owens, M. Kurabayashi, PIAS1 mediates TGFbeta-induced SM alpha-actin gene expression through inhibition of KLF4 function-expression by protein sumoylation, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 99–106.
[37] Y. Liu, S. Sinha, O.G. McDonald, Y. Shang, M.H. Hoofnagle, G.K. Owens, Kruppel-like factor 4 abrogates myocardin-induced activation of smooth muscle gene expression, J. Biol. Chem. 280 (2005) 9719–9727.
[38] J.H. Kim, S.W. Nam, B.W. Kim, W.J. Kim, Y.H. Choi, Astaxanthin improves the proliferative capacity as well as the osteogenic and adipogenic differentiation potential in neural stem cells, Food Chem. Toxicol. 48 (2010) 1741–1745.
[39] K.E. King, V.P. Iyemere, P.L. Weissberg, C.M. Shanahan, Kruppel-like factor 4 (KLF4/GKLF) is a target of bone morphogenetic proteins and transforming growth factor beta 1 in the regulation of vascular smooth muscle cell phenotype, J. Biol. Chem. 278 (2003) 11661–11669.
[40] S. Wassmann, K. Wassmann, A. Jung, M. Velten, P. Knuefermann, V. Petoumenos, U. Becher, C. Werner, C. Mueller, G. Nickenig, Induction of p53 by GKLF is essential for inhibition of proliferation of vascular smooth muscle cells, J. Mol. Cell. Cardiol. 43 (2007) 301–307.
[41] A. Schuetz, D. Nana, C. Rose, G. Zocher, M. Milanovic, J. Koenigsmann, R. Blasig, U. Heinemann, D. Carstanjen, The structure of the Klf4 DNA-binding domain links to self-renewal and macrophage differentiation, Cell. Mol. Life Sci. 68 (2011) 3121–3131.