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An Intact Canonical NF- κ B Pathway Is Required for Inflammatory Gene Expression in Response to Hypoxia

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Hypoxia is a feature of the microenvironment in a number of chronic inflammatory conditions due to increased metabolic activity and disrupted perfusion at the inflamed site. Hypoxia contributes to inflammation through the regulation of gene expression via key oxygen-sensitive transcriptional regulators including the hypoxia-inducible factor (HIF) and NF- κ B. Recent studies have revealed a high degree of interdependence between HIF and NF- κ B signaling; however, the relative contribution of each to hypoxia-induced inflammatory gene expression remains unclear. In this study, we use transgenic mice expressing luciferase under the control of NF- κ B to demonstrate that hypoxia activates NF- κ B in the heart and lungs of mice in vivo. Using small interfering RNA targeted to the p65 subunit of NF- κ B, we confirm a unidirectional dependence of hypoxic HIF-1 α accumulation upon an intact canonical NF- κ B pathway in cultured cells. Cyclooxygenase-2 and other key proinflammatory genes are transcriptionally induced by hypoxia in a manner that is both HIF-1 and NF- κ B dependent, and in mouse embryonic fibroblasts lacking an intact canonical NF- κ B pathway, there is a loss of hypoxia-induced inflammatory gene expression. Finally, under conditions of hypoxia, HIF-1 α and the p65 subunit of NF- κ B directly bind to the cyclooxygenase-2 promoter. These results implicate an essential role for NF- κ B signaling in inflammatory gene expression in response to hypoxia both through the regulation of HIF-1 and through direct effects upon target gene expression. *The Journal of Immunology*, 2011, 186: 1091–1096.

Chronic inflammation underpins a number of important diseases including rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). Chronically inflamed tissues are characterized by a number of environmental features including metabolic acidosis and hypoxia (1–3). It has recently become clear that such features of the microenvironment can affect disease progression (4). For example, hypoxia can alter transcriptional responses in the inflamed tissue (2, 4). Proinflammatory genes increased in response to hypoxia include cytokines, chemokines, adhesion molecules, and enzymes, all of which can contribute to the development of inflammation (5). By developing our understanding of the mechanisms underpinning the regulation of these proinflammatory factors in hypoxic inflammation, we will identify new targets in diseases such as RA and IBD, which have severe morbidity and limited current therapeutic opportunities. The molecular mechanisms underpinning hypoxia-induced expression of inflammatory genes likely involve the activation of two hypoxia-

responsive transcriptional regulators, the hypoxia-inducible factor (HIF) and NF- κ B (6). However, the relative role of each of these in mediating hypoxia-induced inflammatory gene expression is unclear.

HIF is a heterodimeric transcription factor consisting of oxygen-regulated HIF α and constitutively expressed HIF β subunits. The oxygen sensitivity of the HIF pathway has been an area of intense investigation in recent years, and the identification of a family of oxygen-dependent HIF hydroxylases that confer oxygen sensitivity to this pathway (by regulating HIF α stability) has greatly enhanced our understanding of cellular oxygen sensing (7). Although our understanding of HIF-dependent processes in hypoxia is well developed, the role of NF- κ B in the global transcriptional response to hypoxia has only recently begun to become clear. Recent evidence has indicated that the hypoxic responsiveness of the NF- κ B pathway may be conferred by components of this pathway being targets for the same hydroxylases that confer hypoxic sensitivity to HIF. Evidence for this comes from experiments demonstrating that hydroxylase inhibition with either a chemical inhibition (8) or by RNA interference results in enhanced NF- κ B activity (8, 9) and the demonstration by mass spectrometry of hydroxylation of ankyrin repeat domains in various components of the NF- κ B pathway (10). However, the specific hydroxylation targets important in hypoxia-induced NF- κ B signaling remain to be identified.

Recent studies have demonstrated both in vitro and in vivo that NF- κ B and HIF signaling are strongly interdependent with NF- κ B playing an important role in basal and stimulated HIF-1 α mRNA expression (11). Conversely, HIF has been reported to affect NF- κ B signaling in neutrophils (12). Furthermore, a number of proinflammatory genes that contribute to inflammation including inducible NO synthase and cyclooxygenase-2 (COX-2) contain functional response elements for both NF- κ B and HIF in their promoters,

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Abbreviations used in this article: COX-2, cyclooxygenase-2; HIF, hypoxia-inducible factor; IBD, inflammatory bowel disease; MEF, mouse embryonic fibroblast; RA, rheumatoid arthritis; siRNA, small interfering RNA.

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although the relative contribution of these transcriptional regulators remains controversial. For example, whereas it is clear that the prostaglandin-synthesizing enzyme COX-2 is induced at the transcriptional level during hypoxia, this has been variously attributed to being mediated by HIF (13–16) or NF- κ B (14, 17).

In the current study, we have investigated the impact of hypoxia on NF- κ B activity *in vivo* using a luciferase reporter mouse and have shown for the first time, to our knowledge, that hypoxia activates NF- κ B in heart and lungs in mice exposed to normobaric atmospheric hypoxia. Furthermore, we demonstrate that knock-down of the canonical NF- κ B pathway in cultured cells results in suppression of the hypoxic induction of HIF-1 α . Finally, the hypoxic inducibility of inflammatory genes including COX-2 that are under the control of HIF is also strongly dependent upon the presence of an intact canonical NF- κ B signaling pathway. We propose that NF- κ B through both its direct regulation of inflammatory genes and its regulation of HIF is a central mediator and thus a potential therapeutic target in hypoxic inflammation.

Materials and Methods

In vivo imaging

Female mice ubiquitously expressing a transgene encoding firefly luciferase under the control of a concatamer of NF- κ B response elements (Caliper LS) were exposed to either normoxia or normobaric hypoxia (as outlined later). Immediately after treatment, the mice were anesthetized and injected intraperitoneally with luciferin (150 mg/kg). *In vivo* luciferase activity (indicative of NF- κ B activation), measured as photons/s/cm²/steradian, was quantified using Living Image Software (version 3.0.2; Caliper LS). After this, organs were harvested for *ex vivo* imaging. The time between removal of mice from hypoxia and imaging of organs was no more than 15 min. Because of the time taken for gene and subsequent protein expression, this period of reoxygenation is not a confounding factor in these experiments.

Cell culture and hypoxia

HeLa cells were incubated according to standard protocols. Mouse embryonic fibroblasts (MEFs) and Caco-2 cells were cultured with DMEM high-glucose medium supplemented with 10% FCS and 100 U/ml penicillin-streptomycin. Cells were exposed to hypoxia using pre-equilibrated media and maintained in standard normobaric hypoxic conditions (1% O₂, 5% CO₂, and 94% N₂) in a hypoxia chamber (Coy Laboratories). Normoxic controls were exposed to pre-equilibrated normoxic media and maintained at atmospheric O₂ levels (21% O₂, 5% CO₂) in a tissue culture incubator. For tissue COX-2 measurements, adult male pathogen-free C57BL/6J mice were exposed to hypoxia (8% normobaric atmospheric oxygen) in hypoxic incubation cabinets. For *in vivo* imaging experiments, female mice containing the luciferase transgene were exposed to hypoxia (10% normobaric atmospheric oxygen). All *in vivo* experiments were carried out with full institutional ethical approval and according to national guidelines for animal experimentation.

Western blotting

Whole-cell or nuclear extracts were generated in either normoxia or hypoxia according to previously published protocols (18). Protein concentration was quantified using a Bradford assay, and samples were normalized accordingly. Samples were separated by SDS-PAGE and immunoblotted as described previously (8) using the following primary Abs and dilutions: HIF-1 α (1:250; BD Pharmingen), NF- κ B p65 (1:1000; Santa Cruz Biotechnology), COX-2 (1:200; Santa Cruz Biotechnology), IKK α (1:1000; Cell Signaling), IKK β (1:1000; Cell Signaling), TLR-3 (1:1000; eBioscience), and β -actin (1:10,000; Sigma).

PGE₂ assay

Confluent monolayers of HeLa cells were grown on 35-mm dishes in 1 ml medium. Cells were exposed to hypoxia as outlined earlier. At the end of the time course, medium was removed from the cells. PGE₂ levels were measured using PGE₂ express enzyme immunoassay kit (Cayman Chemicals) according to the manufacturer's instructions.

DNA binding assay

HeLa cells were exposed to experimental conditions as outlined earlier. Nuclear lysates were generated, and samples were normalized to protein

content. Nuclear HIF-1 α and p65 levels were determined by measuring interaction with the relevant immobilized DNA response elements using an assay kit according to the manufacturer's instructions (TransAM assay; Active Motif).

RNA interference

Smart pool on-target small interfering RNA (siRNA) oligonucleotides against HIF-1 α or p65 or nontarget siRNA were purchased from Dharmacon. HeLa cells or Caco-2 cells were cultured to ~50% confluence on 35-mm plates. siRNA (5 nM final concentration in HeLa cells or 80 nM final concentration in Caco-2 for HIF-1 α and 20 nM final concentration in HeLa cells or 80 nM final concentration in Caco-2 cells for p65 or equivalent amounts of nontarget control siRNA) transfection was carried out as described previously (8). Cells were exposed to experimental conditions 48 h after this initial siRNA transfection.

Real-time PCR

RNA extraction was carried out using an RNase Mini Kit according to the manufacturer's instructions (Qiagen), and cDNA was synthesized using standard protocols. The expression levels of COX-2, HIF-1 α , and p65 after siRNA or nontarget siRNA treatment under normoxic or hypoxic conditions was compared using real-time PCR. All reactions were carried out in duplicate, and 18S RNA was the endogenous control used to normalize the target gene. Dissociation melt curves were used to confirm that there was specific amplification of the target cDNA and that there was no nonspecific amplification. The relative expression of each of the genes was analyzed using the δ cycle threshold (DCT) method (19). The change in gene expression was then recorded as a fold change and graphed using Microsoft software. The following primers were used: Hif-1 α F, 5'-ACCATGCCAGATTCAGG-3'; Hif-1 α R, 5'-AGTGCTTCCATCGGAAGGACT-3'; RelA F, 5'-ACCAACAACAACCCCTTCCA-3'; RelA R, 5'-GTAGTCCCCACGCTGCTCTT-3'; COX-2 F, 5'-GAGCAGTCCCCTCCCTAGGA-3'; COX-2 R, 5'-TGATTTAGTCGGCCTGGGAT-3'.

Chromatin immunoprecipitation

HeLa cells were grown on 3 \times 145 mm dishes per treatment and exposed to normoxia/hypoxia as outlined above for 0–24 h. At the end of the time course, cells were removed from the hypoxia chamber or the tissue culture incubator, and medium was aspirated. Cells were immediately fixed (1% formaldehyde and Eagle's MEM tissue culture media) for 10 min. Fixation was stopped using glycine solution, and cells were scraped in PBS supplemented with PMSF following a PBS wash step. Cells were pelleted by centrifugation and lysed prior to shearing of chromatin by sonication. After precleaning, chromatin was incubated with a specific Ab, and immunocomplexes were subsequently collected using salmon sperm DNA/protein A agarose (Millipore). After a series of washes, immunocomplexes were eluted using an elution buffer (1% SDS and 0.1 M NaHCO₃), and cross-links were reversed. DNA was then recovered by phenol/chloroform extraction. Purified DNA (3 μ l) was amplified using human COX-2 promoter primers (forward, 5'-GAATTTACCTTTCCGCCTCTC-3'; reverse, 5'-AAGCCCGGTGGGGCAGGGTTT-3') (16) using a thermocycler program (94°C for 3 min; then 36 cycles of 94°C for 20 s, 60°C for 30 s, and 72°C for 30 s; then a hold cycle of 10°C). Samples were run on a 2% agarose gel using ethidium bromide to visualize a 649-bp product.

Statistical analysis

All experiments were carried out a minimum of $n = 3$ independent times. Where indicated, statistical comparisons were made using the Student t test or one-way ANOVA with a Tukey posttest for intergroup comparisons where appropriate with $*p < 0.05$.

Results

Hypoxia activates NF- κ B signaling in vivo and in vitro

Mice expressing a transgene that encodes firefly luciferase under the control of multiple NF- κ B promoter elements were exposed to either normoxia (21% atmospheric oxygen) or hypoxia (10% atmospheric oxygen) for 24 h. Luciferase activity was measured and quantified using Living Image Software. Basal NF- κ B activity in these mice was detected predominately in the thymus, the spleen, and the lymphoid tissue (Peyer's patches) of the intestine (Fig. 1A). Whereas whole-animal NF- κ B activity was not different between normoxic and hypoxic treatments (Fig. 1B, upper panels), a significant increase in cardiac and pul-

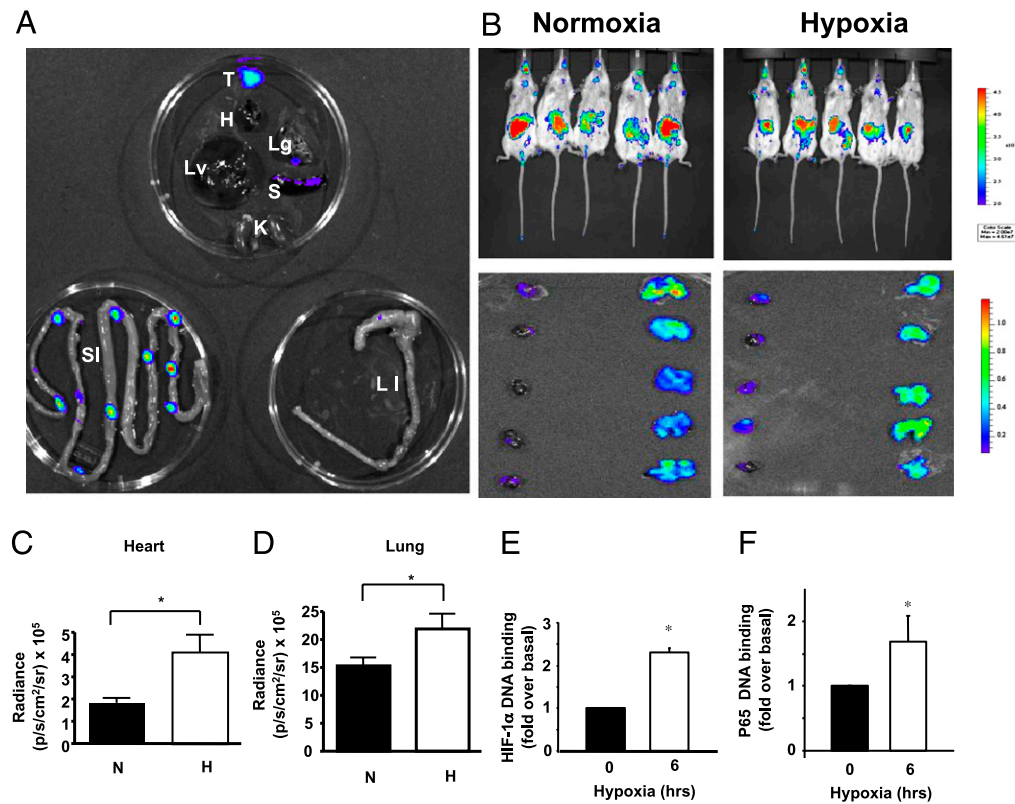
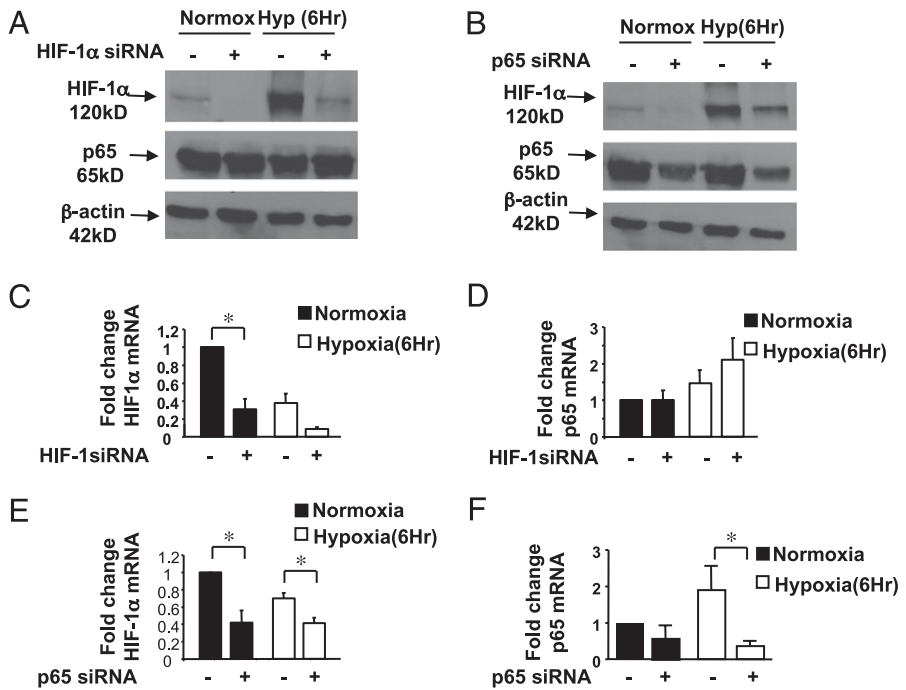


FIGURE 1. Hypoxia activates NF-κB activity in vivo and in vitro. *A*, In vivo imaging of organs harvested from transgenic mice expressing an NF-κB-dependent luciferase transgene demonstrates high basal NF-κB activity in lymphoid tissues in the thymus, spleen, and intestine. *B*, Transgenic NF-κB reporter mice exposed to either normoxia or hypoxia (10% normobaric atmospheric hypoxia, 24 h). Whole-animal in vivo imaging is displayed in the *upper panels*, and ex vivo imaging of the heart and lungs is displayed in the *lower panels*. *C* and *D*, Quantitative imaging for ex vivo heart (*C*) and lungs (*D*) demonstrates a statistically significant increase in NF-κB-dependent luciferase activity in hypoxic mice compared with that in normoxic mice. *E* and *F*, HeLa cells were exposed to hypoxia for 6 h and HIF-1α (*E*) or p65 (*F*) DNA binding was determined by TransAM DNA-binding assay. *n* = 3–4 throughout. **p* < 0.05. H, heart; K, kidney; Lg, lung; LI, large intestine; Lv, liver; S, spleen; SI, small intestine; T, thymus.

monary NF-κB was detected in tissues taken from the hypoxic animals (Fig. 1*B*, *lower panels*) indicating tissue-specific NF-κB activation in response to hypoxia in vivo. Quantitative photometry of the heart and lungs of these mice confirms a

statistically significant degree of NF-κB activation in these tissues with hypoxic treatment (Fig. 1*C*, *1D*). We next investigated the effect of hypoxia on NF-κB activity in vitro. HeLa cells were exposed to hypoxia, and nuclear extracts

FIGURE 2. HIF expression in HeLa cells is dependent upon NF-κB. *A*, HeLa cells were transfected with nontarget siRNA or siRNA targeted against HIF-1α prior to exposure to hypoxia (6 h). Whole-cell extracts were assayed for HIF-1α, p65, and β-actin protein levels by Western blot. *B*, HeLa cells were transfected with nontarget siRNA or siRNA targeted against p65 prior to exposure to hypoxia. Whole-cell extracts were blotted for HIF-1α, p65, and β-actin protein levels. *C*, HIF-1α mRNA levels were measured by real-time PCR in HeLa cells treated with HIF-1α siRNA prior to exposure to normoxia or hypoxia (6 h). *D*, p65 mRNA levels were measured in HeLa cells treated with HIF-1α siRNA prior to exposure to normoxia or hypoxia. *E*, HIF-1α mRNA levels were measured in HeLa cells treated with p65 siRNA prior to exposure to hypoxia. *F*, p65 mRNA levels were measured in HeLa cells treated with p65 siRNA prior to exposure to hypoxia. *n* = 3–4 throughout. **p* < 0.05.



were generated. Hypoxia resulted in a rapid and robust stabilization of HIF-1 α protein that peaked at 6 h (19.96-fold increase by densitometry) and gradually reduced over time thus demonstrating that the cells had indeed perceived hypoxia (Supplemental Fig. 1A). Notably, in these cells HIF-1 α mRNA demonstrated a steady time-dependent decrease in hypoxia (Supplemental Fig. 1B). Nuclear lysates from cells exposed to hypoxia for 6 h demonstrated increased HIF-1 α DNA-binding activity (Fig. 1E). Furthermore, in agreement with previous studies (8), DNA binding assay analysis demonstrated increased nuclear p65 DNA-binding activity confirming that hypoxic exposure activates both HIF and NF- κ B signaling concurrently in cultured cells (Fig. 1F). Taken together, these data demonstrate that hypoxia activates NF- κ B signaling both in vivo and in vitro.

HIF-1 α activity is NF- κ B dependent

Recent work has implicated a high level of cross-talk between NF- κ B and HIF in the cellular response to hypoxia (6). We next investigated whether an intact canonical NF- κ B pathway was required for the regulation of HIF-1 α mRNA and protein expression. HeLa cells were transfected with either siRNA targeted to HIF-1 α or nontarget control siRNA. In the presence of HIF-1 α siRNA, the level of hypoxia-induced HIF-1 α protein expression after 6 h of hypoxic exposure was reduced compared with cells treated with nontarget siRNA (3.43 versus 9.38 fold increase over normoxia, respectively). Protein levels of the p65 subunit of NF- κ B remained unaffected by HIF-1 α siRNA (Fig. 2A). Cells treated with p65-specific siRNA had strongly suppressed p65 and HIF-1 α protein expression (Fig. 2B). These data confirm a unidirectional dependence of HIF expression upon the presence of an intact canonical NF- κ B pathway.

We next examined whether these results were reflected at the level of mRNA expression. Cells treated with HIF-1 α siRNA had reduced HIF-1 α mRNA under both normoxic and hypoxic conditions (Fig. 2C). Consistent with the protein expression data, HIF-1 α siRNA had no effect on p65 mRNA expression (Fig. 2D). In contrast, and also in agreement with the protein measurements, treatment with p65 siRNA reduced the expression of both HIF-1 α (Fig. 2E) and p65 mRNA (Fig. 2F) further supporting a uni-

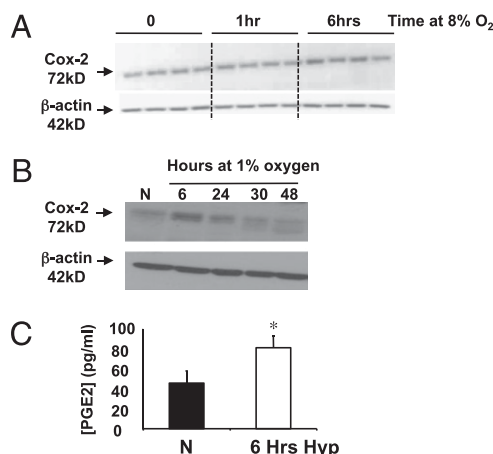


FIGURE 3. Hypoxia increases COX-2 expression and activity. *A*, Mice were exposed to hypoxia (8% oxygen) for 0–6 h, and COX-2 protein expression in kidney-derived lysates was determined by Western blot analysis. *B*, HeLa cells were exposed to hypoxia for 0–48 h, and COX-2 protein expression was determined by Western blot analysis. *C*, PGE₂ levels were measured in conditioned medium from HeLa cells exposed to hypoxia for 6 h. *n* = 3–4 throughout. **p* < 0.05.

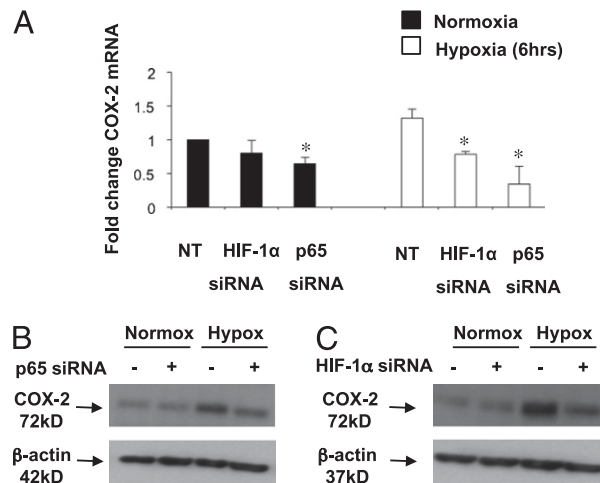


FIGURE 4. Hypoxic activation of COX-2 expression is dependent upon the presence of an intact NF- κ B pathway. *A*, HeLa cells were transfected with p65 siRNA or HIF-1 α siRNA. After exposure to hypoxia for 0–6 h, COX-2 mRNA levels were assessed by real-time PCR. *B* and *C*, HeLa cells were treated with p65 siRNA (*B*), HIF-1 α siRNA (*C*), or control scrambled siRNA. After exposure to hypoxia for 0–30 h, COX-2 protein expression was assessed by Western blot analysis. *n* = 3–4 throughout. **p* < 0.05.

directional dependence of HIF-1 α expression upon the presence of an intact canonical NF- κ B signaling pathway.

Hypoxia increases COX-2 expression

We next investigated the importance of NF- κ B in hypoxia-induced expression of proinflammatory gene expression. In agreement with previous studies, we found that COX-2 protein expression was

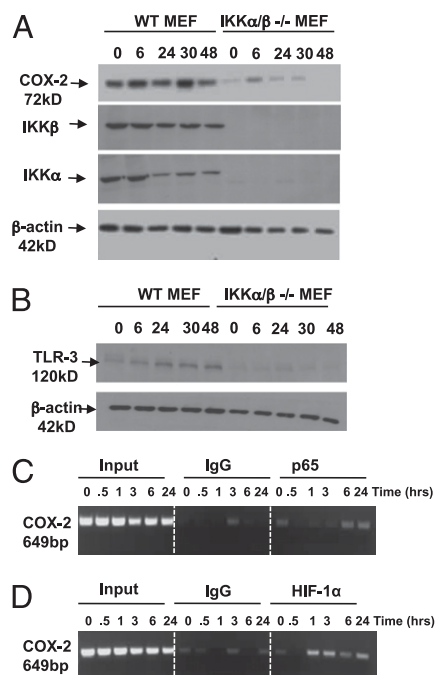


FIGURE 5. Wild-type embryonic fibroblasts and those derived from IKK α /IKK β ^{-/-} mice were exposed to hypoxia (0–48 h), and COX-2 (*A*) or TLR-3 (*B*) protein expression was determined by Western blot analysis of whole-cell protein extracts. Chromatin immunoprecipitation analysis was carried out using an Ab against p65 (*C*) or HIF-1 α (*D*) (or control Abs as indicated) in cells exposed to hypoxia for 0–24 h to assess whether p65 or HIF-1 α binds directly to the COX-2 promoter under conditions of hypoxia. *n* = 3–4 throughout.

significantly increased in response to hypoxia both in vivo and in vitro. First, mice were exposed to normoxia (21% O₂) or atmospheric normobaric hypoxia (8% O₂) for up to 6 h. COX-2 expression levels were quantified by densitometry. COX-2 expression in the kidney was 110.5% of the normoxic expression level at 1 h and 158.5% at 6 h after hypoxic exposure (four animals per group; Fig. 3A). COX-2 was also transiently induced in HeLa cells exposed to hypoxia (maximum 3.65-fold increase at 6 h by densitometry; Fig. 3B). Previous studies have shown that COX-2 activation plays an important role in cell survival in hypoxia via the increased production of PGE₂. We used an ELISA-based approach to measure PGE₂ levels in conditioned media collected from HeLa cells exposed to normoxia or hypoxia. PGE₂ levels were significantly elevated in hypoxia (6 h) reflecting a functional consequence of increased COX-2 expression (Fig. 3C).

We next investigated the role of NF- κ B and HIF signaling in hypoxia-induced COX-2 expression. p65 siRNA and HIF-1 α siRNA both inhibited hypoxia-induced COX-2 mRNA (Fig. 4A) and protein expression (Fig. 4B, 4C). These data reflect roles for both NF- κ B and HIF in the regulation of COX-2 expression.

Further analysis (data not shown) identified a cohort of alternative proinflammatory genes induced in response to hypoxia including TLR-3. To test the dependence of hypoxia-induced proinflammatory genes upon canonical NF- κ B signaling, we exposed IKK α / β ^{-/-} MEFs (which lack an intact canonical NF- κ B pathway) to hypoxia for 0–48 h and investigated the expression of COX-2 and TLR-3 protein levels. COX-2 protein expression was induced in a biphasic manner in wild-type MEFs at 6 h and 30 h (Fig. 5A). However in IKK α / β ^{-/-} MEFs, a dramatic reduction in both basal and hypoxia-inducible COX-2 protein expression was observed (Fig. 5A). Notably, some residual upregulation of COX-2 protein was observed indicating a functional role for some NF- κ B-independent signaling. Similarly, TLR-3 expression was upregulated in hypoxia in wild-type MEFs but not in IKK α / β ^{-/-} MEFs (Fig. 5B). We next investigated whether HIF-1 α and/or the p65 subunit of NF- κ B binds directly to the COX-2 promoter in hypoxia. Using chromatin immunoprecipitation, we demonstrate that both HIF-1 α and p65 bind directly to the COX-2 promoter in a hypoxia-dependent manner (Fig. 5C, 5D) giving evidence for direct roles for both HIF-1 α and the canonical NF- κ B signaling in regulating COX-2 gene expression in hypoxia. From these data, we propose that NF- κ B is important for hypoxia-dependent COX-2 expression both via a direct effect upon the COX-2 promoter and via the facilitation of HIF signaling, which also directly regulates COX-2 promoter activity. Thus, a close relationship exists between the activity of HIF-1 and NF- κ B in the regulation of specific cohorts of inflammatory genes in response to hypoxia.

Discussion

Tissue hypoxia is a common feature during chronic inflammation in a number of conditions including IBD, RA, and cancer (1–3). A number of transcription factors demonstrate oxygen dependence and are activated during hypoxia resulting in the altered expression of genes that can impact directly upon disease progression (5). Principal among hypoxia-sensitive transcription factors is HIF, the oxygen sensitivity of which is conferred by a family of 2-oxoglutarate-dependent dioxygenases (7).

A number of studies have demonstrated hypoxic sensitivity also exists in the NF- κ B transcriptional regulatory pathway (6). Although the mechanisms through which hypoxia activates NF- κ B signaling are less clearly elucidated, it appears that this pathway is also under the regulatory control of hydroxylases. For example, we and others have previously shown that the canonical but not the noncanonical NF- κ B signaling pathway can be positively regu-

lated by hydroxylase inhibition (8, 20). Furthermore, other components of the NF- κ B pathway have been shown to be direct targets for hydroxylation by the asparagine hydroxylase FIH (10).

Rather than being independent arms of a global transcriptional regulatory network, it has recently been appreciated that the NF- κ B and HIF pathways are intimately associated and that a significant level of cross-talk between these pathways exists at a number of levels. A number of stimuli have been shown to upregulate HIF mRNA expression through NF- κ B-dependent mechanisms (21, 22). Indeed, a recent publication has implicated an absolute necessity of basal canonical NF- κ B signaling for HIF expression (23). In agreement with this, we found HIF-1 α levels in IKK α / β ^{-/-} MEFs to be significantly reduced (data not shown). Thus, under conditions where NF- κ B is activated (e.g., inflammation), it is likely that this increases the rate of synthesis of HIF-1 α . Conversely, NF- κ B has been reported in some systems to be subject to regulation by HIF (12). It is possible that interdependent coregulation involving NF- κ B and HIF ultimately determines the profile and magnitude of inflammatory gene expression in hypoxia. Thus, hypoxia likely has a significant impact upon inflammatory events and, ultimately, inflammatory disease progression.

Among the inflammatory genes upregulated during inflammatory hypoxia are TNF- α , IL-6, MIP-2, and COX-2. The mechanisms underlying the expression of inflammatory genes in hypoxia remain controversial and are the topic of our current study. For example, hypoxia-induced COX-2 expression has been suggested to be solely dependent upon the activity of HIF-1 (13, 15, 16), whereas other studies conclude it to be through the NF- κ B pathway (14, 17). Conversely, COX-2 has also been shown to play a role in the regulation of HIF signaling (24, 25).

In the current study, we demonstrate that hypoxia activates NF- κ B signaling both in vivo and in vitro and confirm in our system the previously described dependence of HIF on the presence of an intact canonical NF- κ B pathway (23). Furthermore, we demonstrate that in the case of COX-2 and TLR-3, NF- κ B is central to gene expression in response to hypoxia. With respect to COX-2, we show that this is due both to the regulation of the HIF pathway and to direct binding to the COX-2 promoter. We hypothesize that the coordinated and interdependent activities of HIF and NF- κ B during hypoxia is key to determining which cohorts of genes are activated and to what degree. Thus, hypoxia is an environmental event in a range of chronic inflammatory conditions and may actively contribute to disease progression through the promotion of inflammatory gene expression in a manner that is heavily dependent upon the presence of an intact canonical NF- κ B signaling pathway.

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Disclosures

The authors have no financial conflicts of interest.

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