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NF-κB suppresses HIF-1α response by competing for P300 binding

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ABSTRACT

Hypoxia has emerged as a key determinant of osteogenesis. HIF-1α is the transcription factor mediating hypoxia responses that include induction of VEGF and related bone induction. Inflammatory signals antagonize bone repair via the NF-κB pathway. The present investigation explored the functional relationship of hypoxia (HIF-1α function) and inflammatory signaling (NF-κB) in stem like and osteoprogenitor cell lines. The potential interaction between HIF-1α and NF-κB signaling was explored by co-transfection studies in hFOB with p65, HIF-1α and 9x-HRE-luc or HIF-1α target genes reporter plasmids. Nuclear cross-talk was directly tested using the mammalian Gal4/VP16 two-hybrid, and confirmed by co-immunoprecipitation/western blotting assays. The results show that inflammatory stimulation (TNF-α treatment) causes a marked inhibition of HIF-1α function at the HRE in all cell lines studied. Also, co-transfection with p65 expression vector leads to reduced hVEGFp transcription after DFO-induced hypoxia. However, TNF-α treatment had little effect on HIF-1α mRNA levels. The functional interaction of Gal4-HIF-1α and VP16-p300 fusion proteins is effectively blocked by expression of p65 in a dose dependent manner. It was concluded that NF-κB-mediated inflammatory signaling is able to block HIF-1α transactivation at HRE-encoding genes by direct competition for p300 binding at the promoter. Inflammation may influence the stem cell niche and tissue regeneration by influencing cellular responses to hypoxia.

1. Introduction

Bone formation and repair is dependent on the recruitment and differentiation of mesenchymal stem cells (or osteoprogenitor cells) in spatial and lineage specific ways. The many well orchestrated biological steps involve transcriptional regulation of the mesenchymal stem cell. Multiple environmental signals converge at the site of formation and repair within a systemic biologic context to establish a cell population capable of osteogenesis. Within bone marrow, hypoxia has emerged as one of the key determinants of this specific signaling of osteogenesis. The role of hypoxia on osteogenesis is clearly illustrated by deletion of the von Hippel-Lindau gene (*VHL*) that regulates cellular responses to hypoxia by the Hypoxia-Inducible Factor (HIF) pathway. In the absence of *VHL*, mice develop extremely dense, highly vascularized long bones. Suggested is a critical link between hypoxia, vascularization and osteogenesis [1].

HIF-1 is a heterodimeric transcription factor that is composed of a constitutively expressed HIF-1β subunit (also know as ARNT) and an oxygen-regulated HIF-1α subunit. HIF-1α abundance is controlled by ubiquitination and proteasomal degradation. During normoxia, HIF-1α is continuously synthesized and degraded, and degradation is triggered by binding of the von Hippel-Lindau tumor-suppressor protein (VHL), and posterior degradation by the 26S proteasome. Hypoxia results in HIF-1α stabilization, nuclear translocation and transcription of genes containing hypoxia response elements (HRE (5′-RCGTG-3′)). Bound HIF-1α/HIF-1β interacts with the coactivator protein p300 that results in transactivation at hypoxia responsive genes [2,3]. The VEGF gene promoter exemplifies that this regulation and this hypoxia-mediated mechanism is central to the observations made concerning $VHL^{-/-}$ as well as HIF-$1\alpha^{-/-}$ mice.

Bone repair is antagonized by inflammation. Several different investigations have shown that inflammatory stimuli reduce or preclude bone repair or bone formation. For example, TNF-α treatment of osteoprogenitor cells blocks induced osteoblast differentiation. One possible mechanism is the TNF-α mediated antagonism of bone morphogenetic protein signaling [4]. Another possible effect of inflammatory signaling in osteoblastic cells is the direct inhibition of key transcriptional regulators such as Osterix [5,6]. Although HIF-1α has a well defined role in positive regulation of vascularization through transactivation of VEGF and is further considered important in the process of wound repair, initial studies of inflammatory modulation of osteoblastic differentiation suggested that inflammatory signaling strongly antagonized HIF-1α function

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in osteoprogenitor cells. The aim of this investigation was to investigate how inflammatory signaling inhibited HIF-1α function in different cell lines. These studies demonstrate that inflammatory signaling mediated by NF-κB is able to block transactivation at HRE-encoding genes by HIF-1α by direct competition for p300 binding at the promoter.

2. Materials and methods

2.1. Cell culture and reagents

Human osteoblast-like cells derived from osteosarcoma (MG63), human fetal osteoblasts (hFOB 1.19), mouse osteoblast-like cells (MC3T3-E1), and murine C3H10T½ multipotential mesenchymal cells were obtained from American Type Culture Collection (Manassas, VA). Cells were grown in recommended media, supplemented with 10% FBS and 100 units/ml penicillin/streptomycin, from Sigma–Aldrich (St. Louis, MO). Growth medium was changed every 3 days. Recombinant tumor necrosis factor alpha (TNF-α, 10 ng/ml), lipopolysaccharide (LPS *Escherichia coli*, 1 μg/ml), and desferrioxamine (DFO, 10 μM) were obtained from Sigma.

2.2. Plasmids and luciferase reporter assay

The wild type hemagglutinin (HA) tagged HA-HIF-1α-pcDNA3 (HA-HIF-1α WT), and the constitutively active HA-HIF-1α P402A/P564A-pcDNA3 (HA-HIF-1α-Δpro2) were obtained from Addgene (www.addgene.org) (plasmids 18949 and 18955, William G. Kaelin). The p65-CMV and IκBα-SR-CMV expression vectors were kindly provided by Dr. Albert S. Baldwin (University of North Carolina, Chapel Hill).

The luciferase reporter plasmid 9x-HRE-luc was generated by subcloning nine copies of the Hypoxia Responsive Element (HRE) sequence (5′-GTGACTA**CGTG**CTGCCTAG-3′) [7] into the *SmaI* digested pGL3 promoter vector (Promega, Madison, WI). The human iNOS promoter was amplified by PCR using an upstream primer 5′-CAGGTACCACAAGGCAGAACCAGCTACATC-3′ and a downstream primer 5′-TACTCGAGGCAGGAATGAGGCTGAGTTCT-3′. The region subcloned extended from nucleotides −1659 to +72 and was inserted into KpnI and XhoI digested pGL3 basic vector (Promega). The human VEGF promoter was amplified by PCR using an upstream primer 5′-CAGGTACCAGACGTTCCTTAGTGCT-3′ and a downstream primer 5′-TACTCGAGGCGGACGCTCAGTGAAG-3′. The region subcloned extended from nucleotides −642 to +373 and was inserted into KpnI and XhoI digested pGL3 basic plasmid. Transfections included a constitutively expressed *Renilla* luciferase plasmid (pRL-TK), using the SureFECT reagent (SABiosciences, Frederick, MD). Luciferase activity was determined using the Dual Luciferase assay (Promega) and normalized to *Renilla* luciferase activity. Luminescence was measured in a Lumat LB 9507 (Berthold Technologies, BadWildbad, Germany).

2.3. Mammalian two-hybrid assay

Protein–protein interactions between HIF-1α, p65 and p300 were evaluated with the CheckMate/Flexi Vector Mammalian Two-Hybrid System (Promega). The CAD region of HIF-1α [8] or p65 [9] were amplified by PCR, and cloned into *PmeI/SgfI* digested pFN11A (BIND) flexi vector. The CH1 domain of p300 [10] was amplified by PCR, and cloned into VP16-encoding pFN10A (ACT) flexi vector. All constructs were verified by DNA sequencing. The interactions between HIF-1α and p300 were monitored using pGL4.31[*luc*2P/*GAL4*UAS/Hygro]. Positive and negative controls were used according to manufacturer's instructions. Transfection procedures and assessment of luciferase activity were as described above.

2.4. Adenoviral transduction

High titer adenovirus stocks encoding p65 and IκBα-SR were obtained from the University of North Carolina Vector Core Facility (Chapel Hill, NC, USA). Cells were transduced with adenoviral vectors at a multiplicity of infection (MOI) of 2000 in serum-free culture medium for 60 min, to induce transgene expression. Subsequently, virus was aspirated, and regular growth medium added.

2.5. RNA extraction and real-time PCR

Total RNA from hFOBs was isolated using Trizol Reagent (Invitrogen, Carlsbad, CA) and quantified using Nanodrop (Thermo Scientific, Wilmington, DE). cDNAs were generated using Superscript III Reverse Transcriptase (Invitrogen). Real-Time PCR (Real-Time SYBR Green/ROX PCR master mix, SABiosciences) was used to measure the mRNA expression levels of HIF-1α, GLUT-1, iNOS and VEGF. Relative mRNA abundance was determined by the $-\Delta\Delta$Ct method and reported as fold induction. ACTB abundance was used for normalization.

2.6. Co-immunoprecipitation analysis

Total cell extracts were precleared by preimmune mouse IgG for 30 min with protein A-Agarose. Samples were incubated with anti-VP16 (14-5) antibody overnight at 4 °C and the immune complexes were recovered with protein A-Agarose for 4 h at 4 °C. The beads were then washed three times with PBS, subjected to SDS–PAGE, and immunoblotted with anti-HA-Peroxidase high affinity (3F10) (Roche, Indianapolis, IN), anti-FLAG M2-Peroxidase (Sigma) or anti-VP16. The antibodies against IgG and VP16 and protein-A agarose were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

2.7. Western blot analysis

Proteins were separated by 10% SDS–PAGE and incubated with anti-HA or anti-FLAG. After incubation with the appropriate HRP-conjugated secondary antibody, the antigens were detected using ECL Plus (Amersham Biosciences, Pittsburgh, PA).

2.8. Statistical analysis

For luciferase reporter assay and real-time PCR analysis, each experiment was done in triplicate. The mean and the standard deviation of the results were calculated and plotted. Student's *t* test was used to compare two treatments. The level of significance used was $p \leqslant 0.05$.

3. Results

3.1. Inflammation decreases HIF-1α activity at the HRE on different cell lines

The 9x-HRE-luc reporter vector was responsive to DFO-induced hypoxia (almost 40-fold induction). In normoxia, a slight decrease for the HIF-1α activity at the HRE could be observed when hFOB cells were treated with TNF-α, but not after LPS treatment. Likewise, in the presence of induced hypoxia, TNF-α but not LPS, decreased the HIF-1α activity at the HRE (Fig. 1A). To evaluate the effects of inflammation on hypoxia, stem-like and osteoblast-like cells were co-transfected with HA-HIF1α-Δpro2 and 9x-HRE-luc. Cells were subsequently treated with p65 or IκBα-SR-encoding virus, pro-inflammatory stimuli or cytokines. After DFO-induced hypoxia (H), it was observed an increase in HIF-1α activity at the HRE in all cell types studied. After TNF-α treatment, in normoxic conditions, a decrease in HIF-1α activity was seen for osteoblastic

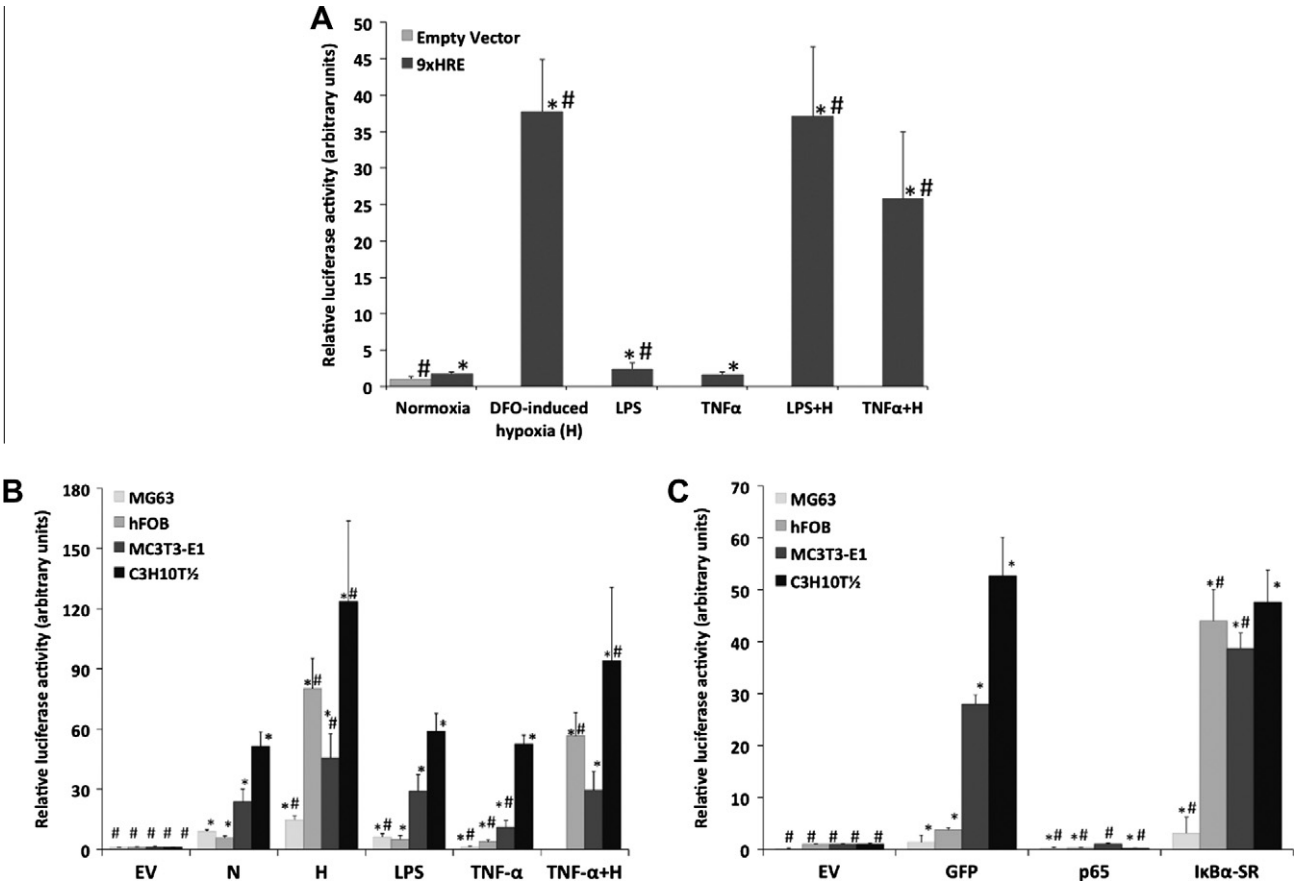

Fig. 1. *Hypoxia-induced HIF-1α activity was reduced by TNF-α.* (A) hFOBs were transfected with 9x-HRE-luc reporter construct. After 18 h, cells were treated with DFO-induced hypoxia (H), LPS, TNF-α, LPS + H or TNF-α + H. Luciferase activity was measured 24 h post-treatment. * Statistically significant difference compared to pGL3p (Empty Vector/Normoxia) ($p \leqslant 0.05$). # Statistically significant difference compared to 9x-HRE-luc (9x-HRE-luc/Normoxia) ($p \leqslant 0.05$). *LPS treatment decreased HIF-1α activity at the HRE.* (B) Osteoblast-like and stem-like cells were co-transfected with HA-HIF-1α-Δpro2 and 9x-HRE-luc. After 8 h, cells were exposed to Normoxia (N), H, LPS, TNF-α or TNF-.α + H. Luciferase activity was measured 36 h post-treatment. * Statistically significant difference compared to pcDNA3.1 + pGL3p (EV, non-stimulation) ($p \leqslant 0.05$). # Statistically significant difference compared to 9x-HRE-luc + HA-HIF-1α-Δpro2 (N) ($p \leqslant 0.05$). *p65 treatment blocked HRE activity.* (C) Osteoblast-like and stem-like cells were co-transfected with HA-HIF-1α-Δpro2 and 9x-HRE-luc. After 8 h, cells were transduced with p65 or IκBα-SR-encoding virus for 60 min. Luciferase activity was measured 36 h post-transduction. * Statistically significant difference compared to pcDNA3.1 + pGL3p + GFP-encoding virus (EV) ($p \leqslant 0.05$). # Statistically significant difference compared to 9x-HRE-luc + HA-HIF-1α-Δpro2 + GFP-encoding virus (GFP) ($p \leqslant 0.05$). The relative activity in all experiments was normalized with *Renilla* luciferase activity. Data are shown as the mean ± SE of three independent experiments performed in triplicate.

cells only. In hypoxia, a decrease in HIF-1α activity at the HRE was noticed for all cell lines after TNF-α treatment. However, after LPS treatment the HIF-1α activity at the HRE decreased slightly for MG63 and hFOB cells, and increased for C3H10T½ and MC3T3-E1 compared to normoxia (Fig. 1B). After p65-encoding virus transduction, the HIF-1α function at the HRE was completely blocked for all cell types. However, when cells were transduced with the IκBα-SR virus, which blocks NF-κB function, the HIF-1α activity was restored to levels that surpassed the control for hFOB, MG63 and MC3T3-E1 cells (Fig. 1C). Transduction with p65-encoding virus indicates that signaling via NF-κB resulted in marked reduction in transcription via the HRE.

3.2. NF-κB caused minor changes in HIF-1α and HIF-1α target genes mRNA expression

To begin to define how p65 reduced HRE-mediated function, the abundance of HIF-1α mRNA was explored. In the presence of DFO-induced hypoxia, increased mRNA levels were observed for HIF-1α target genes in hFOB cells. mRNA levels of HIF-1α, GLUT-1 and VEGF remained at baseline levels after LPS or TNF-α treatment. HIF-1α was responsive to the combination of hypoxia and inflammatory signaling. Likewise, addition of DFO resulted in elevated GLUT-1 and VEGF expression beyond LPS or TNF-α stimulation. For iNOS mRNA level, a 7.1- and 20.4-fold induction was observed when LPS and TNF-α treatment were administered in addition to DFO, respectively. This synergy (DFO plus either LPS or TNF-α) was observed for all genes, especially for iNOS and VEGF (Fig. 2A).

In an attempt to further explore these results, potential NF-κB (κB) and HIF-1α (HRE) binding sites were identified in HIF-1α and its target genes (MatInspector, Genomatix, Munich, Germany). Based on that and to further investigate the mechanism of the synergistic interaction between LPS or TNF-α and hypoxia at the transcriptional level for iNOS and VEGF, functional studies in hFOBs transfected with either the promoter of iNOS or VEGF luciferase reporter plasmids were performed.

3.3. Endogenous NF-κB did not alter DFO-mediated iNOS and VEGF promoter activity

A 6.4- and 20.3-fold increase was observed for iNOS and VEGF promoter activity, respectively, when hFOB cells were exposed to DFO-induced hypoxia (H). Treatment with either LPS or TNF-α did not alter DFO-mediated iNOS and VEGF promoter activity (Fig. 2B).

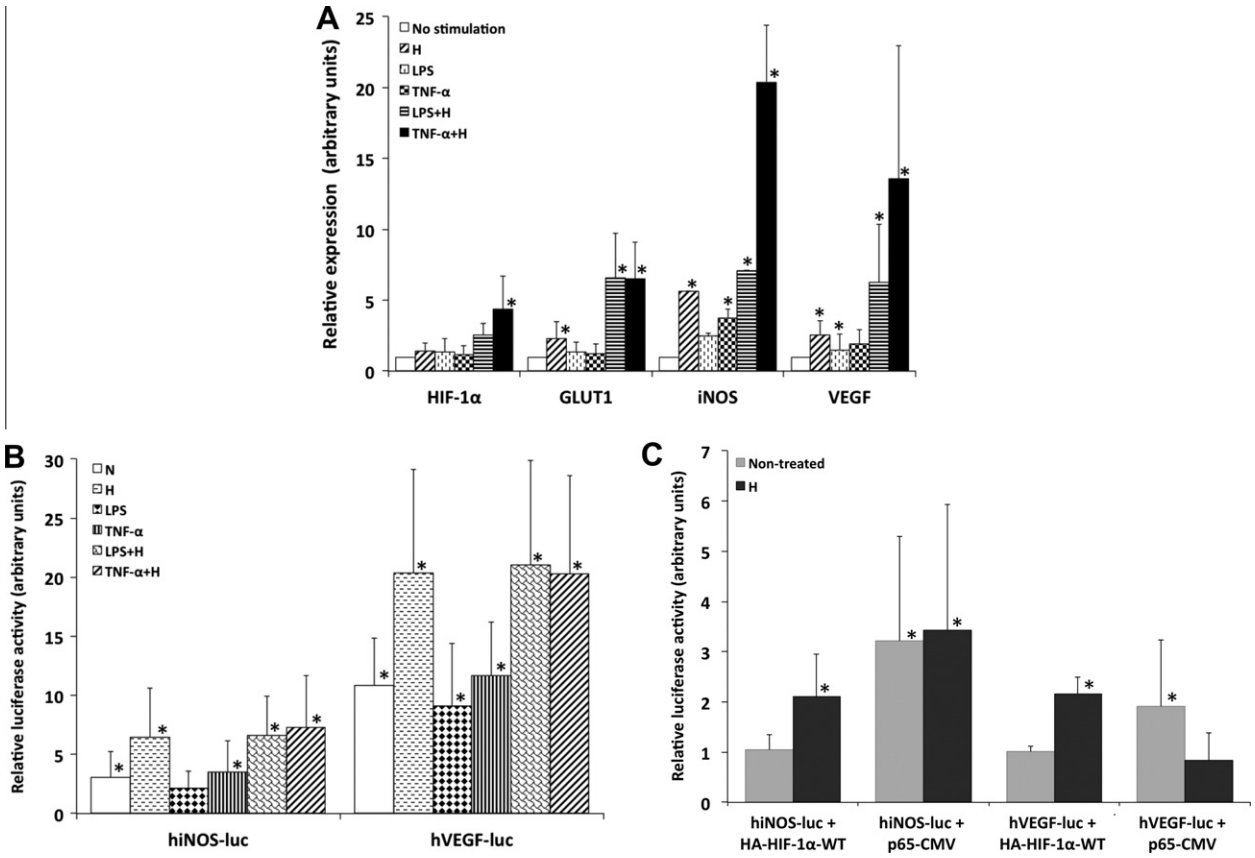

Fig. 2. *p65 caused minor changes in HIF-1α and HIF-1α target genes mRNA expression.* (A) hFOB cells were exposed to H, LPS, TNF-α, LPS + H or TNF-α + H for 6 h. Expression of HIF-1α, GLUT-1, iNOS and VEGF mRNA was examined by quantitative RT–PCR. Data are shown as the mean ± SE of three independent experiments performed in triplicate. * Statistically significant difference compared to control (no stimulation) ($p \leqslant 0.05$). *Endogenous NF-κB did not alter DFO-mediated iNOS and VEGF promoter activity.* (B) hFOBs were transfected with hiNOS-luc or hVEGF-luc reporter constructs. Eighteen hours after transfection, cells were exposed to N, H, LPS, TNF-α, LPS + H or TNF-α + H. Luciferase activity was measured 24 h post-treatment. The relative activity was normalized with *Renilla* luciferase activity. Data are shown as the mean ± SE of three independent experiments performed in triplicate. * Statistically significant difference compared to pGL3p (N = Normoxia = non-treated) ($p \leqslant 0.05$). *NF-κB reduced transcription of VEGF promoter.* (C) hFOBs were co-transfected with hiNOS-luc or hVEGF-luc and HA-HIF-1α WT or p65-CMV. Eighteen hours after transfection, cells were exposed to N or H. Luciferase activity was measured 24 h post-treatment. The relative activity was normalized with *Renilla* luciferase activity. Data are shown as the mean ± SE of three independent experiments performed in triplicate. * Statistically significant difference compared to pcDNA3.1 + pGL3p in normoxia/non-treated ($p \leqslant 0.05$). N = Normoxia = Non-treated, H=DFO-induced hypoxia.

3.4. NF-κB reduced transcription of VEGF promoter

In the presence of hiNOS-luc and p65-CMV co-transfection, hFOBs presented increased hiNOS transcription, compared to cells co-trasfected with hiNOS-luc and HA-HIF-1α-WT. Co-transfection of hFOBs with p65-CMV expression vector led to reduced hVEGF-luc transcription after DFO-induced hypoxia (Fig. 2C).

3.5. p65 Competes with HIF-1α for p300

Given the lack of evidence showing changes in HIF-1α abundance following treatment with LPS or TNF-α, the possibility that p65 interferes with HIF-1α transcriptional activation via p300 was directly investigated. It was hypothesized that the blockade of HRE function by p65 (Fig. 1C) was a result of p65 interference with HIF-1α binding to the p300 coactivator. To gain further insight regarding the possible relationship of p65, HIF-1α and p300, the interactions between p300, HIF-1α and p65 were studied by mammalian Gal4/VP16 two-hybrid system, and confirmed by co-immunoprecipitation/western blotting assays. After co-transfection with VP16-p300, Gal4-HIF-1α-CAD fusion constructs and p65-CMV, HIF-1α transactivation activity was decreased, suggesting that p65 interfered with HIF-1α for p300 binding (Fig. 3A). To check the effect of endogenous p65, hFOB cells were co-transfected with the VP16-p300/ Gal4-HIF-1α-CAD fusion constructs and then subjected to DFO-induced hypoxia (H), LPS or TNF-α treatment for 24 h. Both LPS and TNF-α decreased HIF-1α transactivation (Fig. 3B). When re-ordering the experiment by co-transfection with VP16-p300, Gal4-p65-CAD fusion constructs and HA-HIF1α-WT, p65 transactivation activity was less affected by HIF-1α, suggesting that p300 preferably binds to p65 rather than HIF-1α (Fig. 3C). Consistent with the notion that endogenous p65 affects p300-HIF-1α activity, co-transfection with the dominant-negative regulator IκBα-SR led to increased HRE function (Fig. 1C) as well as increased p300-mediated transcription in the two-hybrid assay (Fig. 3A). To further confirm that the antagonistic response at the HRE was due to competition for the p300 coactivator, HRE-mediated transcription in hFOB was monitored following co-transfection with a constitutively active HIF-1α, and increasing concentrations of p65-CMV expression plasmid. Under hypoxic conditions, increasing amounts of p65 reduced HIF-1α activity, in a dose dependent manner (Fig. 3D, black bars). Increasing the amount of HA-HIF1α-Δpro2 under conditions where p65-CMV was constant did not overcome the observed p65-mediated reduction in HRE transcription (Fig. 3E, black bars).

The physical interaction between p300, HIF-1α and p65 was also confirmed by co-immunoprecipitation/Western Blot assay, and when increasing amounts of FLAG-p65-CMV were used,

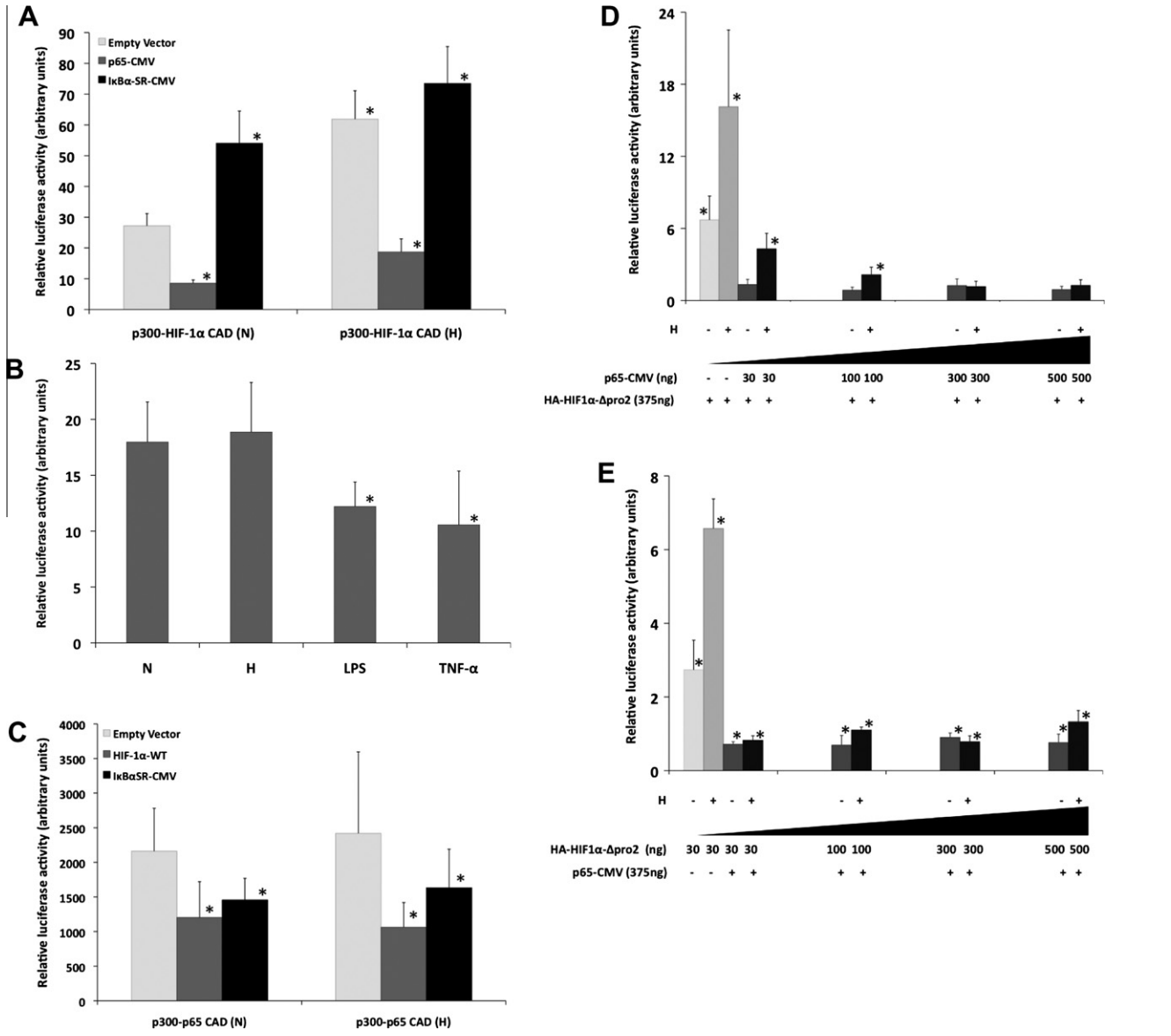

Fig. 3. *p65 competes with HIF-1α for p300.* hFOBs were (A) co-transfected with VP16-p300, Gal4-HIF-1α-CAD fusion constructs (125 ng) and p65- or IκBα-SR-CMV (375 ng) for 18 h. Cells were then exposed to Normoxia (N) or DFO-induced Hypoxia (H). Luciferase activity was measured 24 h post-treatment. * Statistically significant difference compared to p300 + HIF-1α-CAD + Empty Vector (normoxia) ($p \leqslant 0.05$). (B) co-transfected with the fusion constructs described above for 18 h. Then, they were subjected to N, H, LPS or TNF-α treatments. Luciferase activity was measured 24 h post-treatment. * Statistically significant difference compared to normoxia (N) ($p \leqslant 0.05$). (C) co-transfected with VP16-p300, Gal4-p65-CAD fusion constructs (125 ng) and HIF-1α-WT or IκBα-SR-CMV (375 ng) for 18 h. Cells were then exposed to N or H. Luciferase activity was measured 24 h post-treatment. * Statistically significant difference compared to p300 + p65-CAD + Empty vector (N) ($p \leqslant 0.05$). (D) co-transfected with 9x-HRE-luc (125 ng), HA-HIF-1α-Δpro2 at a constant concentration (375 ng) and p65-CMV at increasing concentrations of 30, 100, 300 and 500 ng for 18 h and subsequently subjected to DFO treatment. Luciferase activity was measured 24 h post-treatment. * Statistically significant difference compared to pcDNA3.1 + 9x-HRE-luc (no treatment) ($p \leqslant 0.05$). (E) co-transfected with 9x-HRE-luc (125 ng), p65-CMV at a constant concentration (375 ng) and HA-HIF-1α-Δpro2 at increasing concentrations of 30, 100, 300 and 500 ng for 18 h and subsequently subjected to DFO treatment. Luciferase activity was measured 24 h post-treatment. The relative activity was normalized with *Renilla* luciferase activity. * Statistically significant difference compared to pcDNA3.1 + 9x-HRE-luc (no treatment) ($p \leqslant 0.05$). Data are shown as the mean ± SE of three independent experiments performed in triplicate.

decreased HA-HIF-1α protein levels were observed (Figs. 4A,C). However, FLAG-p65 protein levels remain constant after an increase in HA-HIF-1α double mutant construct (Figs. 4B,D).

4. Discussion

The interactions between NF-κB inflammatory mediators and hypoxia have been investigated in different cell types [11–13]. Bharti et al.[14] have proposed that NF-κB and HIF-1α act at same target genes. Frede et al. [15] showed that LPS induced HIF-1α mRNA expression in monocytes was mediated by a NF-κB site located at +130 bp in the HIF-1α promoter. Functional studies of the iNOS promoter demonstrated that the synergistic interaction between LPS and hypoxia was mediated by the NF-κB and HIF-1α binding sites [11]. The activity of the κB sites in the iNOS promoter could explain elevated iNOS mRNA levels in the presence of LPS and TNFα in this study. Lukiw et al. [16] reported that the human VEGF promoter has at least five HIF-1α DNA-binding sites but no obvious NF-κB DNA-binding consensus sequence. On the other hand, Ramanathan et al. [17] reported the existence of 2 NF-κB binding sites at the murine VEGF promoter. In the present analysis, VEGF transcription was reduced after co-transfection of hFOBs

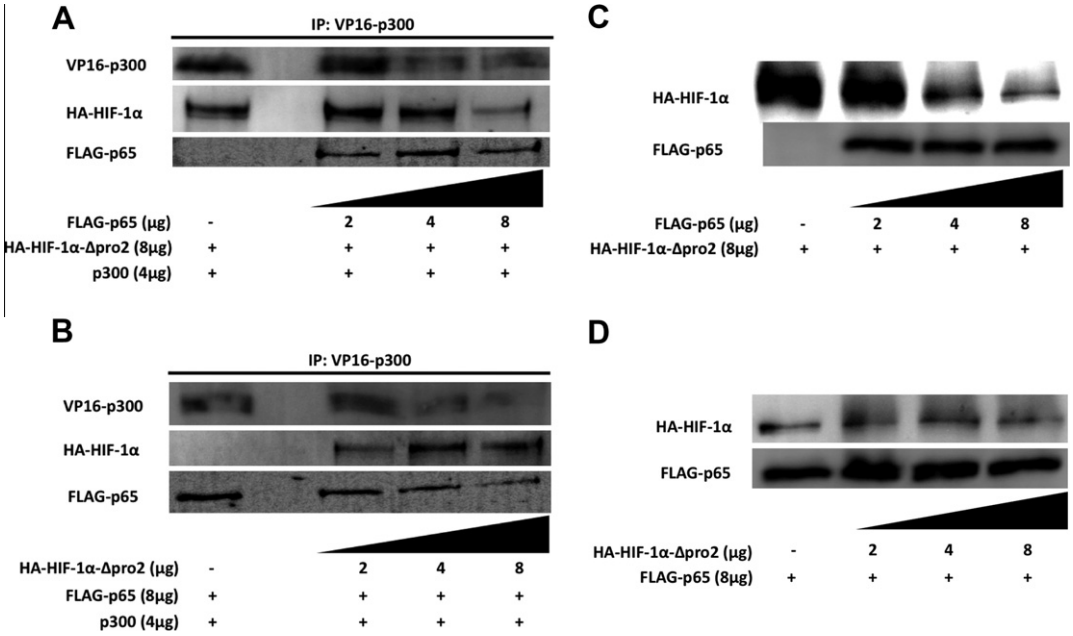

Fig. 4. *p65 competes with HIF-1α for p300.* IP/WB in hFOBs of (A) VP16-p300, FLAG-p65 and HA-HIF-1α following transfection with increasing concentrations of FLAG-p65 (2, 4 and 8 μg) and constant amount of HA-HIF-1α-Δpro2 (8 μg) and VP16-p300 (4 μg). (B) VP16-p300, FLAG-p65 and HA-HIF-1α following transfection with increasing concentrations of HA-HIF-1α-Δpro2 (2, 4 and 8 μg) and constant amount of FLAG-p65 (8 μg) and VP16-p300 (4 μg). WB in hFOBs of (C) FLAG-p65 and HA-HIF-1α after transfection with increasing concentrations of FLAG-p65 (2, 4 and 8 μg) and constant amount of HA-HIF-1α-Δpro2 (8 μg). (D) FLAG-p65 and HA-HIF-1α after transfection with increasing concentrations of HA-HIF-1α-Δpro2 (2, 4 and 8 μg) and constant amount of FLAG-p65 (8 μg).

with p65 expression vector, suggesting that NF-κB interfered with VEGF expression.

Whether LPS or TNF-α leads to an increase in either HIF-1α or HIF-1α target genes mRNA, there exists conflicting results which may be caused by the different cell types and treatment conditions explored by several investigators [11,13,18–20]. This and other investigations demonstrated that TNFα and LPS treatment did not reduce HIF-1α and its target genes at the mRNA level. Such cross-talk between HIF-1α and NF-κB in HIF-1α target genes requires further exploration.

The integration of signaling may occur in the cytoplasm and at multiple levels as described above. However, transcriptional signaling may converge within the nucleus. The p300 is a common coactivator for both HIF-1α [21] and NF-κB pathways [22]. There has not been any exploration of HIF-1α and NF-κB interactions in terms of competition for the p300 co-activator. On the other hand, a similar mechanism has been explored for other genes [23–25]. Yu et al. [26] demonstrated that p300 physically associated with p65 rather than Smad4 in the presence of both TNF-α and TGF-β.

The significance of the interaction between p300-HIF1α-NF-κB should be considered in the context of wound healing, since both transcription factors are essential in this respect. Some reports link NF-κB and HIF-1α to wound healing and most of them used diabetic (*db/db*) mouse model [27–29].

Inflammatory signaling mediated by NF-κB is able to block HIF-1α transactivation at HRE-encoding genes by direct competition for p300 binding at the promoter. Inflammation may influence the stem cell niche and tissue regeneration by influencing cellular responses to hypoxia. Even though the influence of hypoxia on the outcome of inflammation is widely accepted, many molecular and cellular mechanisms mediating this response remain to be elucidated.

Acknowledgments

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