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Transforming growth factor β-activated kinase 1 negatively regulates interleukin-1α-induced stromal-derived factor-1 expression in vascular smooth muscle cells

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ABSTRACT

Stromal-derived Factor-1 (SDF-1) derived from vascular smooth muscle cells (VSMCs) contributes to vascular repair and remodeling in various vascular diseases. In this study, the mechanism underlying regulation of SDF-1 expression by interleukin-1α (IL-1α) was investigated in primary rat VSMCs. We found IL-1α promotes SDF-1 expression by up-regulating CCAAT-enhancer-binding protein β (C/EBPβ) in an IκB kinase β (IKKβ) signaling-dependent manner. Moreover, IL-1α-induced expression of C/EBPβ and SDF-1 was significantly potentiated by knockdown of transforming growth factor β-activated kinase 1 (TAK1), an upstream activator of IKKβ signaling. In addition, we also demonstrated that TAK1/p38 mitogen-activated protein kinase (p38 MAPK) signaling exerted negative effect on IL-1α-induced expression of C/EBPβ and SDF-1 through counteracting ROS-dependent up-regulation of nuclear factor erythroid 2-related factor 2 (NRF2). In conclusion, TAK1 acts as an important regulator of IL-1α-induced SDF-1 expression in VSMCs, and modulating activity of TAK1 may serve as a potential strategy for modulating vascular repair and remodeling.

1. Introduction

Activation of vascular smooth muscle cells (VSMCs) contributes to the pathogeneses of multiple inflammatory vascular diseases [1]. Activated by inflammatory stimuli, VSMCs produce not only extracellular matrix, but also inflammatory modulators that orchestrate vascular repair and remodeling [2].

Among others, stromal cell-derived factor-1 (SDF-1) is a constitutively expressed chemokine markedly up-regulated in multiple vascular diseases [3,4]. Modulating recruitment and function of vascular progenitors, SDF-1 affects vascular repair and remodeling through regulating reendothelialization and neointimal hyperplasia [3,5,6]. However, it remains unclear how SDF-1 expression is regulated in VSMCs, the important sources of SDF-1 in the vessels affected [3].

Transforming growth factor β-activated kinase-1 (TAK1), a member of the mitogen-activated protein kinase kinase kinase

Abbreviations: C/EBPβ, CCAAT-enhancer-binding protein β; ERK, extracellular signal-regulated kinases; IKK, IκB kinase; IL-1, interleukin-1; IκB, inhibitor of nuclear factor-κB; JNK, c-Jun N-terminal kinase; MAP3K, mitogen-activated protein kinase kinase kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; NAC, N-acetyl-L-cysteine; NRF2, nuclear factor erythroid 2-related factor 2; SDF-1, stromal-derived factor-1; TAK1, transforming growth factor β-activated kinase 1; VSMCs, vascular smooth muscle cells.

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(MAP3K) family, acts as a key component of signaling transduction induced by multiple inflammatory factors [7], including interleukin-1 (IL-1), a prototypic inflammatory cytokine broadly involved in various inflammation-related cardiovascular disorders [8,9]. And it was demonstrated that TAK1 regulates not only expression of inflammatory mediators in VSMCs, but also progression of neointimal hyperplasia [10], a key pathological feature seen in multiple vascular diseases [1,4]. As the critical role of SDF-1 in neointimal hyperplasia is recognized [3,4], how SDF-1 expression in VSMCs is regulated by TAK1 deserves further investigation.

In this study, we demonstrated that activation of IκB kinase β (IKKβ)by IL-1α increases SDF-1 expression in rat VSMCs through up-regulating CCAAT-enhancer-binding protein β (C/EBPβ). Importantly, a negative role of TAK1 in IL-1α-induced SDF-1 expression was identified. And we found that inhibition of TAK1/p38 MAPK signaling enhances IL-1α-induced C/EBPβ and SDF-1 expression through potentiating ROS-dependent up-regulation of nuclear factor erythroid 2-related factor 2 (NRF2). These data reveal that TAK1 is an important regulator of SDF-1 expression in VSMCs, and implicate TAK1 as a potential therapeutic target for inflammation-related vascular diseases.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats were purchased from the Center of Experimental Animals (Tongji Medical College, Huazhong University of Science and Technology). Animal protocols were approved by the Animal Care and Use Committee of Tongji Medical College.

2.2. Reagents and antibodies

Medium 231 and smooth muscle growth supplement were purchased from Life Technology (Grand Island, USA). Recombinant rat IL-1α and primary antibodies against HIF-1α were purchased from R&D (Minneapolis, USA). TPCA-1, SP600125, SB239063, 5Z-7-Oxozeaenol and trigonelline were purchased from Sigma–Aldrich (St. Louis, USA). SCH772984 and PX-478 were purchased from Selleck (Houston, USA). HRP-conjugated secondary antibodies, Primary antibodies against TAK1, phospho-TAK1, phospho-IKKα/β, phospho-ERK, phospho-JNK, phospho-p38 MAPK and β-actin were purchased from Cell Signaling Technology (Danvers, USA). Primary antibodies against C/EBPβ and NRF2 were purchased from Abcam (Cambridge, UK).

2.3. Cell culture and treatment

Primary VSMCs were isolated from the aorta of 4-week-old male Sprague–Dawley rats as previously described [3]. VSMCs were subcultured in medium 231 supplemented with smooth muscle growth supplement, and cells at passages 3 to 6 were used for subsequent experiments. Before IL-1α treatment, subconfluent VSMCs were serum-starved for 48 h to achieve quiescence. Prior to treatment with IL-1α (10 ng/ml), quiescent VSMCs were pretreated with 5Z-7-Oxozeaenol (0.3 μM), TPCA-1 (10 μM), SCH772984 (5 μM), SP600125 (10 μM), SB239063 (10 μM), PX-478 (50 μM), or trigonelline (0.5 μM) for 30 min.

2.4. Lentivirus infection

The short hairpin RNA (shRNA) target sequences against rat *Map3k7* (*Tak1*) and *Cebpb* were 5′-GCCCTAGTGTCAGAATGAT-3′ and 5′-CGACTTCCTTTCCGACCTCTT-3′, respectively. A nonspecific sequence, 5′-TTCTCCGAACGTGTCACGT-3′, was used as negative control. Recombinant lentiviruses expressing corresponding shRNA were constructed as previously described [11]. VSMCs were infected with lentiviruses express corresponding shRNA at a multiplicity of infection of 100 in medium containing polybrene (5 μg/ml). Experiments were carried out on quiescent VSMCs at Day 7 after infection.

2.5. Enzyme-linked immunosorbent assay (ELISA)

ELISA kit against SDF-1 was purchased from Cloud-Clone (Houston, USA). Medium from VSMCs was analyzed following the instructions of the manufacturer. And amount of target cytokine in medium was normalized to that of total protein in corresponding cell lysate.

2.6. Quantitative real-time PCR (qRT-PCR)

Total RNA extracted from VSMCs was reverse-transcribed into complementary DNA (cDNA) using PrimeScript RT Master Mix (Takara). Subsequently, qRT-PCR was performed using the SYBR Premix Ex Taq (Takara). The mRNA levels of SDF-1 were normalized by those of β-actin and analyzed for fold change using the ΔΔCt method. Primers used were: SDF-1, 5′-GCTCTGCATCAGTGACGG-TAAG-3′ (forward) and 5′-AGGGCACAGTTTGGAGTGTTGAG-3′ (reverse); β-actin, 5′-TGCTATGTTGCCCTAGACTTCG-3′ (forward) and 5′-GTTGGCATAGAGGTCTTTACGG-3′ (reverse).

2.7. Western blotting

Equal amounts of denatured whole cell lysates of VSMCs were separated by SDS-PAGE. Proteins transferred onto PVDF membranes were probed with corresponding primary antibodies and HRP-conjugated secondary antibodies. Bands of different proteins were visualized and recorded by ChemiDoc imaging system (Bio-Rad). And β-actin served as a loading control.

2.8. Statistical analysis

Images shown are representative of at least 3 independent experiments. All data were expressed as mean ± SEM. Comparisons between three or more groups were performed with analysis of variance (ANOVA) followed by multiple comparisons test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. IL-1α induces SDF-1 expression through up-regulating C/EBPβ in an IKKβ signaling-dependent manner

We initially examined how SDF-1 transcription in rat VSMCs was affected by IL-1α. As shown in Fig. 1A, IL-1α time-dependently increased SDF-1 transcription, and SDF-1 mRNA levels in VSMCs were increased by 1-fold at 24 h. A concomitant increase in C/EBPβ, a transcription factor that regulates SDF-1 expression [12], was also detected (Fig. 1B).

Then, SDF-1 expression was investigated in VSMCs infected with lentiviruses express either non-specific or *Cebpb*-targeting shRNAs (referred as NS-KD and C/EBPβ-KD VSMCs, respectively). In C/EBPβ-KD VSMCs, where IL-1α-induced C/EBPβ up-regulation was significantly inhibited (Fig. 1C), IL-1α-induced, but no basal, SDF-1 mRNA transcription and protein production was significantly decreased (Fig. 1D), indicating that IL-1α induces SDF-1 expression in VSMCs primarily through up-regulating C/EBPβ.

Subsequently, we explored how SDF-1 expression in VSMCs is regulated by IKKβ signaling, which regulates expression of many

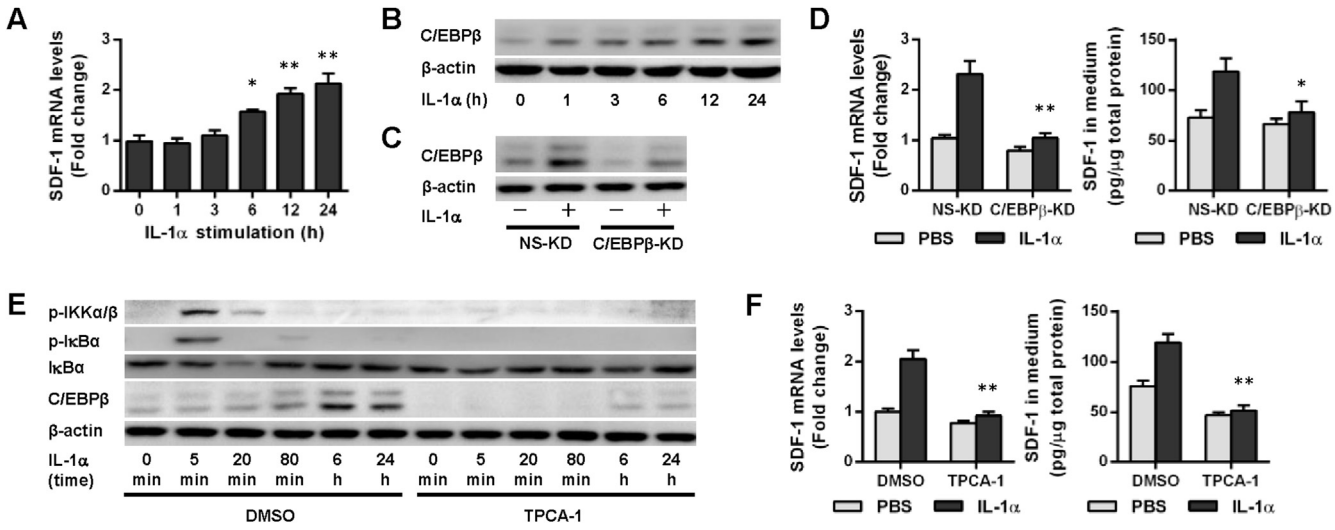

Fig. 1. IL-1α induces SDF-1 expression through up-regulating C/EBPβ in an IKKβ signaling-dependent manner. (A) VSMCs were treated with IL-1α for the times indicated. RNA was analyzed for SDF-1 transcription using qRT-PCR (n = 3; * and **, P < 0.01 and 0.05 versus PBS-treated VSMCs). (B) VSMCs were treated with IL-1α for the times indicated. Whole cell lysates were immunoblotted using anti-C/EBPβ and anti-β-actin. (C and D) NS-KD and C/EBPβ-KD VSMCs were treated with PBS or IL-1α for 24 h. Whole cell lysates were immunoblotted using anti-C/EBPβ and anti-β-actin. RNA and medium were analyzed for SDF-1 expression using qRT-PCR and ELISA (n = 3; * and **, P < 0.05 and 0.01 versus IL-1α-treated NS-KD VSMCs). (E) Following DMSO or TPCA-1 pretreatment, VSMCs were treated with PBS or IL-1α for the times indicated. Whole cell lysates were immunoblotted using anti-IKKα/β, anti-phospho-IκBα, anti-IκBα, anti-C/EBPβ and anti-β-actin. (F) Following DMSO or TPCA-1 pretreatment, VSMCs were treated with PBS or IL-1α for 24 h. RNA and medium from VSMCs was analyzed for SDF-1 expression using qRT-PCR and ELISA (n = 3; **, P < 0.01 versus VSMCs that treated with IL-1α following DMSO pretreatment).

cytokines, including SDF-1 [13]. And we found pretreating VSMCs with TPCA-1, a potent IKKβ inhibitor, not only abolished IL-1α-induced IKKα/β phosphorylation, IκBα phosphorylation, IκBα degradation and C/EBPβ up-regulation (Fig. 1E), but also significantly inhibited IL-1α-induced SDF-1 expression (Fig. 1F). These results suggest IL-1α induces SDF-1 expression in VSMCs through up-regulating C/EBPβ in an IKKβ signaling-dependent manner.

3.2. TAK1 negatively regulates IL-1α-induced SDF-1 expression through attenuating C/EBPβ up-regulation

To evaluate the role of TAK1 in SDF-1 expression, VSMCs infected with lentiviruses expressing *Map3k7*-targeting shRNA (TAK1-KD VSMCs) were used. And we found that, in IL-1α-treated TAK1-KD VSMCs, TAK1 phosphorylation, a critical event for TAK1 activation, was dramatically attenuated, suggesting effective inhibition of TAK1 activation by TAK1 knockdown (Fig. 2A). Unexpectedly, though TAK1 is an upstream activator of IKKβ, TAK1 knockdown, as well as TAK1 inhibitor (5Z-7-Oxozeaenol) pretreatment, did not significantly affect basal SDF-1 production, but did enhance IL-1α-induced SDF-1 production in VSMCs (Fig. 2B and C).

Considering the critical role of C/EBPβ in IL-1α-induced SDF-1 expression, TAK1 might negatively regulate IL-1α-induced SDF-1 expression through attenuating C/EBPβ up-regulation. To test this hypothesis, regulation of SDF-1 expression by IL-1α was further examined in VSMCs where TAK1 and C/EBPβ were simultaneously knocked down (TAK1-C/EBPβ-KD VSMCs). As showed in Fig. 2D, IL-1α-induced C/EBPβ up-regulation was abolished in both C/EBPβ-KD and TAK1-C/EBPβ-KD VSMCs. Similarly, IL-1α-induced SDF-1 expression in TAK1-C/EBPβ-KD VSMCs was also decreased to a level comparable to that in C/EBPβ-KD VSMCs (Fig. 2E). These data reveal that TAK1 negatively regulates IL-1α-induced SDF-1 expression primarily through attenuating C/EBPβ up-regulation. Notably, TPCA-1 pretreatment still eliminated IL-1α-induced expression of C/EBPβ and SDF-1 even in TAK1-KD VSMCs (Fig. 2D and E), suggesting IKKβ signaling in TAK1-KD VSMCs is still activated by IL-1α and critically contributes to expression of C/EBPβ and SDF-1.

3.3. Activation of p38 MAPK is responsible for negative regulation of IL-1α-induced SDF-1 expression by TAK1

Given that TAK1 activates not only IKKβ but also MAPKs, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK [14], and that IKKβ signaling is critical for SDF-1 expression, the negative effect of TAK1 on IL-1α-induced SDF-1 expression might be mediated by one or more of these MAPKs. As shown in Fig. 3A, IL-1α-induced phosphorylation of MAPKs, which was typically observed within 1 h after IL-1α treatment, was greatly impaired in TAK1-KD VSMCs. On the other hand, in IL-1α-treated TAK1-KD VSMCs, though IKKα/β phosphorylation was also decreased, phosphorylation and degradation of IκBα were still detected. These results indicate that TAK1 knockdown may unevenly affect IKK and MAPK pathways in IL-1α-treated VSMCs.

Then, VSMCs pretreated with inhibitors of the three MAPKs separately (i.e. SCH772984 for ERK, SP600125 for JNK and SB239063 for p38 MAPK) were used. We found IL-1α-induced SDF-1 expression was significantly enhanced in VSMCs pretreated with SB239063, but not in those pretreated with SCH772984 or SP600125 (Fig. 3B). And further examination showed that the positive effect of SB239063 pretreatment on IL-1α-induced expression of C/EBPβ and SDF-1 is dramatically diminished in C/EBPβ-KD VSMCs (Fig. 3C and D). Moreover, IL-1α-induced expression of C/EBPβ and SDF-1 in TAK1-KD VSMCs was comparable to that in SB239063-pretreated VSMCs, and was not further significantly by SB239063 pretreatment (Fig. 3E and F). Consequently, TAK1 may exert its negative effect on IL-1α-induced expression of C/EBPβ and SDF-1 primarily through p38 MAPK signaling.

3.4. TAK1/p38 MAPK signaling negatively regulates ROS-dependent SDF-1 expression in IL-1α-treated VSMCs through counteracting NRF2 up-regulation

As TAK1 is implicated in regulation of ROS production [15,16], which also affects SDF-1 expression [17], we examined whether TAK1 regulates SDF-1 expression in VSMCs through interfering

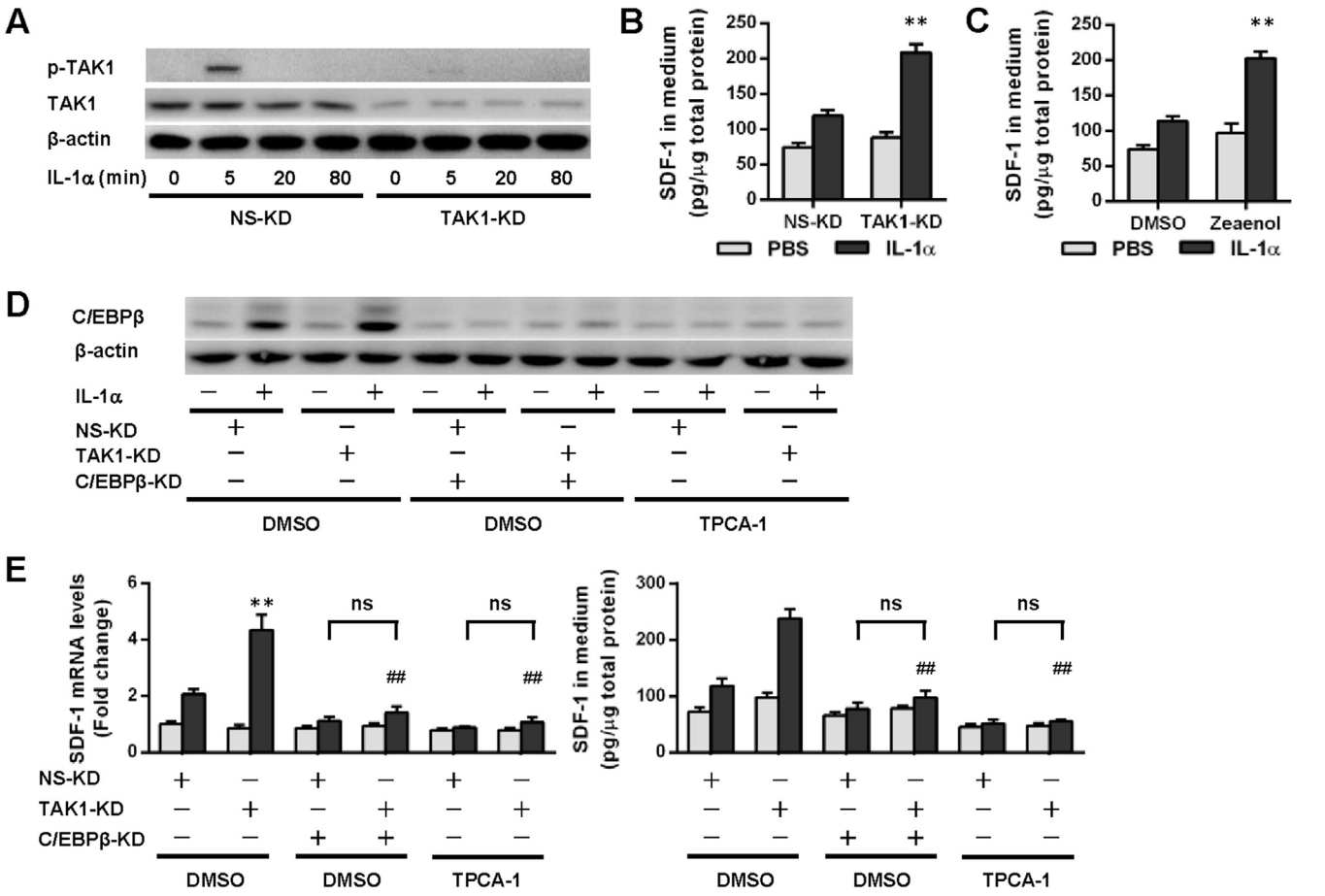

Fig. 2. TAK1 negatively regulates IL-1α-induced SDF-1 expression through attenuating C/EBPβ up-regulation. (A) NS-KD and TAK1-KD VSMCs were treated with PBS or IL-1α for the times indicated. Whole cell lysates were immunoblotted with anti-phospho-TAK1, anti-TAK1 and anti-β-actin. (B) NS-KD and TAK1-KD VSMCs were treated with PBS or IL-1α for 24 h. Medium was analyzed for SDF-1 production using ELISA. (n = 3; **, P < 0.01 versus IL-1α-treated NS-KD VSMCs). (C) Following DMSO and 5Z-7-Oxozeaenol (Zeaenol) pretreatment, VSMCs were treated with PBS or IL-1α for 24 h. Medium was analyzed for SDF-1 production using ELISA. (n = 3; **, P < 0.01 versus VSMCs that treated with IL-1α following DMSO pretreatment). (D and E) Pretreated as indicated, VSMCs were then treated with PBS or IL-1α for 24 h. While whole cell lysates were immunoblotted with anti-C/EBPβ and anti-β-actin, RNA and medium were analyzed for SDF-1 expression using qRT-PCR and ELISA (n = 3; **, P < 0.01 versus NS-KD VSMCs that treated with IL-1α following DMSO pretreatment; ##, P < 0.01 versus TAK1-KD VSMCs that treated with IL-1α following DMSO pretreatment; ns, not significant).

with ROS-related signaling. Indeed, pretreatment with N-acetyl-L-cysteine (NAC), a ROS scavenger, significantly decreased IL-1α-induced SDF-1 expression in TAK1-KD and SB239063-pretreated VSMCs, but not in NS-KD or DMSO-pretreated VSMCs. And TAK1 knockdown and SB239063 pretreatment failed to enhance IL-1α-induced SDF-1 expression in the presence of NAC (Fig. 4A and B). Similarly, NAC pretreatment also attenuates the positive effects of TAK1 knockdown and SB239063 pretreatment on IL-1α-induced C/EBPβ up-regulation (Fig. 4C). Therefore, inhibition of TAK1/p38 MAPK signaling enhances IL-1α-induced expression of C/EBPβ and SDF-1 in VSMCs in a ROS-dependent manner.

Indeed, as shown in Fig. 4D, moderate increases in hypoxia-inducible factor-1α (HIF-1α) and NRF2, two redox-sensitive transcription factors, were observed in VSMCs around 6 h after IL-1α treatment. Moreover, while TAK1 knockdown and SB239063 pretreatment significantly enhanced and prolonged IL-1α-induced NRF2 up-regulation, NAC pretreatment abolished IL-1α-induced up-regulation of HIF-1α and NRF2 even in TAK1-KD and SB239063-pretreated VSMCs. Therefore, we hypothesized that inhibition of TAK1/p38 signaling may regulate SDF-1 expression in IL-1α-treated VSMCs through enhancing up-regulation of HIF-1α and (or) NRF2. To test this hypothesis, VSMCs were pretreated with HIF-1α inhibitor (PX-478) and NRF2 inhibitor (trigonelline) separately before IL-1α treatment. As showed by our results (Fig. 4E and F), PX-478 and trigonelline both showed inhibitory effect on IL-1α-induced expression C/EBPβ and SDF-1 in TAK1-KD VSMCs, but not in NS-KD VSMCs. Notably, in subgroup pretreated with PX-478, significantly higher levels of IL-1α-induced expression of C/EBPβ and SDF-1 were still observed in TAK1-KD VSMCs. In contrast, trigonelline pretreatment eliminated the differences between NS-KD and TAK1-KD VSMCs in IL-1α-induced expression of C/EBPβ and SDF-1. In summary, these results demonstrate that TAK1/p38 MAPK signaling negatively regulates IL-1α-induced expression of C/EBPβ and SDF-1 in rat VSMCs primarily through counteracting ROS-dependent NRF2 up-regulation.

4. Discussion

The effect of inflammatory stimuli on expression of SDF-1, an evolutionarily conserved chemokine, is controversial, and positive [18] and negative [13] regulation of SDF-1 expression by inflammatory cytokines have both been reported previously. Among others, a previous study showed that activation of IKKβ signaling by tumor necrosis factor inhibits SDF-1 expression in human umbilical vein endothelial cells [13], which is in sharp contrast to our observation that IL-1α promoted SDF-1 expression in rat VSMCs in

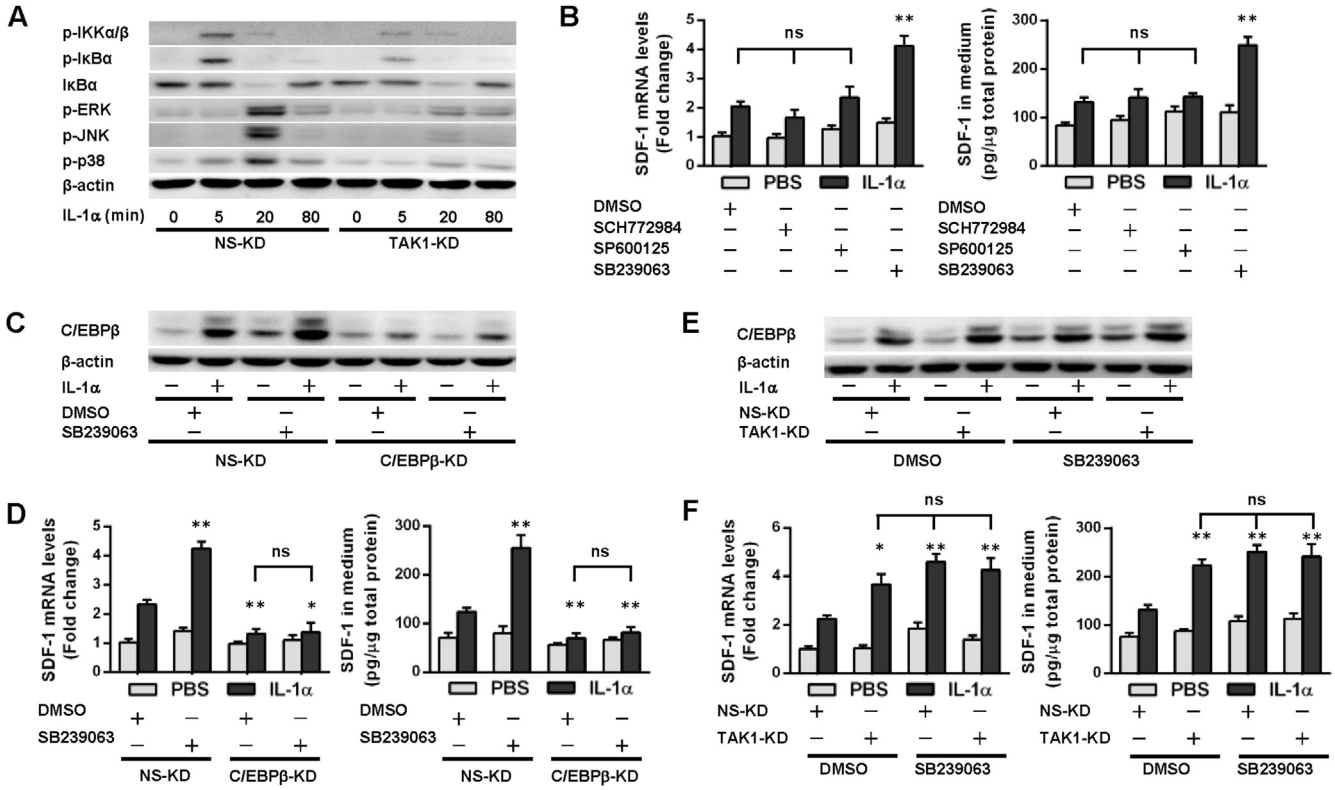

Fig. 3. Activation of p38 MAPK is responsible for negative regulation of IL-1α-induced SDF-1 expression by TAK1. (A) NS-KD and TAK1-KD VSMCs were treated with PBS or IL-1α for the times indicated. Whole cell lysates were immunoblotted with antibodies as indicated. (B) Following pretreatment with DMSO, SCH772984, SP600125, or SB239063, VSMCs were treat with PBS or IL-1α for 24 h. RNA and medium was analyzed for SDF-1 expression using qRT-PCR and ELISA (n = 3; **, P < 0.01 versus VSMCs that treated with IL-1α following DMSO pretreatment; ns, not significant). (C and D) NS-KD and C/EBPβ-KD VSMCs that pretreated with DMSO or SB239063 were then treated with PBS or IL-1α for 24 h. While whole cell lysates were immunoblotted with anti-C/EBPβ and anti-β-actin, RNA and medium were analyzed for SDF-1 expression using qRT-PCR and ELISA (n = 3; * and **, P < 0.05 and 0.01 versus VSMCs that treated with IL-1α following DMSO pretreatment; ns, not significant). (E and F) Following DMSO or SB239063 pretreatment, NS-KD and TAK1-KD VSMCs were treated with PBS or IL-1α for 24 h. While whole cell lysates were immunoblotted with anti-C/EBPβ and anti-β-actin, RNA and medium was analyzed for SDF-1 expression using qRT-PCR and ELISA (n = 3; * and **, P < 0.05 and 0.01 versus VSMCs that treated with IL-1α following DMSO pretreatment; ns, not significant).

an IKKβ signaling-dependent manner. Notably, consistent with the notion that SDF-1 primarily functions as a homeostatic factor rather than an inflammatory factor [4], to date, no typical binding site of classical inflammatory transcription factor (e.g. NF-κB) has been found in the promoter region of SDF-1 gene [12,19]. Therefore, IKKβ seems to affect SDF-1 expression indirectly, probably through regulating other transcription factors, such as HIF-1α [20] and C/EBPβ [12].

In our study, activation of IKKβ by IL-1α promoted SDF-1 expression in VSMCs primarily through up-regulating C/EBPβ in both ROS-dependent and -independent manners. While ROS-dependent C/EBPβ up-regulation, which was only evident in TAK1/p38 MAPK signaling-incompetent VSMCs, primarily resulted from ROS-dependent NRF2 up-regulation, how ROS-independent C/EBPβ up-regulation was affected by IKKβ signaling remains obscure. Though IKKβ may promote C/EBPβ autoregulation through activating NF-κB [21], SN50, a cell-permeable peptide that inhibits NF-κB nuclear translocation, did not significantly inhibit expression of C/EBPβ and SDF-1 (data not shown). As cAMP-dependent protein kinase signaling, which also affects C/EBPβ expression [22], can also be regulated by IKKβ in an NF-κB-independent manner [23], whether similar NF-κB-independent effects of IKKβ contribute to IL-1α-induced expression of C/EBPβ and SDF-1 in VSMCs deserves further investigation.

Importantly, we also demonstrated that TAK1 exerts negative effect of on IL-1α-induced SDF-1 expression, though it was seemingly contradictory to the positive role of IKKβ in SDF-1 expression in VSMCs. And we propose this may be explained by the uneven inhibition of IKKβ and p38 MAPK by TAK1 knockdown. Probably due to the activation of residual TAK1 or other functionally overlapping MAP3Ks (such as MAP3K1 [24], MAP3K3 [25] and MAP3K5 [15]), in TAK1-KD VSMCs, IL-1α-induced phosphorylation of IKKs was only moderately inhibited. Because IL-1α-induced SDF-1 expression in TAK1-KD VSMCs, as well as that in NS-KD VSMCs, was abolished by TPCA-1 pretreatment, therefore, the compromised IKKβ signaling in IL-1α-treated TAK1-KD VSMCs might have been sufficient for inducible SDF-1 expression. Additionally, as IL-1α-induced activation of p38 MAPK, which counteracts ROS-dependent SDF-1 expression, was also inhibited by TAK1 knockdown, enhanced IL-1α-induced SDF-1 expression in TAK1-KD VSMCs can thus be anticipated.

NRF2 is a redox-sensitive transcription factor whose abundance is tightly regulated by proteasome-dependent degradation and significantly increased by oxidative stress [26]. In addition to genes involved in antioxidant defense, NRF2 also regulates expression of various transcription factors [26], including C/EBPβ [27,28], which is in consistence with our observation that NRF2 inhibitor and ROS scavenger both suppressed ROS-dependent C/EBPβ up-regulation. Notably, though the underlying mechanism remains controversial, loss of TAK1 tends to induce ROS accumulation [15,16]. And in IL-1α-treated VSMCs, p38 MAPK inhibition, as well as TAK1 knockdown, significantly enhances ROS-dependent NRF2 up-regulation. Given that p38 MAPK also regulates genes that promote ROS clearance [29,30], and that inhibition of p38 MAPK can

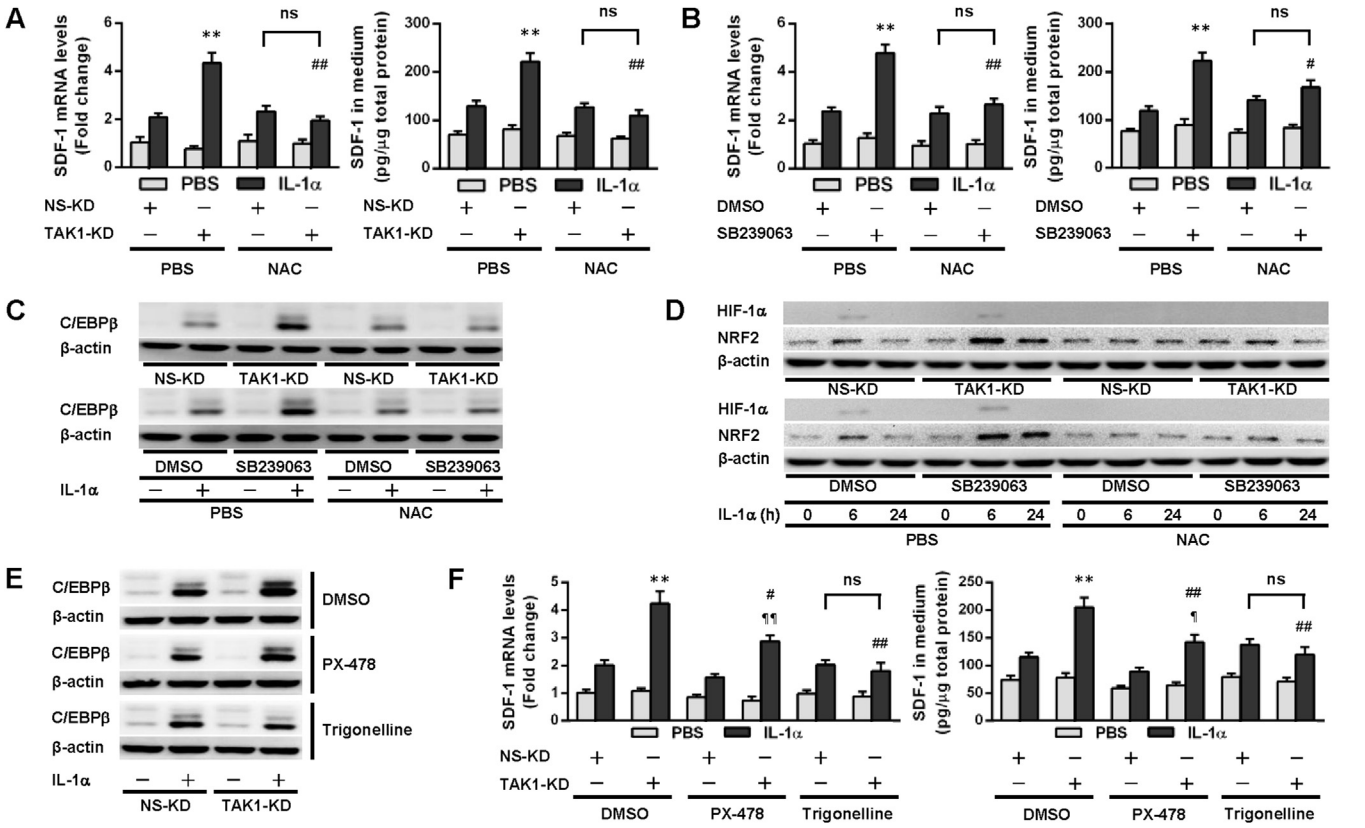

Fig. 4. TAK1/p38 MAPK signaling negatively regulates ROS-dependent SDF-1 expression in IL-1α-treated VSMCs through counteracting NRF2 up-regulation. (A, B and C) NS-KD and TAK1-KD VSMCs, as well as VSMCs that pretreat with DMSO or SB239063, were treated with PBS or IL-1α in the absence or presence of NAC. While RNA and medium was analyzed for SDF-1 expression using qRT-PCR and ELISA, whole cell lysates were immunoblotted with anti-C/EBPβ and anti-β-actin (n = 3; **, P < 0.01 versus NS-KD VSMCs treated with IL-1α in the absence of NAC; # and ##, P < 0.05 and 0.01 versus TAK1-KD VSMCs treated with IL-1α in the absence of NAC; ns, not significant). (D) NS-KD and TAK1-KD VSMCs, as well as VSMCs that pretreat with DMSO or SB239063, were treated with IL-1α for times indicated in the absence or presence of NAC. Whole cell lysates were immunoblotted with anti–HIF–1α, anti-NRF2 and anti-β-actin. (E and F) Following pretreatment with DMSO, PX-478 or trigonelline, NS-KD and TAK1-KD VSMCs were treated with PBS or IL-1α for 24 h. While whole cell lysates were immunoblotted with anti-C/EBPβ and anti-β-actin, RNA and medium was analyzed for SDF-1 expression using qRT-PCR and ELISA (n = 3; **, P < 0.01 versus NS-KD VSMCs treated with IL-1α following DMSO pretreatment; # and ##, P < 0.05 and 0.01 versus TAK1-KD VSMCs treated with IL-1α following DMSO pretreatment; ¶ and ¶¶, P < 0.05 and 0.01 versus NS-KD VSMCs treated with IL-1α following PX-478 pretreatment; ns, not significant).

increase ROS production in specific cell types [31], coordinate activation of IKKβ and p38 MAPK by TAK1 may serve as an inherent mechanism to prevent excessive ROS accumulation in VSMCs treated with IL-1α. Therefore, TAK1 may negatively regulate IL-1α-induced SDF-1 expression through activating p38 MAPK, which counteracts ROS-dependent up-regulation of NRF2 and C/EBPβ.

In conclusion, our results identified IKKβ and p38 MAPK as two signaling components that exert distinct effects on SDF-1 expression in IL-1-treated VSMCs. And acting upstream of IKKβ and p38 MAPK, TAK1 functions as an important regulator of SDF-1 expression in inflammatory condition. Considering the important role of SDF-1 in vascular progenitor recruitment and function, regulating activity of TAK1 may serve as a potential strategy for modulating vascular repair and remodeling, and, therefore, optimizing treatment of inflammation-related vascular diseases.

Conflict of interest

The authors declare no conflict of interest.

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Transparency document

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