Bach1 Functions as a Hypoxia-inducible Repressor for the Heme Oxygenase-1 Gene in Human Cells*

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Heme oxygenase 1 (HO-1) catalyzes heme breakdown, eventually releasing iron, carbon monoxide, and bilirubin IXa. HO-1 is induced by its substrate heme and various environmental factors, which represents a protective response against oxidative stresses. Here we show that hypoxia represses HO-1 expression in three human cell types but induces it in rat, bovine, and monkey cells, indicating the inter-species difference in the hypoxic regulation of HO-1 expression. The hypoxia-mediated repression of HO-1 expression is consistently associated with the induction of Bach1, a heme-regulated transcriptional repressor, in human cells. Bach1 is a basic leucine zipper protein, forming a heterodimer with a small Maf protein. Expression of HO-1 was also reduced in human cells when exposed to interferon-γ or an iron chelator desferrioxamine, each of which induced Bach1 expression. In contrast, induction of HO-1 expression by CoCl₂ is associated with reduced expression of Bach1 mRNA. Thus, expression of HO-1 and Bach1 is inversely regulated. We have identified a Maf recognition element in the human HO-1 gene that is required for repression of a reporter gene by hypoxia and targeted by Bach1. Therefore, Bach1 functions as a hypoxia-inducible repressor for the HO-1 gene, thereby contributing to fine-tuning of oxygen homeostasis in human cells.

Heme oxygenase (EC 1.14.99.3) is the rate-limiting enzyme in heme catabolism that cleaves heme at the α-methene bridge to form biliverdin IXα, carbon monoxide, and iron (1, 2). Biliverdin IXα is immediately converted by biliverdin reductase to bilirubin IXα that is transported to the liver for conjugation and excretion into bile (3). There are two isozymes of heme oxygenase, heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2) (4, 5). HO-1 is inducible whereas HO-2 is constitutively expressed in human cells (6). Expression of HO-1 mRNA is highly increased in human cells by the substrate heme (7), heavy metals (8, 9), UV irradiation (10), and nitric oxide donors (11–14). Because bilirubin IXα functions as a natural radical scavenger (15, 16), induction of HO-1 probably represents a protective response against oxidative stress. The physiological importance of HO-1 has been confirmed by the phenotypic consequences of the HO-1-deficient mice (17) and a patient with HO-1 deficiency (18).

Induction of HO-1 has been extensively studied for the last few decades by many investigators. In contrast, repression of HO-1 expression has been largely ignored, despite its physiological importance (3). We have shown that HO-1 is not induced or rather reduced by heat shock in human cells (19), whereas rat HO-1 is a heat shock protein (20, 21). The expression levels of HO-1 are also decreased in human glioblastoma cells by the treatment with interferon-γ (22). In addition, hypoxia represses HO-1 mRNA expression in primary cultures of human umbilical vein endothelial cells (HUVECs), human astrocytes, and human coronary arterial endothelial cells (23). On the other hand, hypoxia increased HO-1 expression in rat liver (24) and heart (25) and in various cultured animal cells, including Chinese hamster ovary cells (26), rat ventricular smooth muscle cells (27, 28), and rat myocytes (29). These results suggest the inter-species difference in the regulation of HO-1 gene expression by hypoxia between human and animal cells.

The inter-species variations in the hypoxic response are of clinical significance because hypoxia is involved in the pathophysiology of various disorders, including ischemic heart disease, cerebrovascular disease, cancer, sleep apnea syndrome, and chronic obstructive pulmonary disease, which account for common causes of death and disability in the developed world. Mammalian cells respond to hypoxia in part by increased expression of several genes coding for erythropoietin (30), vascular endothelial growth factor (31), adrenomedullin (32, 33), and glycolytic enzymes (34, 35), all of which cooperate to protect cells and tissues against the hypoxic state. Hypoxia-inducible

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factor-1 (HIF-1) serves as a key regulator that induces the expression of most of these genes (36).

Transcription repressor Bach1 is a basic leucine zipper (bZIP) protein and forms heterodimers with one of the small Maf proteins (i.e. MafK, MafF, and MafG) that bind to the Maf recognition element (MARE) (37, 38), which may repress transcription of certain target genes. Recently, Sun et al. (39) have shown that ho-1 is constitutively expressed at higher levels in many tissues of bach1-deficient mice, indicating that Bach1 acts as a negative regulator of transcription of the mouse ho-1 gene. In fact, the Bach1-MafK heterodimer binds to the MAREs of the ho-1 gene enhancers, thereby repressing transcription (39). Importantly, heme abrogated the repressor function of Bach1 by inhibiting its binding to the MAREs of the ho-1 gene, which is consistent with the notion that the in vitro DNA-binding activity of Bach1 is negatively regulated by direct Bach1-heme interaction (40). Furthermore, Bach1 is widely expressed in human tissues that have been analyzed (41).

This study was aimed to clarify the mechanism by which hypoxia represses the HO-1 expression in human cells. Here we show that hypoxia induces the expression of Bach1, which is consistently associated with the repression of HO-1 expression in human cells. Furthermore, we have identified a MARE in the human HO-1 gene enhancer that is responsible for the repression by hypoxia and is targeted by Bach1. Thus, Bach1 is a newly recognized hypoxic regulator and functions as an inducible repressor for the HO-1 gene in several human cell types.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures**—A human glioblastoma cell line, T98G, was obtained from the American Type Culture Collection and was cultured in minimum essential medium supplemented with 5% fetal bovine serum (FBS) at 37 °C under 5% CO2/95% room air. To examine the effect of hypoxia on the expression of heme oxygenase-1, T98G glioblastoma cells were placed in a chamber filled with 5% CO2/94% N2/1% O2 as hypoxia on the expression of heme oxygenase-1, T98G glioblastoma cells were placed in a chamber filled with 5% CO2/94% N2/1% O2, as described previously (33). The cells were cultured for 6–48 h and were harvested for RNA extraction. In parallel, T98G cells were cultured under 5% CO2/95% room air for the same periods and used as normoxia control. In another series of experiments, T98G cells were exposed to 260 μM desferrioxamine or 150 μM CoCl2, under normoxia for 6, 12, 18, and 24 h, and were harvested for total RNA extraction. A549 human lung cancer cells were obtained from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan), and were cultured in RPMI 1640 medium containing 2% FBS. C6 rat glioma cells were obtained from Cell Resource Center for Biomedicine, and pHHO1 (7). The northern blot analysis and subcloning of a human heme oxygenase-1 cDNA, pHHO1 (7), and the supernatant (10%). The Nuclear extract (5 μg of protein) of T98G cells were also used for Western blot analysis to confirm the expression of Bach1 protein. The proteins in the gel were treated with 20% methanol buffer containing 48 μg Tris, 39 μg glycine, and 0.037% SDS and electrophotographed transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore Corporation), which was pretreated with the same buffer. Expression of HO-1 and Bach1 was determined with anti-HO-1 antibody (6) and with anti-Bach1 antibody (37), respectively. The specific immunocomplexes were detected with a Western blot kit (ECL Plus, Amersham Biosciences).

**HO-1 mRNA Stability Assays**—T98G cells and C6 rat glioma cells were cultivated for 6 h in RPMI supplemented with 5% fetal bovine serum (FBS) or normoxia, in the presence or absence of CoCl2, followed by addition of actinomycin D (1 μg/ml). The cells were further incubated for 2, 4, and 6 h after the addition of actinomycin D and harvested at each time point for RNA extraction.

**Transient Transfection Assays**—A549 lung cancer cells were seeded in a 6-cm diameter dish 24 h before DNA transfection. Cells were transfected by the calcium phosphate method with modifications, as described previously (43). Reporter plasmids used were pPHIO1 constructs containing the promoter region of the human HO-1 gene upstream of the Firefly luciferase gene (43). Two constructs, pHOHOL14 and pHIOHOL1, contain the (GT)15AT(GT)14 repeat in the proximal promoter region (44, 45). The internally deleted construct pPHIOHOL20 and its derivatives were described previously (43). Base changes were introduced into the MARE of pPHIOHOL20 by the method based on polymerase chain reaction (46), yielding pHIOHOL20 (see Fig. 7B). A reporter plasmid, pSv40 promoter-Epo HRE-Luc, contains four copies of hypoxia-response element (5′-GATCGCCCTAACGTGCTGTCATCA-3′), the core sequence underlined) and was used as a positive control for hypoxic induction (47). A549 cells were incubated for 24 h with 8.0 μg of reporter plasmid DNA and 0.5 μg of internal control DNA (pRL-TK), then re-fed with fresh medium and incubated for 12 h. The pRL-TK contains the thymidine kinase promoter region upstream of Renilla luciferase (Promega). Following the 12-hour incubation, cells were treated for 12 h with 25 μM 2-deoxyuridine. The transfected cells and assayed for luciferase activity by Dual-Luciferase reporter assay system (Promega). The luciferase activity was measured with Lumat LB9507 (Berthold).

**Electrophoretic Mobility Shift Assay** (EMSA)—T98G glioblastoma cells were incubated under hypoxia or normoxia for 3–24 h, and their nuclear extracts described previously were prepared for EMSA. EMSA was a synthetic double-stranded hHO-1 MARE (5′-GATTTTG-CGTAGTCACACTGGCCTTCAG-3′), the core sequence underlined), and its derivatives were described previously (43). A probe was incubated with the labeled DNA fragment containing 20 nt HEPES (pH 7.9), 0.1 μg/ml poly(dI-dC), 0.1 μg/ml salmon sperm DNA, 0.5 μg/ml poly[dT]12–18, 0.5 μg/ml heparin, 0.5 μg/ml poly(A), 20 μM KCl, and 4% glycerol (40). The binding reaction was also performed with the nuclear extracts prepared from untreated T98G cells in the presence of unlabeled competitors, the probe (hHO-1 MARE), or hHO-1 MARE mut (5′-GATTTCCTAGGTACACTGGCC-

9126
RESULTS

Repression of Heme Oxygenase-1 by Hypoxia Preceded by Induction of Bach1 in Human Cells—We analyzed the effect of hypoxia on the expression of HO-1 and Bach1 mRNAs in T98G human glioblastoma cells by Northern blot analysis, because T98G cells are able to respond to hypoxia by inducing adrenomedullin and express mRNAs for HIF-1α and HIF-β that constitute HIF-1 (33). Hypoxia decreased expression levels of HO-1 mRNA and conversely increased the expression of Bach1 mRNA (Fig. 1A). In contrast, hypoxia had no noticeable effect on the expression levels of β-actin mRNA. Under the hypoxic conditions employed, the growth or survival rate of T98G cells remained unchanged by 48 h (data not shown). The levels of HO-1 mRNA were decreased by 12 h after exposure to hypoxia and reached the lowest level by 48 h (about 36% of the parallel control) (Fig. 1B). The hypoxia-mediated reduction of HO-1 mRNA expression is associated with the reduction of HO-1 protein levels (data not shown), as observed in HUVECs (23). Induction of Bach1 mRNA expression was detectable at 2 h after exposure to hypoxia (Fig. 1A) and reached the maximum by 12 h (Fig. 1C). The expression levels of Bach1 mRNA remained at the increased levels by 48 h.

To explore whether the repression of HO-1 expression and induction of Bach1 expression by hypoxia seen in T98G glioblastoma cells represents a general response, we repeated similar experiments in A549 human lung cancer cells and primary culture of HUVECs. It is noteworthy that HIF-1 is functionally activated in HUVECs under our hypoxic conditions (23). In both cell types, the reduction of HO-1 mRNA levels was detectable at 12 h after exposure to hypoxia, when the levels of Bach1 mRNA were higher than those under normoxia (Fig. 2, A and B). The expression levels of HO-1 mRNA were reduced to the lowest level by 48 h after exposure to hypoxia, whereas the expression levels of Bach1 mRNA were maintained at higher levels under hypoxia. The effect of hypoxia on expression pro-
files of HO-1 and levels of Bach1 mRNA were similar to the results observed in T98G cells although less marked. Thus, hypoxia induces expression of Bach1 mRNA in three types of human cells, which is consistently associated with the hypoxia-mediated repression of HO-1 mRNA expression.

Hypoxic Induction of HO-1 mRNA Expression in Animal Cells—We then analyzed the effects of hypoxia on the expression of HO-1 mRNA in C6 rat glioma cells, BBMVECs, and COS7 monkey kidney cells (Fig. 3) as well as in NIH3T3 mouse fibroblasts (data not shown). Hypoxia consistently induced the HO-1 mRNA expression in these cells, suggesting the species difference in the regulation of HO-1 expression by hypoxia. Expression levels of HO-1 mRNA in C6 rat cells were noticeably increased by 48 h of incubation even under normoxia, which may be due to a certain type of metabolic stress generated during the long-term culture. In fact, rat HO-1 is a heat shock protein (20). Bach1 mRNA expression was induced by hypoxia in C6 rat cells and COS7 monkey kidney cells (Fig. 3, A and C). Bach1 mRNA expression was undetectable in BBMVECs with a human cDNA probe.

Induction of Bach1 Expression by Other Repressors for HO-1 Expression—We next examined the effects of interferon-γ and an iron chelator, desferrioxamine, on Bach1 mRNA expression in T98G glioblastoma cells, as both reagents repress the HO-1 expression in human cells (22, 23). We also included CoCl2 as a positive control for the induction of HO-1. Hypoxia and CoCl2 exerted the opposing effects on the HO-1 mRNA expression in HUVECs, despite that both activated the function of HIF-1.
compared with normoxia. Each condition were considered to be intensities representing HO-1 mRNA at the time of addition of actinomycin D (1 \mu M) and harvested for RNA preparation. Other conditions are the same as in Fig. 1. A and C, Northern blot analysis (left panel, normoxia; center, hypoxia; right, CoCl$_2$). The data shown are from one of three independent experiments with similar results. B and D, relative expression levels of HO-1 mRNA. The intensities representing HO-1 mRNA at the time of addition of actinomycin D under each condition were considered to be 100%. The data shown are mean ± S.E. (n = 3). In D, *, p < 0.05; **, p < 0.01 compared with normoxia.

(23). HO-1 mRNA expression levels were decreased time-dependently by the treatment with interferon-\(\gamma\) (Fig. 4A) or desferrioxamine (Fig. 4B), but remarkably increased by the treatment with CoCl$_2$ (C). The repression of HO-1 mRNA expression by interferon-\(\gamma\) or desferrioxamine was consistently associated with the induction of Bach1 mRNA. Thus, interferon-\(\gamma\) and desferrioxamine exhibited the effects similar to those of hypoxia on the expression of HO-1 and Bach1 mRNAs (see Fig. 1). In contrast, Bach1 mRNA levels were reduced time-dependently during the treatment with CoCl$_2$ and reached the lowest levels at 24 h. Thus, the induction of HO-1 mRNA expression by CoCl$_2$ was associated with the repression of Bach1 mRNA expression. Taken together, these results indicate that the expression levels of HO-1 and Bach1 mRNA are inversely regulated.

**Stability of HO-1 mRNA under Hypoxia**—We then analyzed the effects of hypoxia on the stability of HO-1 mRNA in T98G human glioblastoma cells and that in C6 rat glioma cells (Fig. 5), because the hybridization signal representing human HO-1 gene transcripts is too low under the basal conditions to detect its reduction by nuclear run-on assays (43, 50). In T98G cells, the half-life of HO-1 mRNA was about 3 h under normoxia, which is similar to the value determined in other human cells (12, 23). Hypoxia appeared to reduce the half-life of HO-1 mRNA, and CoCl$_2$ prolonged the half-life of HO-1 mRNA, although these effects were not statistically significant (Fig. 5B). Thus, the observed repressive effect of hypoxia on HO-1 mRNA expression is mainly due to the decreased transcription of the HO-1 gene. It is noteworthy that the half-life of Bach1 mRNA is much shorter than 2 h.

In C6 rat glioma cells, the half-life of HO-1 mRNA was about 2.5 h under normoxia. CoCl$_2$ induced HO-1 mRNA expression in C6 cells, as did hypoxia (Fig. 5C). There was no significant change in the half-life of HO-1 mRNA under hypoxia, but CoCl$_2$ prolonged the half-life of HO-1 mRNA to −4.5 h. Thus, the induction of HO-1 mRNA expression in C6 cells by hypoxia is mainly due to the increased transcription, and the induction by CoCl$_2$ is in part due to the reduced degradation rate.

**Bach1 as a Repressor for the Human HO-1 Gene**—We next studied the role of Bach1 in the promoter activity of the human HO-1 gene by transient co-transfection assays in A549 human lung cancer cells. Co-expression of Bach1 caused significant reduction of the expression of a reporter plasmid pH10L15 carrying the 4.5-kb upstream region (Fig. 6A). Deletion studies have localized the cis-acting region (positions −4.5 to −4 kb) that is required for the basal promoter activity and for the Bach1-mediated repression. Incidentally, this cis-acting region contains the composite enhancer (14) that consists of the cadmium-responsive element (43, 51) and a putative MARE. To localize a cis-acting element that is responsible for the Bach1-mediated repression we used the internally deleted construct pH10L20 that contains the upstream cis-acting region (−4.5 to −4 kb) at the position −283 of the human HO-1 gene (Fig. 6B). Bach1 reduced the expression levels of pH10L20 and its derivative pH10L23 carrying the MARE, but not a construct pH10L22, lacking the MARE. Together with the fact that Bach1 represses the mouse ho-1 gene transcription through MAREs (39), these results suggest that the identified MARE may be involved in the repression of the human HO-1 gene by Bach1. It is noteworthy that the basal expression level of pH10L23, lacking the 5′ portion of the upstream region, is lower than that of pH10L20, suggesting that the entire upstream region is required for the efficient transcription of the HO-1 gene.

**Repression of the Human HO-1 Gene Promoter Activity by Hypoxia**—We then studied the effect of hypoxia on the promoter activity of the human HO-1 gene by transient expression assays. Hypoxia significantly reduced the expression of pH10L15 but not pH10L14 (Fig. 7A), whereas hypoxia consistently induced expression of a construct containing four copies of hypoxia-response element by more than 3-fold (data not shown). Thus, the cis-acting region (positions −4.5 to −4 kb) is required for the reduction of reporter expression by hypoxia. Moreover, expression of the internally deleted constructs, pH10L20 and pH10L23, carrying the MARE was reduced under hypoxia, but not pH10L22 (Fig. 7B). Importantly, the
hypoxia-mediated repression was not detectable with a construct, pHHOL\(20\), carrying a mutated MARE, suggesting that the MARE located at \(4\) kb may be required for the hypoxic repression of \(HO-1\) gene expression. It is also noteworthy that the basal promoter activity of pHHOL\(20\) is lower than that of pHHOL\(20\). Thus, the MARE is also required for the efficient transcription of the \(HO-1\) gene under basal conditions.

**Induction of the MARE-binding Activity by Hypoxia**—Hypoxia increased the amounts of immunoreactive Bach1 protein in nuclear extracts of T98G glioblastoma cells in a time-dependent manner (Fig. 8A). Unexpectedly, Bach1 protein was also increased after a 12–24-h incubation even under normoxia, which may reflect a preferential nuclear translocation of Bach1 under a certain metabolic stress generated during the long-term culture of T98G cells. Bach1 binds to a MARE as heterodimers with small Maf proteins (37) that are ubiquitously expressed. EMSA showed that a MARE of the human \(HO-1\) gene was bound \textit{in vitro} by nuclear proteins prepared from the T98G cells exposed to hypoxia (Fig. 8B), indicating that the nuclear extracts also contain a member of Maf proteins. Importantly, the MARE-binding activities increased in parallel with the hypoxic induction of Bach1 protein. In addition, the MARE-binding activities increased in nuclear extracts prepared from cells after the 12-h incubation under normoxia, which appears in parallel with the induction of Bach1 protein. Attempts to demonstrate the supershifted complex of the detected band with the anti-Bach1 antibody were unsuccessful, probably due to the properties of the antibody or inaccessibility of the antibody to the Bach1-Maf complex on the DNA under the conditions employed. Consequently, we used recombinant Bach1 and MafK proteins to confirm the involvement of Bach1 in the binding to the MARE of the human \(HO-1\) gene, because a heterodimer of Bach1 and MafK binds to a MARE consensus \textit{in vitro} (37, 40). As shown in Fig. 8C, the MARE was specifically bound by the complex of recombinant Bach1 and MafK.

**DISCUSSION**

The expression levels of \(HO-1\) mRNA are decreased in human cells by the treatment with hypoxia, desferrioxamine, or interferon-\(\gamma\), each of which consistently induces Bach1 mRNA expression. In addition, expression levels of Bach1 mRNA are decreased by the treatment with CoCl\(_2\) that remarkably induces \(HO-1\) expression. Thus, there is an inverse relationship in the expression levels between \(HO-1\) and Bach1. These results suggest that Bach1 may function as a metabolic sensor. Importantly, the present study identifies Bach1 as a hypoxia-inducible regulator that represses the transcription of the \(HO-1\) gene in human cells; Bach1 represents a component of the hypoxia-inducible repressor because Bach1 functions as heterodimers with one of small Maf proteins (37–40). Under
our hypoxic conditions, HIF-1, a key regulator in hypoxic response, is functionally activated in A549 human lung cancer cells as well as in T98G glioblastoma cells (33) and HUVECs (23). Human cells therefore fine-tune oxygen homeostasis by inducing Bach1 and HIF-1 in response to hypoxia.

The MARE located at -4 kb is required for the hypoxic repression of the human \textit{HO-1} gene expression and is bound by Bach1. Importantly, MARE is also bound by nuclear factor erythroid 2 (NF-E2) that is a heterodimer of an erythroid-specific subunit (p45) and the small Maf proteins (48, 52–54). Furthermore, heterodimers of NF-E2-related factor 2 and one of the small Maf proteins activate transcription of the mouse \textit{ho-1} gene by binding to the MAREs (55–57). Taken together with the overexpression of \textit{ho-1} in many tissues of the \textit{bach1}-deficient mouse (39), these results suggest that transcription of the \textit{ho-1} gene may vary depending on the availability of transcriptional activators, such as NF-E2-related factor 2, and the repressor Bach1. In fact, expression of HO-1 mRNA is induced by hypoxia in C6 rat glioma cells (Fig. 3A) and monkey kidney cells (Fig. 3C), in which Bach1 mRNA is also induced by hypoxia.

An important question that remains to be answered is the physiological implication for the hypoxia-mediated repression of \textit{HO-1} expression that is usually maintained at relatively low levels. We provide three explanations that are not mutually exclusive. First, the repression of \textit{HO-1} expression reduces energy expenditure consumed for heme catabolism because the reaction catalyzed by HO-1 requires at least 3 mol of oxygen and 4 mol of NADPH to cleave 1 mole of heme (1, 2). Second, it prevents the local accumulation of carbon monoxide, iron, and bilirubin IX\(_{b}\) beyond certain threshold levels in the HO-1-expressing cells and their surroundings, thereby preventing the tissue damage, as the heme breakdown products are potentially toxic to cells (58). For example, bilirubin IX\(_{a}\) causes bilirubin encephalopathy in certain newborns (3). The repression of \textit{HO-1} expression could also restrict iron supply to cancer cells or certain pathogens that might be carried by a host because HO-1 is important in the turnover of iron that is an essential requirement for cell proliferation. This possibility is plausible for the repression of \textit{HO-1} expression mediated by interferon-\(\gamma\) and desferrioxamine. Third, it represents a mechanism by which the intra-cellular heme level is maintained at the narrow range by the balance between HO-1 and Bach1. Importantly, Bach1 functions as a heme-regulated transcriptional repressor and loses its DNA-binding activity when bound by heme (39, 40); namely, heme inhibits the DNA-binding activity of Bach1 (40), thereby leading to de-repression of the downstream target genes of Bach1. Consistent with this unique property of Bach1, heme oxygenase activity is inducible by hemin in all the cultured cells examined, including human, monkey, mouse, pig, and rat cells (59, 60). Identification of the target genes of Bach1 other than \textit{HO-1} will give further insights into the host defense mechanism against metabolic stress.

The inter-species variations in the hypoxic regulation of \textit{HO-1} gene expression are of particular significance, even
extracts of untreated T98G cells were incubated with the 32P-end labeled probe in the absence or presence of an indicated competitor. The competitors used are the probe itself (hHO-1 MARE) and a mutated hHO-1 MARE. The first lane shows the MARE-binding activity detected in untreated T98G cells (0 h). An arrow indicates the specific protein-DNA complex. C, EMSA with recombinant Bach1 and MaFK. Lane 1 represents a buffer control lacking proteins. The digoxigenin-labeled hHO-1 MARE was incubated with recombinant Bach1 and MaFK in the absence (lane 2) or the presence of indicated competitors (lanes 3–10). The competitors used were the probe itself (lanes 3 and 4), the mutated hHO-1 MARE (lanes 5 and 6), chicken \( \beta \)-globin enhancer (C\( \beta \)E) (lanes 7 and 8), and a control MARE (lanes 9 and 10). The protein-DNA complex is indicated with an arrow.

though we cannot generalize experimental findings in cultured cells or animal models to the human condition. A simple question has been provoked why hypoxia induces HO-1 expression in animal cells, if the repression of HO-1 is so important in the host defense, as discussed for human cells. Many studies have established that hypoxia induces HO-1 expression in animal cells (see Introduction). Here we also show the hypoxic induction of HO-1 in rat, bovine, and monkey cells (Fig. 3). Likewise, hypoxia induces HO-1 mRNA expression in NIH3T3 mouse fibroblasts (data not shown). Thus, the differential regulation of HO-1 expression between human and these animal cells may reflect the differences in the availability of certain transcription factors for the HO-1 gene. The inter-species variation might reflect the defense strategy uniquely developed in humans (19), and its implications have been discussed in the relevance to the pathogenesis of cerebral malaria (3).

Importantly, there is at least one report that shows the induction of HO-1 expression by hypoxia in cultured human dermal fibroblasts (61). In this case, dermal fibroblasts were exposed to hypoxia in the culture medium containing 0.5% FBS instead of the maintenance culture medium that contains high glucose and 10% FBS, suggesting that these cells might be exposed to the combined stresses of nutrients deprivation and hypoxia. Moreover, the stabilization of HO-1 mRNA appears to be responsible for the hypoxic induction of HO-1 in these human dermal fibroblasts. It is therefore conceivable that expression of HO-1 mRNA is differentially regulated in human cells by hypoxia depending on cell types.

In summary, hypoxic repression of HO-1 gene expression is consistently associated with the induction of Bach1 expression in several human cell types. Induction or down-regulation of HO-1 in human cells by pharmacological means will be a promising strategy for the treatment of various disorders. Bach1 is an alternate target to modulate the expression of HO-1. The present study suggests that Bach1 serves as a component of hypoxia-inducible repressor in human cells. Future studies will be aimed at analyzing whether expression of the Bach1 gene is under the regulation of HIF-1.

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