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ORIGINAL ARTICLE

The Glycolytic Enzyme PFKFB3 Controls TNF-α-Induced Endothelial Proinflammatory Responses

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Abstract—Endothelial cells play an important role in health and a variety of diseases. Recent evidences show that endothelial cells rely on glycolysis rather than on oxidative phosphorylation to generate energy to support cellular functions such as angiogenesis. However, the effect of endothelial glycolysis on vascular inflammation remains little known. Here, we investigate the role of key glycolytic enzyme PFKFB3 in tumor necrosis factor-α (TNF-α)-induced endothelial proinflammatory responses. siRNAs were used to knockdown the expression of PFKFB3. In some experiments, PFKFB3 inhibitors were also used. TNF-α at 20 ng/ml was added to confluent endothelial cells for different time period of stimulation. PFKFB3 expression was examined by RT-PCR and western blotting. Cytokine antibody panel membranes were employed to detect different cytokines/chemokines in culture supernatant of endothelial cells. The determination of monocyte adhesion to endothelial cells after TNF-α treatment was conducted using THP-1 cells. The monocyte attraction was performed using Transwell filters. For further mechanisms, NF-κB-p65 localization was examined by immunofluorescence. Expression of total IκB, phospho-IκB, phospho-NF-κB-p65, and Ikkβ was detected by western blotting. DNA-binding activity of NF-κB was assessed using electrophoretic mobility shift assay. We found that TNF-α increased endothelial PFKFB3 expression. Knockdown of PFKFB3 almost blocked all TNF-α-induced release of the proinflammatory cytokines/chemokines (MCP-1, IL-8, CXCL1, GMCSF, RANTES, TNF-α) and ICAM-1. PFKFB3 knockdown also significantly inhibited TNF-α-induced monocyte adhesion and transmigration. Furthermore, inhibition of PFKFB3 inhibited TNF-α-induced Ikkβ phosphorylation, IκBα phosphorylation and degradation, NF-κB-p65 phosphorylation, nuclear translocation, and DNA-binding activity. Thus, our results demonstrate that glycolytic enzyme PFKFB3 plays a critical role in TNF-α-induced endothelial inflammation.

KEY WORDS: endothelial cells; inflammation; glycolysis; PFKFB3; TNF-α.

INTRODUCTION

The endothelium forms the inner cellular lining of blood vessels separating blood from surrounding tissue. The endothelium plays an important role in many physiological functions, including the control of barrier function, inflammatory cell trafficking, vasomotor tone, hemostatic balance, angiogenesis, and immunity. There is even a concept that future breakthroughs in biomedicine are

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contingent on acceptance of the endothelium as an organ system [2]. The endothelial activation and dysfunction is involved in a number of diseases including acute and chronic inflammatory disorder such as sepsis and atherosclerosis, either as a primary determinant of pathophysiology or as a victim of collateral damage [1, 3, 13, 14, 22]. During inflammation, endothelial cells control inflammatory cells recruitment and adhesion to endothelial cells and actively participate in inflammation through differential expression of inflammation-associated genes, including cytokines, chemokines, and adhesion molecules [20].

Endothelial cells are not inert but rather are highly metabolically active. The phenomenon of aerobic glycolysis that cancer cells exhibit a high rate of glycolysis even in the presence of oxygen (Warburg effect) is widely observed among cancer cells. The advantages of enhanced glycolysis in cancer remain controversial [12, 15, 19]. Many normal proliferating cells and fast-growing unicellular organisms also rely primarily on glucose fermentation during proliferation regardless of oxygen availability. Recent evidences show that endothelial cells rely on glycolysis rather than on oxidative phosphorylation to generate energy to support cellular functions [9–11, 17, 18]. The amount of ATP generated by glycolysis in endothelial cells is up to 85% of the total cellular ATP content [9, 10]. This finding is surprising, because quiescent endothelial cells are in immediate contact with oxygen in the blood and are exposed to sufficient oxygen for oxidative metabolism, yet they prefer aerobic glycolysis, although the reason for this remains unknown.

A central control point of glycolysis is the negative allosteric regulation of a rate-limiting enzyme, phosphofructokinase-1 (PFK-1) by ATP. Phosphofructokinase-2/fructose-2,6-bisphosphatase (F2,6BP) is an allosteric activator of PFK-1 that overrides the inhibitory influence of ATP on PFK-1, thus the most potent stimulator of glycolysis. The cellular concentration of F2,6BP is dependent on the activities of bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB), which are encoded by four independent genes (PFKFB1-4). Of all PFKFB isoenzymes, PFKFB3/PFK2 has a much higher kinase activity than bisphosphatase activity (700-fold), thus favoring the production of intracellular F2,6P_2 levels [10, 27]. Thus, activation of PFKFB3 drastically accelerates glycolytic flux through PFK1. PFKFB3 is highly expressed in many tumors and endothelial cells [5, 8, 10]. Inhibition of PFKFB3 in tumor cells and endothelial cells reduces cell glycolysis and growth [8, 10]. Endothelial PFKFB3 inhibition also impairs vessel formation [10, 26], attenuates pathological angiogenesis [24], and induces tumor vessels normalization [6]. Thus, manipulating endothelial metabolism by targeting PFKFB3 provides a potential opportunity to affect their function.

Although the endothelial dysfunction underlies many pathological processes and diseases, aside from several reports of glycolysis on angiogenesis, there is few study about the role of endothelial glycolysis in inflammation. Tumor necrosis factor-α (TNF-α) is a key proinflammatory cytokine. One of the primary targets is vascular endothelial cells. Here, we investigate the role of glycolytic enzyme PFKFB3 in TNF-α-induced endothelial proinflammatory responses.

MATERIALS AND METHODS

Antibodies and Reagents

Recombinant human TNF-α was purchased from Peprotech. Proteome Profiler Human Cytokine Array was obtained from R&D Systems. Rabbit anti-IκB, anti-phospho-IκBα (Ser32) (14D4), anti-NF-κB-p65, anti-phospho-NF-κB-p65 (Ser536), and anti-phospho-IKKα/β (Ser176/180) (16A6) were purchased from Cell Signaling. Rabbit anti-PFKFB3 was purchased from Proteintech. Secondary anti-rabbit IgG-Alexa Fluor 568 antibody was purchased from Molecular Probes.

Cell Culture

Human endothelial cell line EA.hy926 cells (ATCC) were used and cultured in DMEM plus 10% FBS. THP-1 cells (ATCC), a human monocyte cell line, were cultured in RPMI-1640 medium containing 10% FBS. All cells were maintained at 37 °C in a 5% CO_2 incubator in the corresponding medium.

RNA Interference

PFKFB3 siRNA and scrambled siRNA were synthesized by GenePharma Co., Ltd. (GenePharma, Shanghai, China). The previously validated siRNA sequence targeting PFKFB3 was shown below [28]: 5′-AGUUGUAGGAGCUGUACUG-3′. siRNAs (100 nM) were introduced into cultured cells with HiPerfect transfection reagent (Qiagen) after reaching 70% confluence. The transfected cells were used for subsequent experiments after 48 h. The silencing effects of siRNAs were confirmed by RT-PCR analysis or western blotting.

RNA Isolation and Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was extracted from endothelial cells using Trizol reagent in accordance with the manufacturer's instructions. RNA manipulation and qPCR analysis were performed as described in [26]. The primer pair for PFKFB3 was F: ATTGCGGTTTTCGATGCCAC, R: GCCACAACTGTAGGGTCGT.

Measurement of Cytokines/Chemokines in Culture Supernatant

After 48 h of siRNA transfection, the culture medium was changed to DMEM without serum. The cells were then cultured in the serum-free DMEM for another 24 h in the presence or absence of TNF-α (20 ng/ml). Culture supernatant was collected for subsequent experiments. For cytokines/chemokines measurement, supernatants were analyzed by the Human Cytokine Array kit.

Monocyte Adhesion Assay

The adhesion of monocyte to endothelial cell was determined using THP-1 cells. Endothelial cells were grown to confluence in 24-well and treated with TNF-α (20 ng/ml) for 6 h. Endothelial cells were then gently washed with serum-free medium and labeled with CytoTracker (Invitrogen). Fluorescent green-labeled THP-1 cells (1×10^6/ml RPMI1640 medium containing 1% FBS) were then added to endothelial cell monolayer. After 1 h incubation, endothelial monolayer was gently washed with DMEM to remove unbound monocytes. The adhered monocytes were determined by immunofluorescence microscopy, and the number of cells was counted using ImageJ software (National Institutes of Health).

Monocyte Migration Assay

After siRNA transfection for 48 h, endothelial cells were treated with vehicle or TNF-α (20 ng/ml) for 24 h. Cell culture medium was collected. To examine the effects of culture supernatants derived from different conditions on monocyte attraction, THP-1 cell migration assay was performed using 5.0 μm pore-size Transwell filters (24-well insert, Corning Costar) with THP-1 cells (2×10^5 cells in 0.2 ml RPMI-1640) in the top compartment and culture supernatants (1.2 ml) in the bottom compartment. After 1 h of incubation with the conditioned media, cells migrated to the lower chamber were collected by centrifugation (1000 *g* for 10 min) and resuspended in 0.1 ml PBS, and the number of cells was counted using a hemocytometer.

Immunofluorescence Staining

Cells were seeded onto 8-well chamber slides. After indicated time of treatment, the cells were washed with PBS, fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X100, and incubated with 1% BSA. Rabbit anti-NF-κB-p65 was then used for 2 h at room temperature. Secondary anti-rabbit IgG-Alexa Fluor 568 antibody was incubated for 1 h at room temperature. Localization of NF-κB-p65 was examined by immunofluorescence.

Western Blotting

After indicated time of treatment, cells were washed and lysed by RIPA buffer (Pierce) in the presence of mixture protease inhibitor. Equal amounts of proteins were loaded on gels, separated by SDS-PAGE, and transferred to a nitrocellulose membrane. After incubation with primary and HRP-linked secondary antibodies, specific bindings were detected by a chemiluminescence system. Band intensities were quantified with the ImageJ.

Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assay (EMSA) was performed using a non-radioactive EMSA kit in accordance with the manufacturer's instructions (Pierce, Rockford, IL). The sequence of the oligonucleotide, which was biotinylated at its 5′ end, was as follows: 5′-CAGAGGGA CTTTCCGAGA-3′. Nuclear protein samples (10 μg) were incubated for 20 min at room temperature in a binding reaction mixture containing 1.5 μl 10 × binding buffer, 1.5 μl poly(dI-dC) (1.0 μg/μl), and ddH_2O to a final volume of 15.0 μl. After 0.5 μl of the probe was added, the reaction was incubated for 20 min at room temperature. Protein-DNA complexes were resolved on 5.5% non-denaturing polyacrylamide gel at 180 V in 0.25 × TBE at 4 °C for 1 h. The gels were transferred to the bonding membrane at 390 mA in 0.5 × TBE at room temperature for 40 min. After UV cross-linking for 10 min, the membrane was blocked, streptavidin-HRP labeled, washed again, and equilibrated; images were then subjected to chemiluminescence and quantified with the ImageJ.

Statistical Analysis

Student's two-tailed *t* test was used to determine statistical significance. The significance level was set at $p < 0.05$.

RESULTS

TNF-α Increased PFKFB3 Expression in Endothelial Cells

To determine the effect of TNF-α on the expression of PFKFB3, endothelial cells were cultured in the presence of TNF-α for different period of time. The mRNA level of PFKFB3 was increased by threefold as analyzed with RT-PCR (Fig. 1a). The upregulation of PFKFB3 protein was further confirmed using western blotting (Fig. 1b). Interestingly, while mRNA level of PFKFB3 was time-dependently increased, the expression of PFKFB3 protein was first increased and then decreased, indicating that PFKFB3 was regulated differently at mRNA and protein level.

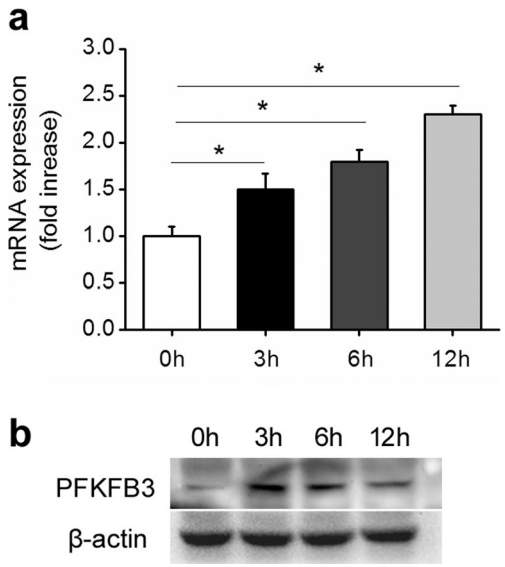

Fig. 1. TNF-α increased PFKFB3 expression in endothelial cells. Endothelial cells were incubated with or without recombinant TNF-α (20 ng/ml) for up to 12 h. **a** RT-PCR-analyses of PFKFB3 mRNA. Data are expressed as the mean ± SD of three experiments. **b** Western blotting analysis of PFKFB3 protein expression. Representative immunoblot of three experiments is shown. $*p < 0.05$.

Knockdown of PFKFB3 Almost Blocked all TNF-α-Induced Release of the Proinflammatory Cytokines/Chemokines

First, we confirmed the efficiency of PFKFB3 siRNA after 48 h transfection by RT-PCR (Fig. 2a). To identify whether and which inflammatory cytokines/chemokines induced by TNF-α in endothelial cells will be affected after PFKFB3 knockdown, we performed a non-biased, high-throughput approach using human cytokine antibody array containing 36 cytokine capture antibodies immobilized on membrane. As shown in Fig. 2b, c, among 36 cytokines on the array, culture of endothelial cells in basic DMEM medium for 24 h contains little cytokine. However, TNF-α induced markedly release of several inflammatory cytokines/chemokines, including monocyte chemoattractant protein-1 (MCP-1), IL-8, CXCL1, GM-CSF (granulocyte-macrophage colony-stimulating factor), RANTES, TNF-α, and adhesion molecule ICAM-1, confirming the effects of TNF-α proinflammatory response. In contrast, knockdown of PFKFB3 almost blocked all these cytokine/chemokines array signals. To note, siPFKFB3 alone had no effect on cytokines/chemokines release.

PFKFB3 Knockdown Significantly Inhibited TNF-α-Induced Monocytes Adhesion and Transmigration

One of the main roles of endothelial cells in inflammation is to regulate the tethering, rolling, activation, arrest, and extravasation of immune cells from the bloodstream into the inflamed site. This mechanism involves expression of adhesive molecules on endothelial cells. As demonstrated by the previous results, there is a decrease expression of adhesion molecule ICAM after PFKFB3 knockdown in TNF-α-stimulated endothelial cells. Thus, we next investigated whether TNF-α-induced monocyte adhesion to endothelial cells could be affected by PFKFB3 interference. After the confirmation of knockdown efficacy (Fig. 3a), while TNF-α increased significant amount of monocytes adhesion to endothelial cells, siPFKFB3 alleviated this effect (Fig. 3b, c). This result showed that PFKFB3 regulates the TNF-α-induced monocyte adhesion to endothelial cells.

To further examine whether PFKFB3 knockdown can inhibit monocytes transmigration, migration of monocytes through Transwell filters in response to culture supernatants of TNF-α-conditioned medium was examined. Supernatants of TNF-α-conditioned medium attracted significantly more monocytes than control medium alone. In contrast, PFKFB knockdown inhibited this TNF-α-induced effects (Fig. 3d). This result shows that inhibition of PFKFB3 can decrease migration of monocytes efficiently.

Inhibition of PFKFB3 Reduced TNF-α-Induced NF-κB Pathway Activation

Numerous studies have demonstrated the activation of NF-κB is central to inflammatory response elicited by TNF-α [29]. To further investigate whether the above

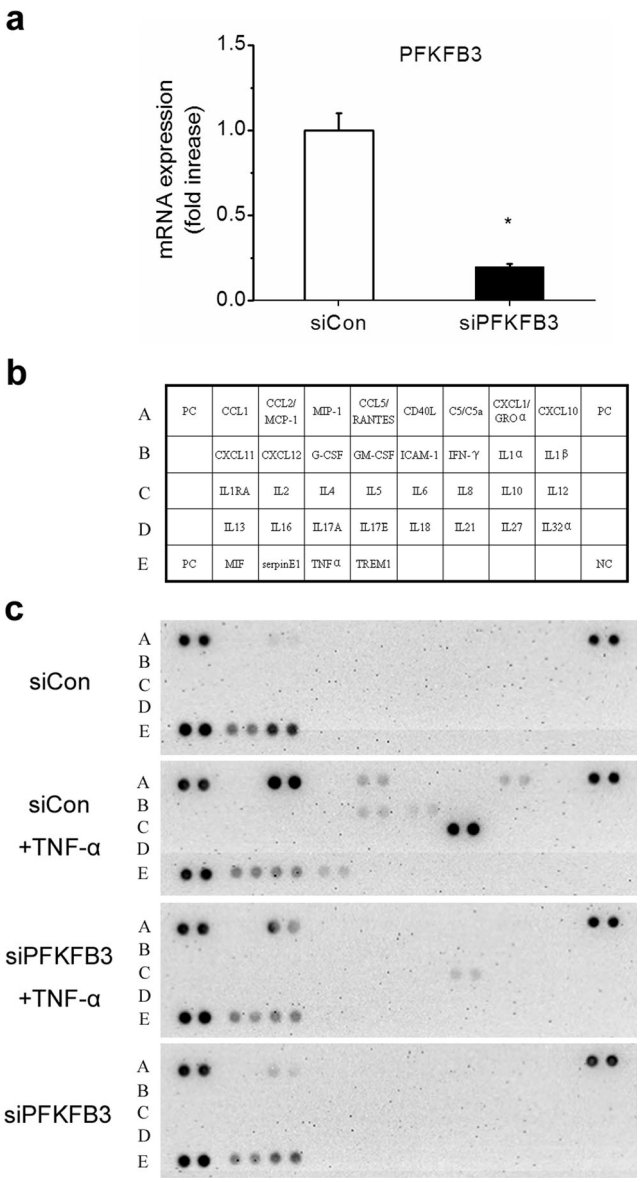

Fig. 2. Knockdown of PFKFB3 blocked TNF-α-induced release of the proinflammatory cytokines/chemokines. After siRNA transfection for 48 h, endothelial cells were treated with vehicle or TNF-α (20 ng/ml) for 24 h. Cell culture medium was collected, centrifuged, and applied to a human cytokine antibody array as described in "Materials and Methods" section. Each spot is spotted on the array membrane in duplicate. **a** Confirmation of PFKFB3 knockdown by RT-PCR. siCon, siControl. $^{*}p < 0.05$. Data are expressed as the mean ± SD of three experiments. **b** Map of the cytokine antibody array. **c** Relative expression of cytokine in the supernatant of different groups detected by cytokine antibody array. PC, positive control; NC, negative control. Representative immunoblot of three experiments is shown.

observed effects of PFKFB3 inhibition on TNF-α-induced endothelial inflammation involve NF-κB pathway, NF-κB-p65 localization was evaluated by immunofluorescence. As shown in Fig. 4a, NF-κB-p65 nuclear translocation induced by TNF-α was almost prevented by PFKFB3 knockdown. To further confirm this, we used two pharmacological inhibitors of PFKFB3, 3PO, and PFK15. The results showed that both these two inhibitor prevented TNF-α-induced NF-κB-p65 nuclear translocation (Fig. 4b).

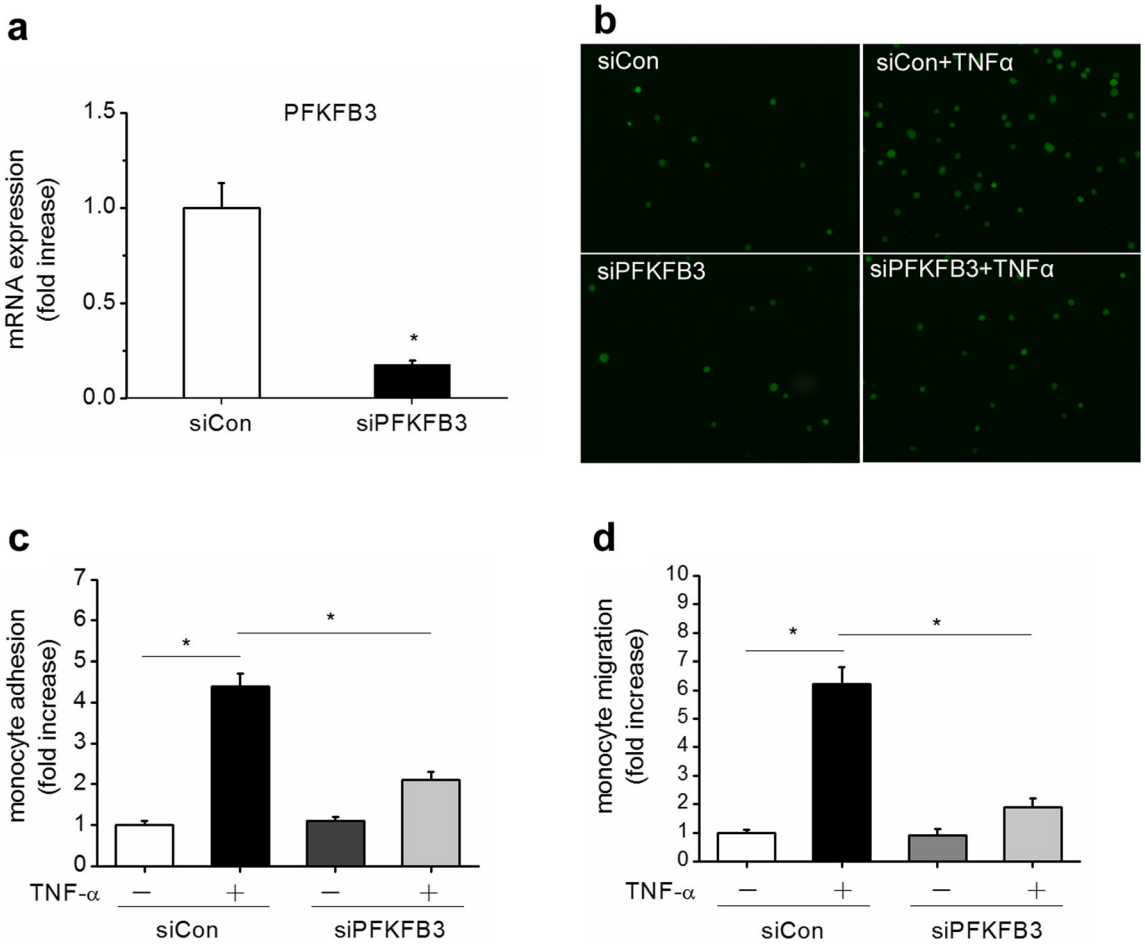

Fig. 3. PFKFB3 knockdown inhibited TNF-α-induced binding of THP-1 cells to endothelial monolayer and transmigration. **a** Confirmation of PFKFB3 knockdown by RT-PCR. **b** Immunofluorescence staining of monocyte adhesion to endothelial cells. After siRNA transfection for 48 h, endothelial cells were treated with vehicle or TNF-α (20 ng/ml) for 6 h. After wash, fluorescence probe-labeled THP-1 cells were incubated with endothelial monolayer. The adhered monocytes were determined by immunofluorescence. **c** Quantitative analysis of monocyte adhesion to endothelial cells. **d** Transmigration of monocyte through Transwell filters. Data are expressed as the mean ± SD of three experiments, $*p < 0.05$. Representative immunofluorescences of three experiments are shown.

In unstimulated cells, IκB proteins bind and mask the nuclear localization sequence on NF-κB, resulting in the retention of the transcription factor in the cytosol. Phosphorylation and degradation of IκB releases NF-κB binding making it translocate to the nucleus and induce transcription [29]. Our results showed that both genetic (Fig. 4c) and pharmacological (Fig. 4d) inhibition of PFKFB3 significantly decreased TNF-α-induced IκB protein. To note, inhibition of PFKFB3 also decreased NF-κB-p65 (Ser536) phosphorylation (Fig. 4c, d).

Stimulus-induced degradation of IκB proteins is initiated through phosphorylation by the IκB kinase β (IKKβ) [29]. To determine if the upstream-signaling events were involved, we used an earlier time point at 5 min to detect the phosphorylation state of IκB and IKKβ. The results showed that inhibition of PFKFB3 using both pharmacological and genetic approach decreased TNF-α-induced phosphorylation of IκB and IKKβ (Fig. 4e, f).

To examine the role of PFKFB3 on downstream of NF-κB-signaling pathway, protein-DNA complex from nuclear extracts was used to assess NF-κB DNA-binding activity by EMSA. As expected, constitutive NF-κB DNA-binding activity was low in control cells. TNF-α treatment greatly stimulated NF-κB DNA-binding activity. However, TNF-α-induced high NF-κB DNA-binding activity was significantly reduced upon PFKFB3 RNA interference

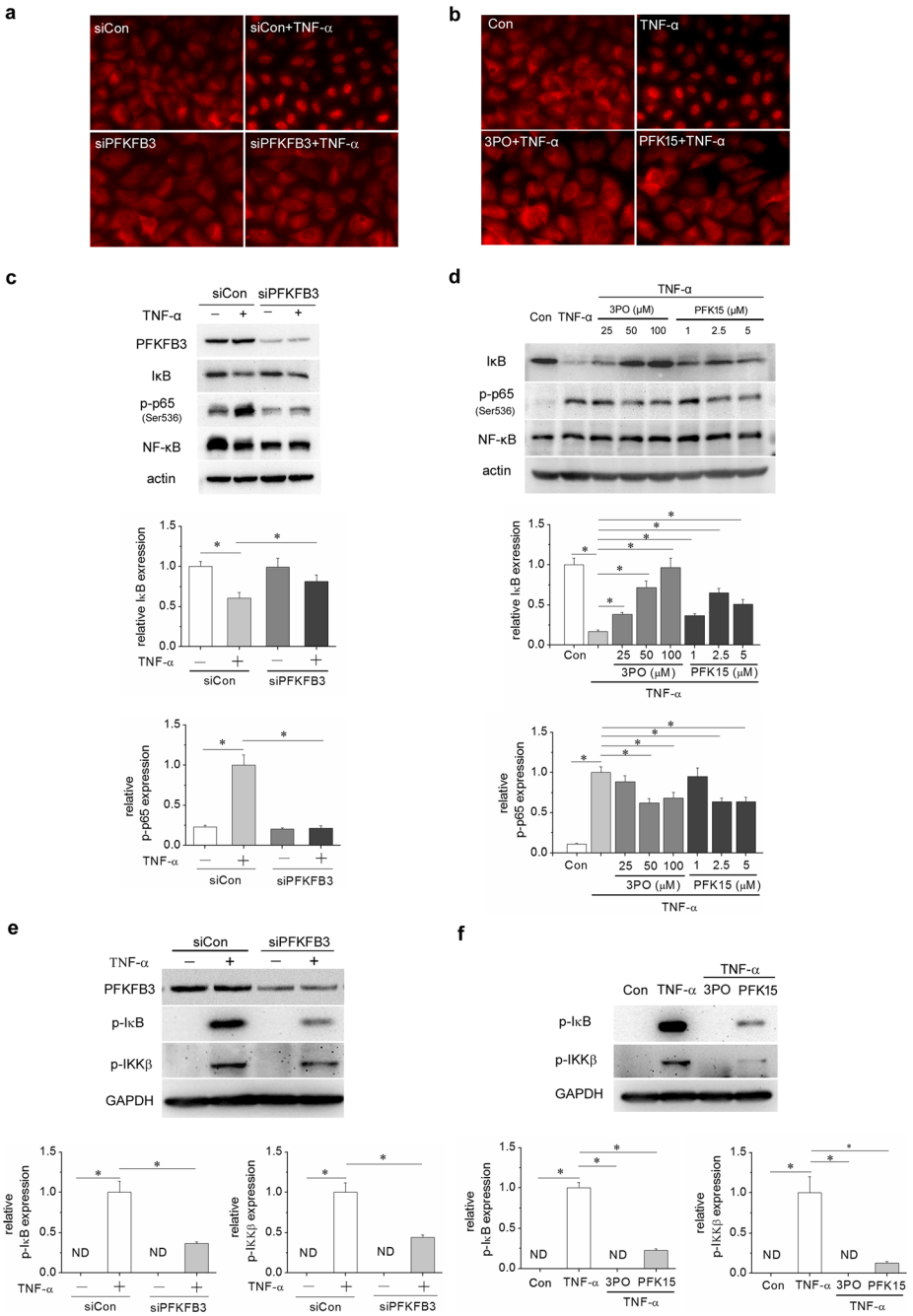

Fig. 4. Inhibition of PFKFB3 reduced TNF-α-induced activation of NF-κB pathway. In the following experiments, siRNA transfection was for 48 h, or pharmacological pretreatment with 3PO (50 μM) or PFK15 (2.5 μM) was for 1 h. After that, cells were stimulated with TNF-α (20 ng/ml) for 30 min unless otherwise indicated. **a** Genetic inhibition of PFKFB3 prevented TNF-α-stimulated NFκB nuclear translocation as dectected by immunofluorescence. **b** Pharmacological inhibition of PFKFB3 prevented TNF-α-stimulated NFκB nuclear translocation. **c** TNF-α-induced IκB degradation and NF-κB-p65 (Ser536) phosphorylation were reduced by PFKFB3 siRNA. **d** The effects of pharmacological PFKFB3 inhibition with different concentrations of 3PO or PFK15 on TNF-α-induced IκB degradation and NF-κB-p65 (Ser536) phosphorylation. **e** Genetic inhibition of PFKFB3 alleviated TNF-α-induced phosphorylation of IκB and IKKβ. TNF-α (20 ng/ml) stimulation for 5 min. ND, not detectable. **f** Pharmacological inhibition of PFKFB3 reduced TNF-α-induced phosphorylation of IκB and IKKβ. TNF-α (20 ng/ml) stimulation for 5 min. **g** Genetic PFKFB3 inhibition decreased TNF-α-induced NF-κB DNA-binding activity as determined by EMSA. **h** Pharmacological inhibition of PFKFB3 decreased TNF-α-induced NF-κB DNA-binding activity. Con, control. *$p < 0.05$. Representative immunofluorescences, immunoblots, and of three experiments are shown.

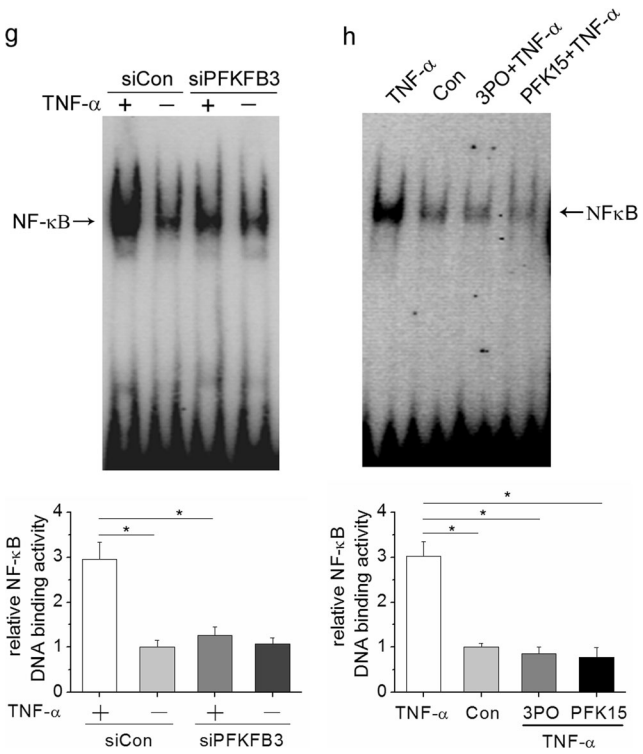

Fig. 4. continued.

(Fig. 4g). This inhibitory effect was further confirmed when cells were treated with PFKFB3 inhibitor 3PO or PFK15 (Fig. 4h). These results indicated that inhibition of PFKFB3 decreases TNF-α-induced NF-κB DNA-binding activity in endothelial cells.

Taken together, both RNA interference and pharmacological inhibition experiments showed that the effects of PFKFB3 inhibition on TNF-α-induced endothelial inflammation may involve NF-κB pathway.

DISCUSSION

The endothelium lining the luminal surface of all blood vessels functions as a highly metabolically active organ spatially distributed throughout the body. The endothelium is involved in most, if not all, disease [3]. Alongside the cancer research field, recent studies demonstrated that endothelial cells rely primarily on glycolysis for ATP production [9–11]. At a first glance, the finding that endothelial cells use aerobic glycolysis as their major energy source in the presence of oxygen is surprising [9–11]. The reason is unclear, but several possible explanations have been proposed [10]: (1) when sprouting into avascular tissues with low levels of oxygen, endothelial cells can still rely on anaerobic glycolysis to form vessels, (2) by consuming little oxygen, they can preserve more oxygen to perivascular cells, (3) glycolysis generates ATP faster than oxidative metabolism, and (4) the glycolysis side pathways are necessary for the biosynthesis of macromolecules needed for cell replication. Several groups further demonstrated that glycolytic regulator PFKFB3 determines angiogenesis [10, 24, 26] and tumor vessels normalization [6]. The role of endothelial cells is central to inflammation [20]. In this study, we demonstrated that TNF-α increased PFKFB3 expression in endothelial cells. By using an unbiased high-throughput antibody array, we found that PFKFB3 inhibition prevented the release of almost all cytokine/chemokines induced by TNF-α, including MCP-1, IL-8, CXCL1, GM-CSF, RANTES, TNF-α, and adhesion molecule ICAM-1. Furthermore, PFKFB3 inhibition alleviated monocyte adhesion to endothelial cells and transmigration. Thus, to extend the recent finding that PFKFB3 plays a critical role in angiogenesis, our results identified endothelial PFKFB3 as a novel target to inhibit TNF-α-induced vascular inflammation and may be a potential therapeutic target in many inflammatory disorders involving endothelial activation and dysfunction.

The underlying mechanisms for PFKFB3 in control of TNF-α-induced endothelial inflammation may be related to NF-κB pathway. NF-κB is known to play an important role in the regulation of inflammatory responses, including cytokines/chemokines, adhesion protein synthesis, and release. In unstimulated cells, NF-κB is mainly cytoplasmic due to the binding of inhibitory protein IκB. After stimulation with proinflammatory cytokines such as TNF-α, IκB is degraded by ATP-dependent proteolytic 26S proteasome [4, 7]. Then, the released NF-κB translocates to the nucleus, leading to stimulate transcription of target genes [29]. Interestingly, the promoter of PFKFB3 also contains binding sites for NF-κB [16]. All these process including the upstream signaling events and IκB degradation are energy consumption. Recent data demonstrated that PFKFB3-mediated glycolysis is the major source of ATP production in endothelial cells [9, 10] and that > 70% of total ATP-consuming processes of endothelial cells can be attributed to specific cellular processes, among which protein synthesis (23%) comprises the largest fraction [9]. Our results showed that both genetic and pharmacological inhibition of PFKFB3 (3PO and PFK15) prevented TNF-α-induced degradation of IκB and nuclear translocation of NF-κB-p65. Besides the phosphorylation and subsequent degradation of IκB, phosphorylation of NF-κB-p65 is also required for optimal NF-κB activation [23, 29]. Consistent with previous reports, we observed that in unstimulated endothelial cells, phospho-Ser536 levels were very low but were upregulated remarkably after TNF-α stimulation. However, this phosphorylation levels were significantly decreased by PFKFB3 inhibition. We also demonstrated that PFKFB3 inhibition reduced TNF-induced phosphorylation of IκB and IKKβ. Finally, inhibition of PFKFB3 decreased TNF-α-induced NF-κB DNA-binding activity as evaluated by EMSA. Thus, our results showed that PFKFB3 inhibition can reduce TNF-α-induced NF-κB pathway activation in endothelial cells.

Several alternative mechanisms and questions may also exist. First, PFKFB3 inhibition might reduce TNF-α-induced endothelial proinflammatory response *via* involvement of lactate. Previous study established that lactate has a signaling role in endothelial cells and can modulate the endothelial phenotype [25]. Second, could non-enzymatic properties of PFKFB3 contribute to the observed TNF-α-mediated proinflammatory response? Although PFKFB3 is a major regulator of glycolysis and glycolysis occurs mostly in the cytosol, recent report indicated glycolysis-independent nuclear roles for PFKFB3 [27]. Finally, how TNF-α signaling in endothelial cells is coupled to PFKFB3, thus, enhancing glycolysis to provide more ATP is another interesting question. Numerous works have identified that TNF-α signaling network involves IκB kinase (IKK) complex that mediates phosphorylation and degradation of IκB. Interestingly, in the most recently published data, Reid et al. uncovered a previously unidentified role of IKK complex catalytic subunit IKKβ in regulating glycolysis by directly interacting with PFKFB3 in cancer cells [21]. However, their data showed that IKKβ has negative regulation on PFKFB3 enzymatic activity and protein stability. Our results showed that PFKFB3 regulates IKKβ activation and downstream of NF-κB pathway. Whether IKKβ interacts directly with PFKFB3 in endothelial cells and other non-cancerous cell lines, and if so, what is the nature of the relation, remain unknown. All these possibilities may be studied in future experiments.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interests. The authors declare that they have no conflict of interest.

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