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ORIGINAL ARTICLE

Role of c-Jun in Tumor Necrosis Factor-alpha Inhibition of Activin A-mediated Erythroid Gene Expression in Erythroleukemia K562 Cells

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Background: The activation of the tumor necrosis factor-alpha (TNF-α)/nuclear factor kappa B (NF-κB) pathway inhibits the expression of erythroid genes, whereas activin A, a member of the transforming growth factor-β superfamily, induces erythroid differentiation. The effect of TNF-α on activin A-induced erythroid gene expression has not been elucidated.
Methods: Luciferase reporter assay and reverse transcription-polymerase chain reaction (PCR) or quantitative PCR were used to investigate globin promoter activity and globin gene expression in the hematopoietic progenitor cell line K562, respectively.
Results: TNF-α inhibited the activin A-induced promoter activity of α-globin and ζ-globin in a concentration-dependent manner in K562 cells. Activin A could reverse the TNF-α-inhibited promoter activity of α-globin and ζ-globin in a concentration-dependent manner. TNF-α decreased the mRNA levels of α-globin, ζ-globin, GATA-1, and NF-E2 p45 induced by activin A. The NF-κB inhibitor, Bay117082, inhibited the TNF-α-increased c-Jun level. NF-κB p65 overexpression increased c-Jun protein and enhanced the TNF-α-increased c-Jun level. Furthermore, TNF-α inhibition of activin A-induced promoter activity and mRNA expression of α-globin and ζ-globin were abolished in cells expressing dominant-negative c-Jun. TNF-α inhibition of activin A-induced mRNA expression of GATA-1 and NF-E2 p45 was also abolished in cells expressing dominant-negative c-Jun.
Conclusion: TNF-α may inhibit activin A-induced erythroid gene expression via increases of c-Jun in K562 cells.

1. Introduction

Erythroid differentiation is regulated by multiple cytokines, such as erythropoietin (EPO), activin A, and tumor necrosis factor-alpha (TNF-α). Activin A, a member of the transforming growth factor-β superfamily, plays an important role in modulating the proliferation and differentiation of erythroid progenitor cells.[1,2] Our previous study showed that activin A induces hemoglobin synthesis in the human K562 erythroleukemia cell line.[3] We have also reported that activin A can induce the promoter activation and mRNA expression of α-globin and ζ-globin in K562 cells.[4] Activin A in cooperation with EPO increases the number of hemoglobin-synthesizing colonies derived from CFU-E progenitors and promotes the formation of BFU-E.[5,6]

The proinflammatory cytokine, TNF-α, plays a critical role in regulating the hematopoietic system and inflammatory responses. In addition, TNF-α can inhibit the erythroid differentiation of hematopoietic progenitor cells.[7] A previous study showed that TNF-α may directly inhibit erythroid differentiation via signaling through TNF-α receptors.[8] TNF-α and nuclear factor kappa B (NF-κB) p65 inhibit erythroid gene expression in K562 cells.[9] That study suggested that NF-κB acts as an erythroid differentiation inhibitor in TNF-α signaling. TNF-α suppresses erythroid differentiation of K562 cells by negatively regulating erythroid-specific transcription factors, such as GATA-1 and NF-E2 p45.[7] However, the mechanisms of TNF-α-inhibited expression of erythroid genes remain unknown. In addition, how activin A and TNF-α interact together to regulate erythroid differentiation has not been elucidated.

The proto-oncogene, c-Jun, is a major component of the activating protein (AP)-1 transcription factor, which forms homo- and heterodimers with other AP-1 family members.[10] It has been shown that NF-κB p65 transactivates and increases expression of AP-1-

Conflicts of interest: The authors have declared that there are no conflicts of interest.

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responsive genes.[11] Those results suggest that NF-κB can induce c-Jun expression. Similar to NF-κB, c-Jun was identified as participating in the inhibition of erythroid differentiation.[12] c-Jun can block hemoglobin synthesis during dimethyl sulfoxide-induced erythroid differentiation of murine erythroleukemia cells.[13] c-Jun also blocked the hemoglobin synthesis of primary human $CD34^+$ hematopoietic progenitors and K562 cells.[14] Because c-Jun proteins mediate some of the effects of TNF-α in different types of cells,[15,16] the above-mentioned studies provide an important link for c-Jun in TNF-α/NF-κB-inhibited erythroid differentiation.

Therefore, the present study was designed to test whether TNF-α inhibits activin A-induced erythroid gene expression and investigate the mechanisms of TNF-α inhibition. We used K562 cells as a model because this cell line can be triggered to undergo erythroid differentiation.[17,18] Our findings demonstrated that TNF-α can induce c-Jun expression through the NF-κB pathway. c-Jun participates in TNF-α inhibition of activin A-induced erythroid gene expression in K562 cells.

2. Methods

2.1. Cell line and reagents

The human K562 erythroleukemia cell line was purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan). K562 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum in a 5% CO_2 incubator at 37°C. Recombinant human activin A and TNF-α were purchased from R&D Systems (Minneapolis, MN, USA). Bay117082 was purchased from Sigma (St. Louis, MO, USA). Antibodies specific for NF-κB p65, c-Jun, and B23 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and α-tubulin monoclonal antibodies were obtained from Abcam (Cambridge, MA, USA).

2.2. Transfection of K562 cells

For K562 cell transfection, 2×10^6 cells were transfected with pCMV4-NF-κB p65 plasmids (2 μg) or an empty vector using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Forty-eight hours after transfection, cells were harvested to analyze c-Jun expression using Western blotting. Dr Warner C. Greene provided pCMV4-NF-κB and p65.[19] For the luciferase reporter assay, transfection of K562 cells was also accomplished using lipofectamine 2000. Two micrograms of plasmids and 0.05 μg of the pRL-TK internal control vector were used in each transfection experiment.

2.3. Luciferase reporter assay

Promoter activity was detected with a reporter assay using previously described procedures.[4] After transfection of cells for 5 hours, the indicated cytokines were added to the culture medium, incubated for 24 hours, and then harvested. Luciferase activity was determined using the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA). Relative luciferase activity was adjusted by normalizing the ratio of firefly luciferase activity to Renilla luciferase activity generated by pRL-TK (Promega). Human c-Jun mutant cDNA was generated by deleting the transactivation domain (TAM-67).[22] Dr Michael J. Birrer provided pCMV-c-Jun and pCMV-TAM-67.[20,21]

2.4. Reverse transcription and quantitative real-time polymerase chain reaction

Total RNA was isolated from K562 cells using the TRIzol reagent (Gibco, Life Technology, Grand Island, NY, USA) according to the manufacturer's instructions. After purification, 1 μg of RNA was reverse-transcribed at 42°C for 60 minutes with an oligo dT_{18} primer, followed by enzyme inactivation at 70°C for 15 minutes. The resulting cDNA was amplified and quantified by performing a quantitative polymerase chain reaction (qPCR). The primers used here were as follows: α-globin sense strand 5′-GACAAGACCAACGTCAAGGCCGCC-3′ and antisense strand 5′-CAGGAACTTGTCCAGGGAGGC-3′; ζ-globin sense strand 5′-GCCACCCGCAGACCAAGACC-3′ and antisense strand 5′-TAGGCGTGCAGCTCGCTCAG-3′; Bmi1 sense strand 5′-CAGCT CATCCTTCTGCTGATGC-3′ and antisense strand 5′-CATTGCTGCTGGG CATCGTAAG-3′; MDR1 sense strand 5′-GCCCTGTTTGCGGTGATCTC-3′ and antisense strand 5′-CGCCTCCTTCTCAGTCTCTG-3′; and CDCP1 sense strand 5′-CTTCAGCCTGGACGAGGATG-3′ and antisense strand 5′-CGAGGGCAGACAGCAGTAAG-3′. qPCR was performed using a QuantiFast SYBR Green PCR Kit (Qiagen, Valencia, CA, USA) on a Roter-Gene Q real-time PCR machine (Qiagen). The normalized gene expression was calculated relative to GAPDH. GAPDH primers were purchased from Qiagen (cat. no.: QT01192646).

2.5. Subcellular fractionation

Cells pellets were resuspended in hypotonic buffer [20mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.4, 1mM $MgCl_2$, 10mM KCl, 0.5% NP-40, 0.5mM dithiothreitol, 1mM phenylmethylsulfonyl fluoride (PMSF), and 2 μg/mL leupeptin] at 4°C for 30 minutes. After centrifugation at 4000 × *g* for 10 minutes at 4°C, supernatants contained the cytoplasmic proteins. Pellets containing the nuclei were resuspended in hypertonic buffer (20mM HEPES at pH 7.4, 20% glycerol, 0.4M NaCl, 1mM $MgCl_2$, 10mM KCl, 0.5mM dithiothreito, 1mM PMSF, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 100mM sodium fluoride) and then incubated at 4°C for 15 minutes. After centrifugation at 8000 × *g* for 15 minutes at 4°C, the supernatants contained the nuclear proteins.

2.6. Western blot analysis

Cells were lysed in lysis buffer (1% Triton X-100, 20mM Tris at pH 7.5, 150mM NaCl, 1mM ethylene glycol tetraacetic acid, 1mM EDTA, 2.5mM sodium pyrophosphate, 1mM β-glycerolphosphate, 1mM PMSF, 1 μg/mL of leupeptin, and 1mM Na_3VO_4) at 4°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to resolve protein lysates, the protein bands were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA), and the membranes were probed with primary antibodies. After binding with the appropriate secondary antibodies (GE Healthcare, Waukesha, WI, USA), the blots were visualized using an enhanced chemiluminescence detection system (Perkin Elmer Life and Analytical Sciences, Waltham, MA, USA).

2.7. Statistical analysis

Quantitative data are presented as the mean ± standard error. Statistically significant differences between groups were identified using the Student *t* test. A p value <0.05 was considered statistically significant.

3. Results

3.1. TNF-α inhibited activin A-mediated promoter activation and gene expression of globins

Our previous study showed that activin A induced erythroid differentiation of K562 cells.[3] Erythroid differentiating cells express globins, an erythroid marker. We first analyzed whether TNF-α inhibited activin A-induced α-globin and ζ-globin promoter activity

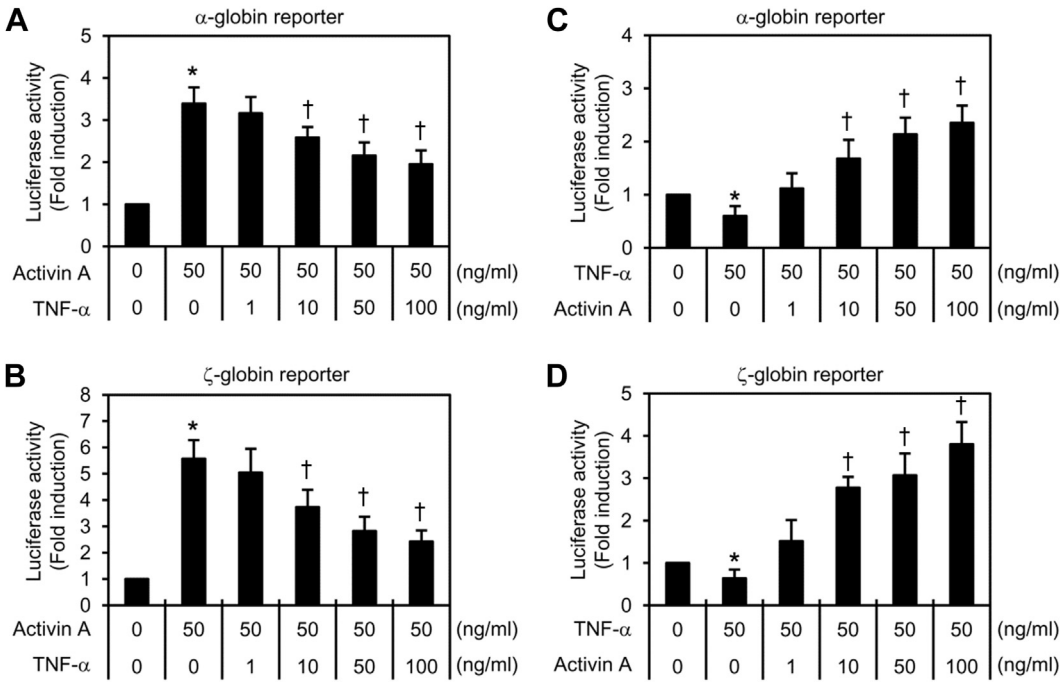

Figure 1 Effects of activin A and TNF-α on luciferase activity of the α-globin and ζ-globin promoters in K562 cells. Cells were transfected with either the (A and C) pHS40-α590 Luc plasmid or (B and D) pHS40-ζ597 Luc plasmid. After 5 hours of transfection, the cells were treated with: (A and B) 50 ng/mL of activin A alone or activin A with various concentrations of TNF-α; (C and D) 50 ng/mL of TNF-α alone or TNF-α with various concentrations of activin A. Luciferase activity was measured 24 hours after adding the cytokines. Values are expressed relative to the untreated control (normalized as 1). Data from four independent experiments are shown as the mean ± SE. $^{*}p < 0.05$ versus untreated cells; $^{\dagger}p < 0.05$ versus activin A or TNF-α treatment. SE = standard error; TNF-α = tumor necrosis factor-alpha.

in K562 cells. Cells were transfected with a reporter plasmid encoding luciferase under the control of an α-globin promoter (pHS40-α590 Luc) or ζ-globin promoter (pHS40-ζ597 Luc).[4] Cells were then stimulated for 24 hours with activin A alone or with activin A combined with TNF-α. Figure 1A and B show that TNF-α concentration-dependently inhibited the activin A-mediated promoter activation of α-globin and ζ-globin genes. Reciprocally, activin A decreased the inhibitory effect of TNF-α on the promoter activation of α-globin and ζ-globin genes in a concentration-dependent manner (Figure 1C and D). To assess whether TNF-α inhibited activin A-mediated globin gene expression, K562 cells were treated for 3 days with activin A, TNF-α, or activin A and TNF-α. The qPCR data indicated that activin A increased, whereas TNF-α decreased, the mRNA levels of α-globin and ζ-globin (Figure 2). TNF-α significantly decreased the activin A-induced expression of α-globin and ζ-globin genes (Figure 2).

3.2. *Effects of TNF-α and activin A on erythroid-specific transcription factor expression*

To explore whether TNF-α suppressed the activin A-induced expression of erythroid-specific transcription factors, GATA-1 and NF-E2 p45, K562 cells were treated for 3 days with activin A, TNF-α,

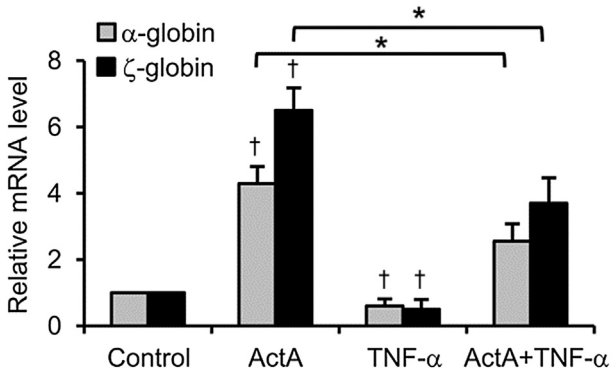

Figure 2 Effects of activin A and TNF-α on globin gene expression in K562 cells. Cells were incubated with or without (control) 50 ng/mL activin A (ActA), 50 ng/mL TNF-α, or both activin A and TNF-α for 72 hours. α-Globin and ζ-globin expression were analyzed using a qPCR. Values are shown as the mean ± SE of three experiments.$^{*}p < 0.05$; $^{\dagger}p < 0.05$ versus the untreated control. qPCR = quantitative polymerase chain reaction; SE = standard error; TNF-α = tumor necrosis factor-alpha.

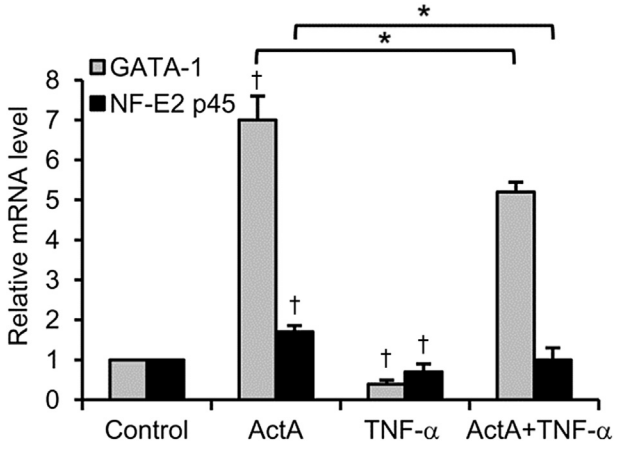

Figure 3 Effects of activin A and TNF-α on expression of GATA-1 and NF-E2 p45 in K562 cells. Cells were treated with or without (control) 50 ng/mL activin A (ActA), 50 ng/mL TNF-α, or both activin A and TNF-α for 72 hours. A qPCR analysis was performed. Values are shown as the mean ± SE of three experiments.$^{*}p < 0.05$; $^{\dagger}p < 0.05$ versus the untreated control. qPCR = quantitative polymerase chain reaction; SE = standard error; TNF-α = tumor necrosis factor-alpha.

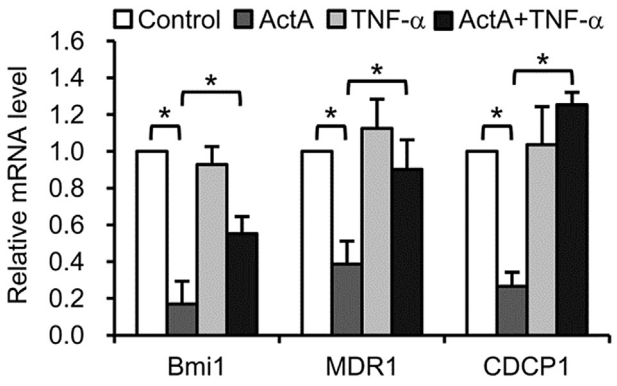

Figure 4 Effects of activin A and TNF-α on expression of the stem/progenitor cell markers in K562 cells. Cells were treated with or without (control) 50 ng/mL activin A (ActA), 50 ng/mL TNF-α, or both activin A and TNF-α. After 72 hours, RNA was isolated from cultured cells. Stem/progenitor cell marker expression was analyzed using a qPCR. Values are shown as the mean ± SE of three experiments. $^{*}p < 0.05$. qPCR = quantitative polymerase chain reaction; SE = standard error; TNF-α = tumor necrosis factor-alpha.

or activin A and TNF-α. The present qPCR data indicated that activin A increased, whereas TNF-α decreased, the mRNA levels of GATA-1 and NF-E2 p45 (Figure 3). TNF-α significantly decreased the activin A-induced expression of the GATA-1 and NF-E2 p45 genes (Figure 3).

3.3. Effects of TNF-α and activin A on stem/progenitor cell marker expression

Because the above results suggested that TNF-α inhibited activin A-mediated erythroid differentiation, we explored the effects of TNF-α and activin A on stem/progenitor cell marker expression. The presence of the stem/progenitor cell markers, Bmi1, MDR1, and CDCP1, within K562 cells was analyzed using a qPCR. Figure 4 shows that activin A reduced the expression of stem/progenitor cell markers in K562 cells. Cotreatment with activin A and TNF-α increased the expression of these markers, compared with activin A alone (Figure 4). These results indicate that TNF-α inhibited the activin A-induced reduction of stem/progenitor cell marker expression.

3.4. TNF-α increased c-Jun protein levels via the NF-κB pathway

NF-κB p65 and c-Jun were identified as erythroid differentiation inhibitors.[8,14] Because a previous report indicated that NF-κB p65 transactivates and increases the expression of AP-1-responsive genes,[11] we explored whether TNF-α influenced c-Jun expression in K562 cells via NF-κB p65. We used subcellular fractionation and Western blot analysis to examine whether TNF-α elicits the nuclear translocation of NF-κB p65 in K562 cells. As shown in Figure 5A, nuclear translocation of NF-κB p65 began to increase after 1 hour and peaked 24 hours after TNF-α treatment. The cytosolic GAPDH protein was solely detected in the cytosolic fraction, whereas the nuclear B23 protein was only present in the nuclear fraction, reflecting the purity of protein isolation (Figure 5A). The NF-κB inhibitor, Bay117082, suppressed TNF-α-mediated increases in c-Jun levels (Figure 5B). The inductive effect of TNF-α on c-Jun levels was significantly enhanced by NF-κB p65 in K562 cells (Figure 5C). These results suggest that TNF-α increased c-Jun levels via the NF-κB pathway.

3.5. Role of c-Jun in TNF-α-induced inhibition of activin A-mediated promoter activation and gene expression of globins

The luciferase activity of α-globin and ζ-globin reporters was analyzed to verify whether c-Jun affected the TNF-α-induced inhibition of activin A-induced globin promoter activation. K562 cells were cotransfected with the reporter plasmid, pHS40-α590 Luc (or

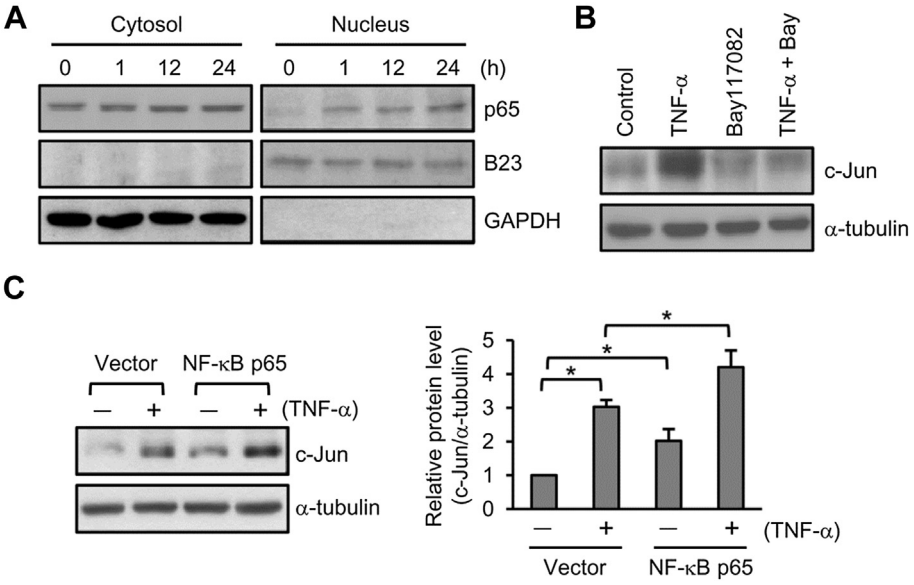

Figure 5 TNF-α increased c-Jun levels via NF-κB p65. (A) K562 cells were cultured with 50 ng/mL TNF-α for the indicated times. Subcellular fractionation was prepared, and protein extracts of the cytosol and nuclei were subsequently analyzed using Western blotting with antibodies against NF-κB p65 (p65), B23, and GAPDH. (B) Cells were pretreated with 5 μM Bay117082 for 30 minutes and then stimulated with 50 ng/mL of TNF-α. After 24 hours, the c-Jun level was measured. α-Tubulin was used as a loading control. (C) Cells were transfected with either a control plasmid (vector) or a plasmid encoding NF-κB p65 and then stimulated with 50 ng/mL TNF-α. After 24 hours, the c-Jun level was measured. α-Tubulin was used as a loading control (left panel). Immunoblots are representative of three experiments, which are shown as the mean ± SE. $^{*}p < 0.05$ (right panel). GAPDH = glyceraldehyde-3-phosphate dehydrogenase; NF-κB = nuclear factor kappa B; qPCR = quantitative polymerase chain reaction; SE = standard error; TNF-α = tumor necrosis factor-alpha.

pHS40-ζ597 Luc), and a plasmid encoding the c-Jun dominant-negative (DN) mutant, TAM-67. Figure 6A and B show that TNF-α inhibited the activation of the α-globin and ζ-globin promoters induced by activin A in the vector control group. However, inhibition of c-Jun transcriptional activity by TAM-67 abolished TNF-α inhibition of activin A-mediated promoter activation of α-globin and ζ-globin in K562 cells (Figure 6A and B). A qPCR analysis demonstrated that the c-Jun DN mutant abolished TNF-α-induced inhibition of activin A-mediated mRNA levels of α-globin and ζ-globin in K562 cells (Figure 6C). These results suggest that c-Jun participates in TNF-α-inhibited activin A-mediated globin promoter activation and gene expression.

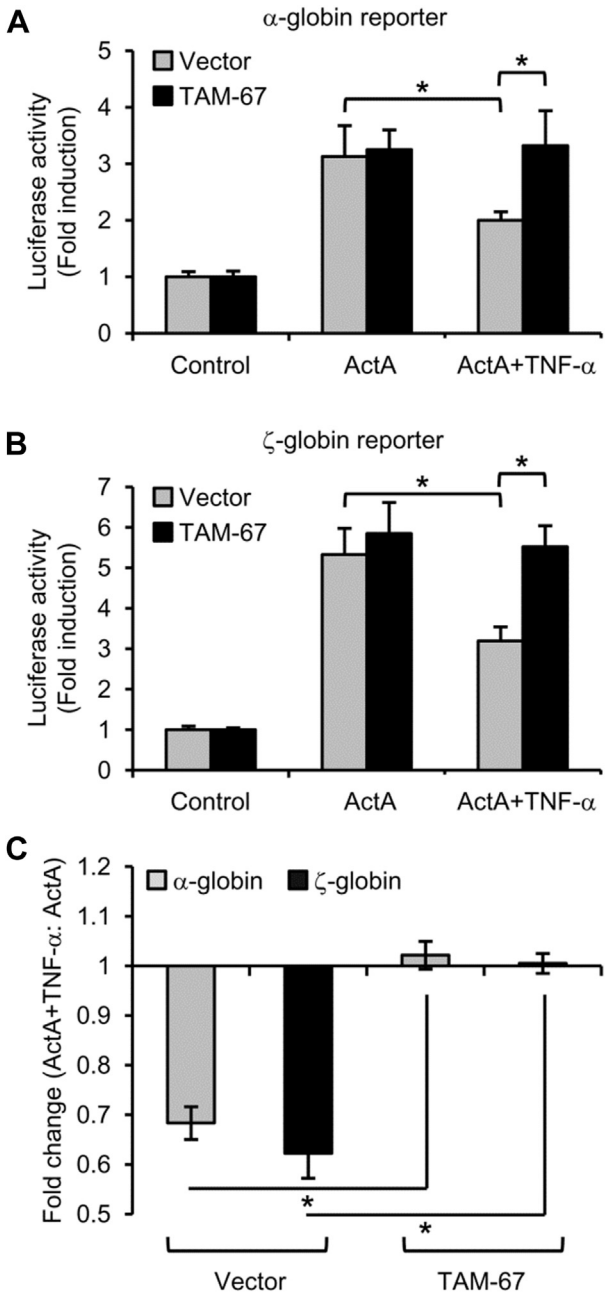

Figure 6 Effects of the c-Jun DN mutant, TAM-67, on TNF-α-inhibited promoter activity and expression of α-globin and ζ-globin. K562 cells were cotransfected with the pCMV-TAM-67 plasmid and reporter construct (A) pHS40-α590 Luc or (B) pHS40-ζ597 Luc. After 5 hours of transfection, cells were treated with or without (control) 50 ng/mL TNF-α. Luciferase activity was measured 24 hours after adding TNF-α. Values are expressed relative to the untreated control (normalized as 1). (C) Cells were transfected with the pCMV-TAM-67 plasmid. After 5 hours of transfection, cells were treated with 50 ng/mL activin A with or without 50 ng/mL TNF-α for 72 hours. qPCR results showed a significant fold change in mRNA levels after TNF-α treatment in activin A-induced cells compared with treatment with activin A alone. Values are shown as the mean ± SE of three experiments. $^*p < 0.05$. DN = dominant-negative; qPCR = quantitative polymerase chain reaction; SE = standard error; TNF-α = tumor necrosis factor-alpha.

3.6. *Role of c-Jun in TNF-α-induced inhibition of activin A-mediated erythroid-specific transcription factor expression*

We also analyzed the role of c-Jun in TNF-α-induced inhibition of activin A-mediated expression of GATA-1 and NF-E2 p45. A qPCR analysis showed that the c-Jun DN mutant abolished TNF-α-induced inhibition of activin A-mediated mRNA levels of GATA-1 and NF-E2 p45 in K562 cells (Figure 7). These results suggest that c-Jun participates in TNF-α-inhibited activin A-mediated erythroid-specific transcription factor expression.

4. Discussion

The present results show that TNF-α increased c-Jun levels through NF-κB (p65) activation. TNF-α-increased c-Jun inhibited activin A-mediated promoter activation of α-globin and ζ-globin. Furthermore, TNF-α also inhibited activin A-mediated expression of the erythroid genes, α-globin, ζ-globin, GATA-1, and NF-E2 p45 in K562 cells.

The anti-TNF-α antibody treatment increases hemoglobin levels in patients with anemia due to chronic disease.[20] An *in vitro* study showed that TNF-α can inhibit hematopoietic stem cell differentiation into erythroid-lineage cells.[23] In addition, TNF-α inhibits EPO-induced erythroid differentiation of TF-1 cells.[24] TNF-α can also inhibit aclacinomycin A-induced erythroid differentiation of K562 cells.[25] In the present study, we showed that TNF-α inhibited activin A-mediated expression of erythroid genes in K562 cells. We also found that activin A-induced erythroid gene expression was accompanied by decreased expression of stem/progenitor cell markers, whereas TNF-α directly inhibited the effects of activin A. These results suggest that TNF-α plays a negative role in erythroid differentiation, regardless of what differentiation inducers are

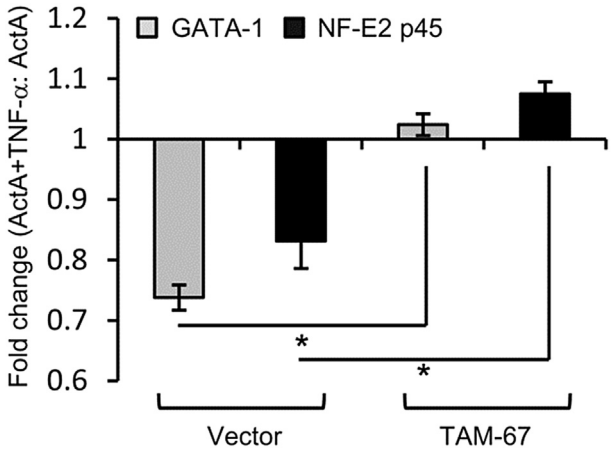

Figure 7 c-Jun participated in TNF-α-inhibited activin A induction of GATA-1 and NF-E2 p45 expression in K562 cells. Cells were transfected with the pCMV-TAM-67 plasmid. After 5 hours of transfection, cells were treated with 50 ng/mL activin A with or without 50 ng/mL TNF-α for 72 hours. qPCR results showed a significant fold change in mRNA levels after TNF-α treatment in activin A-cultured cells compared with treatment with activin A alone. Values are shown as the mean ± SE of three experiments. $^*p < 0.05$. qPCR = quantitative polymerase chain reaction; SE = standard error; TNF-α = tumor necrosis factor-alpha.

used. Furthermore, we demonstrated that an increasing concentration of activin A might reverse TNF-α-inhibited promoter activation of α-globin and ζ-globin genes. It was shown that bone marrow stromal cells exhibit regulatory effects on hematopoietic stem/progenitor cell differentiation and produce activin A.[26] Therefore, whether the interplay between TNF-α and activin A regulates erythroid differentiation in a bone marrow microenvironment will be further examined.

High levels of NF-κB expression has been observed in early erythroid progenitor cells, and this expression decreased with further differentiation.[27] NF-κB binds to promoters of *c-myb* and *c-myc* genes in early erythroid progenitor cells, suggesting that c-myb and c-myc may participate in NF-κB-mediated erythropoiesis inhibition.[27] However, downstream target genes of NF-κB that participate in erythropoiesis inhibition have not been identified. A previous study revealed that c-Jun inhibits erythroid differentiation of hematopoietic stem/progenitor cells.[14] NF-κB p65 can induce expression of AP-1-responsive genes.[11] It remains unknown whether NF-κB induces c-Jun expression, and whether c-Jun is essential for TNF-α-mediated inhibition of erythroid gene expression. Herein, we revealed that TNF-α induced c-Jun levels in K562 cells through the NF-κB pathway. Furthermore, TNF-α inhibited activin A-mediated erythroid gene expression, which was abolished by DN c-Jun. These results indicate that TNF-α inhibits erythroid gene expression via NF-κB/c-Jun.

Previous studies showed that c-Jun can inhibit dimethyl sulfoxide-induced erythroid differentiation of murine erythroleukemia cells.[13] c-Jun also inhibits STI571-induced erythroid differentiation of K562 cells.[28] In addition to inhibiting erythroid differentiation, c-Jun can also induce macrophage differentiation of U-937 cells[29] and monocytic differentiation of hematopoietic progenitor cells.[30] Elagib et al[14] have showed that c-Jun inhibits erythroid differentiation through its target gene, HERP2. Choi et al[31] have reported that c-Jun transactivates expression of the orphan nuclear receptor, SHP, to induce monocytic differentiation by the same receptor. These results suggest that the c-Jun transcription factor regulates different genes to regulate cell differentiation of specific hematopoietic lineages. In addition to HERP2, c-Jun's other target genes must be further screened and identified to determine how c-Jun inhibits erythroid differentiation.

In conclusion, the present study provides additional evidences that TNF-α inhibits activin A-mediated expression of the erythroid genes, α-globin, ζ-globin, GATA-1, and NF-E2 p45, through the increase of proto-oncogene c-Jun. In addition, our data suggest that the K562 cell line may be a valuable cell model for studying the mechanisms of TNF-α-inhibited activin A induction of erythroid differentiation.

Acknowledgments

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