NF-κB–Inhibited Acute Myeloid Leukemia Cells Are Rescued from Apoptosis by Heme Oxygenase-1 Induction

Stuart A. Rushworth¹, Kristian M. Bowles², Prahlad Raninga¹, and David J. MacEwan¹

Abstract

Despite high basal NF-κB activity in acute myeloid leukemia (AML) cells, inhibiting NF-κB in these cells has little or no effect on inducing apoptosis. We previously showed that heme oxygenase-1 (HO-1) underlies this resistance of AML to tumor necrosis factor–induced apoptosis. Here, we describe a mechanism by which HO-1 is a silent antiapoptotic factor only revealed when NF-κB is inhibited, thus providing a secondary antiapoptotic mechanism to ensure AML cell survival and chemoresistance. We show that inhibition of NF-κB increased HO-1 expression in primary AML cells compared with that of nonmalignant cells. In addition, we observed this suppressed HO-1 level in AML cells compared with CD34+ nonmalignant control cells. Using chromatin immunoprecipitation assay and small interfering RNA knockdown, we showed that the NF-κB subunits p50 and p65 control this suppression of HO-1 in AML cells. Finally, we showed that inhibition of HO-1 and NF-κB in combination significantly induced apoptosis in AML cells but not in noncancerous control cells. Thus, NF-κB inhibition combined with HO-1 inhibition potentially provides a novel therapeutic approach to treat chemotherapy-resistant forms of AML. Cancer Res 70(7); 2973-83. ©2010 AACR.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic stem cells (HSC), characterized by proliferation and differentiation of abnormal cells, causing accumulation of immature myeloid cells in bone marrow and blood. Despite considerable advances in diagnosis of AML, initial chemotherapies cure <20% of sufferers (1). Relapse rates following treatment are high, and current treatments offer little chance of long-term survival for most patients, as they develop drug-resistant forms of AML.

NF-κB is a family of transcription factors that share homology to the retroviral oncprotein v-Rel. The five members of the mammalian NF-κB family consist of the class I proteins NF-κB1 (p105→p50) and NF-κB2 (p100→p52) and class II proteins RelA (p65), RelB, and c-Rel. Inhibitors of NF-κB have emerged as potential therapies against AML (2). This is because AML blasts have constitutive NF-κB activation (3). Such abnormal levels of constitutively activated NF-κB have been detected in other hematopoietic cancers, including Hodgkin’s lymphoma (4) and acute lymphoid leukemia (5), and in various solid tumors (6). Previously, we have highlighted the limited effect of NF-κB inhibition on apoptosis of AML (7). We showed in AML-derived cell lines that NF-κB inhibition alone or combined with tumor necrosis factor (TNF) activation (a classic apoptotic stimulus for normal inflammatory cells) had no effect on cell death. In addition, the cytoprotective gene heme oxygenase-1 (HO-1) was induced in AML cell lines in response to NF-κB inhibition and superinduced when in combination with TNF activation. Furthermore, when HO-1 superinduction was inhibited using small interfering RNA (siRNA)–targeted knockdown, the AML cell lines became susceptible to TNF-induced apoptosis, proving a crucial role for HO-1 in apoptotic-resistant forms of AML.

HO-1 is the rate-limiting enzyme of heme catabolism. It catalyzes the breakdown of heme into equimolar amounts of carbon monoxide (CO), iron, and biliverdin, with biliverdin rapidly metabolized to bilirubin (8). HO-1 is critical in two important physiologic processes: recycling iron molecules for erythropoiesis and maintaining homeostasis under stressful conditions (9). The latter property, a manifestation of the potent antioxidant, anti-inflammatory, and signaling activities of CO and bilirubin, is important in a wide range of human pathologies, including inflammatory and cardiovascular diseases (10–12). In cancer, growing evidence indicates that HO-1 may play a role in carcinogenesis (13). That is, HO-1 is often upregulated in tumors, and its expression is further increased in response to therapies (14, 15). Although the exact effect can be tissue specific, HO-1 can be regarded as an enzyme that facilitates tumor progression.

In addition to its substrate heme, a variety of conditions and agents, both physiologic and nonphysiologic, including UV
irradiation, hyperthermia, inflammatory cytokines, bacterial endotoxins, and heavy metals, potently stimulate HO-1 (16–20). This is due to the 5′-flanking region of the HO-1 gene containing binding sites for several different transcription factors that regulate inflammation and apoptosis, including NF-κB, activator protein-1 (AP-1), and NF-E2-related factor 2 (Nrf2; refs. 21–23). The function of NF-κB in regulating HO-1 expression in human cells is controversial because more than one study suggests a direct relationship between NF-κB and HO-1 activity (24, 25); however, other studies investigating expression in human cells is controversial because more than one study suggests a direct relationship between NF-κB and HO-1 activity (24, 25); however, other studies investigating expression in human cells is controversial because more than one study suggests a direct relationship between NF-κB and HO-1 activity (24, 25); however, other studies investigating expression in human cells is controversial because more than one study suggests a direct relationship between NF-κB and HO-1 activity (24, 25). The present study was undertaken to further define the role of NF-κB and Nrf2 in regulating HO-1 gene expression in AML.

Materials and Methods

Materials. AML-derived cell line THP-1 was obtained from the European Collection of Animal Cell Cultures. Anti-human HO-1 antibody was purchased from Assay Designs. Anti–NF-κB antibodies p50, p52, p65, and c-Rel were obtained from Cell Signaling Technology. All other antibodies were obtained from Santa Cruz Biotechnology. BAY 11-7082 was procured from Calbiochem. Control, HO-1, p50, and p65 siRNAs were from Applied Biosystems. Zinc protoporphyrin IX (ZnPP IX; HO-1 inhibitor) and copper protoporphyrin IX (CuPP IX; control) were obtained from Frontier Scientific. All other reagents were obtained from Sigma-Aldrich, unless indicated.

Cell culture. Primary AML cells were obtained under local ethical approval (LREC ref 07/H0310/146). For primary cell isolation, heparinized blood was collected from volunteers as described previously (27) using radioimmunoprecipitation assay–extracted whole-cell lysates. Nuclear extracts were prepared as previously described (28). NF-κB DNA binding was measured using the NF-κB p65 transcription factor ELISA kit (Panomics). Flow cytometry for measuring apoptosis was performed on a Beckman Coulter Epics XL. A dichlorofluorescein (DCF) assay was used to determine cellular reactive oxygen species (ROS) generation (29, 30).

Western immunoblotting, binding assay, and flow cytometry. SDS-PAGE and Western blot analyses were performed as described previously (27) using radioimmunoprecipitation assay–extracted whole-cell lysates. Nuclear extracts were prepared as previously described (28). NF-κB DNA binding was measured using the NF-κB p65 transcription factor ELISA kit (Panomics). Flow cytometry for measuring apoptosis was performed on a Beckman Coulter Epics XL. A dichlorofluorescein (DCF) assay was used to determine cellular reactive oxygen species (ROS) generation (29, 30).

Transfections. Cells (10⁶ per well) were transfected using Amaxa Nucleofector using equivalent molar concentrations of siRNAs (to yield final concentrations of 30 nmol/L). PGL3 reporter and pRL-CMV control constructs (0.5 μg) were cotransfected into THP-1. Transfected cells were incubated 24 h before indicated treatments. Reporter assays were measured with Dual-Luciferase Reporter Assay System (Promega).

Proliferation/death assays. Cells were treated with different doses of CuPP IX, ZnPP IX, and BAY 11-7082 (10 μmol/L) for up to 72 h. Cell number was measured with MTS One Solution Assay (Promega) before reading 960-nm absorbance in quadruplicate.

Statistical analyses. Student’s t test was performed to assess statistical significance among groups. Results with P < 0.05 were considered statistically significant. Results represent the mean ± SEM of at least three independent experiments. For Western blotting experiments, data are representative of three independent experiments.
Results

**High basal expression of NF-κB subunits p50 and p65 in AML.** To understand the function of NF-κB in AML cells, we examined the nuclear profile of NF-κB subunits in human AML cells compared with nonmalignant control cells. To do this, we obtained nuclear extracts from primary monocytes, primary AML blasts, and an AML-derived cell line (THP-1). Protein expression was examined using Western blot for the NF-κB family members p50, p52, p65, and c-Rel. Figure 1A shows that AML blasts and THP-1 human monocytic cell line have increased levels of nuclear p50 and p65 but not c-Rel and p52 when compared with nonmalignant control cells. To determine the κB-binding potential of the increased p50 and p65 levels in AML cells, we used the Transfactor ELISA from Panomics. This showed increased binding of both p50 and p65 in nuclear extracts of all AML samples tested when compared with noncancerous control cells (Fig. 1A). We also showed by immunohistochemistry that basal p65 nuclear translocation...
was higher in AML samples but not in control cells (Supplementary Fig. S1).

**AML cells are not killed by NF-κB inhibition.** To understand the mechanism by which AML protects against apoptosis in response to NF-κB inhibition, we fully analyzed the response of 17 AML patient samples (Table 1), AML cell lines, and nonmalignant control cells to NF-κB inhibition by using BAY 11-7082 (an inhibitor of IκBα phosphorylation, IC50 of 10 μmol/L). We observed in these cells a small and insignificant amount of cell death in response to NF-κB inhibition by BAY 11-7082 up to 10 μmol/L (Fig. 1B). At the highest concentration of BAY 11-7082 (20 μmol/L), we see some cell death in AML blasts and control nonmalignant cells, suggesting some nonspecific toxic effect at higher doses. To determine if NF-κB activity was inhibited by BAY 11-7082, we examined nuclear extracts from THP-1 cells treated with BAY 11-7082 for various time points. Figure 1C shows that both p50 and p65 were inhibited from 4 to 16 hours after treatment. Previously, we have shown that AML-derived cell lines but not noncancerous control cells are resistant to TNF-induced cell death signals (7). The model used for studying TNF-induced cell death was NF-κB inhibition in conjunction with TNF activation (a well-established means for testing NF-κB–driven antiapoptotic mechanisms). Here, we determined if primary AML cells are resistant to such signals. Figure 1D shows that AML cells are resistant to TNF-induced apoptotic signals, unlike primary monocytes that display normal TNF-induced death.

**Inhibiting NF-κB in AML cells induces HO-1 expression.** Our research has shown that NF-κB activity in conjunction with TNF can cause AML cells to superinduce the cytoprotective gene HO-1 (7). Here, we wanted to know if inhibiting NF-κB alone could induce HO-1 expression in AML cells. We tested AML blasts, AML cell lines, and nonmalignant control cells (CD34+ HSCs and human monocytes). Compared with nonmalignant control cells, all but one AML sample and all AML-derived cell lines displayed increased HO-1 mRNA induction by BAY 11-7082 (Fig. 2A), which was matched in terms of HO-1 protein levels (Fig. 2B). However, these Western blot analyses surprised us that the basal protein levels of HO-1 in untreated AML samples were undetectable compared with untreated nonmalignant control cells. This led us to compare basal levels of HO-1 mRNA in untreated AML samples to nonmalignant control cells. Figure 2C shows that basal HO-1 mRNA levels are much lower in AML blasts compared with untreated nonmalignant control cells. This led us to consider HO-1 upregulation in nonmalignant control cells.

### Table 1. Anonymized AML patient sample information

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<th>Number</th>
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<th>WHO diagnosis</th>
<th>Cytogenetics</th>
<th>% Blasts</th>
<th>Previous treatment</th>
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**NOTE:** Nature of the AML disease including WHO diagnosis and cytogenetics. Percent blast denotes the percentage AML blasts after purification using density gradient centrifugation, and in some instances, asterisk denotes samples that have been CD34+ selected.

*Isolated through CD34+ selection.

†Percentage of blasts and promyelocytes.
cells at 8 hours by 22.4 ± 4.7-fold over control in AML blasts and by 16.7 ± 3.2-fold over control in primary monocytes (means ± SEM, n = 8–17).

**NF-κB suppresses HO-1 in AML.** Because inhibition of NF-κB induces HO-1 expression, we determined if NF-κB subunits p50 and p65 were involved in the observed suppression of HO-1 in AML. Here, we used siRNA constructs to knock down p50 and p65 in AML-derived cell line THP-1. This experiment was also initiated to negate any nonspecific effects of BAY 11-7082 accounting for our observations. The THP-1 AML-derived cell line has been used throughout this study with similar results obtained by primary AML blasts, suggesting that THP-1 represents a valid model in which to further study the role of NF-κB in regulating HO-1 in human AML. The individual siRNA constructs were tested for their ability to knock down the expression of their respective proteins, p50 and p65 siRNAs knocked down their respective target proteins with no obvious nonspecific knockdown effects (Fig. 2D). Furthermore, knockdown of p50 or p65 by siRNA transfection of THP-1 cells resulted in increased HO-1 expression. This further suggests that both p50 and p65 are involved in suppressing HO-1 levels in human AML.

**Figure 2.** Inhibiting NF-κB in AML cells induces HO-1 expression. A, human AML and control cells were treated with 10 μmol/L BAY 11-7082 for various times. RNA was extracted and HO-1 mRNA was measured using real-time PCR, with expression normalized to GAPDH mRNA levels. B, Western blot analysis was performed on nonmalignant primary monocytes, AML103, and THP-1 in response to BAY 11-7082 incubation to confirm RNA results. C, RNA was extracted from human AML cells and control cells; HO-1 mRNA was measured and normalized to GAPDH levels. *, statistical significance of P < 0.05 exists between the different treatment groups. D, THP-1 cells were transfected with 30 nmol/L of either control, p50, or p65 siRNA and incubated for 24 and 48 h. Extract p50, p65, and HO-1 protein levels were analyzed by Western blot. Blots were reprobed with β-actin to confirm equal loading.
**HO-1 gene promoter is negatively controlled by NF-κB in AML cells.** We and others have shown that the human HO-1 promoter contains several transcription factor–binding sites, including two κB-binding sites located downstream of the ARE (Fig. 3A; refs. 22, 31). To determine if any of these sites are important in the basal regulation of HO-1 in AML cells, we created site-directed mutants of the human HO-1 promoter, which removed one or other of the κB sites (Fig. 3A). Such site-directed mutants of the two κB sites were also constructed within the pHO1 mutARE mutated ARE construct (Fig. 3A). To examine the functional role of these mutants compared with the wild-type promoter, we transfected THP-1 cells with each individual construct and examined basal expression levels of luciferase report. The construct with mutated κB1 site (construct B) had increased basal levels of promoter activity compared with wild-type promoter construct (Fig. 3B). Furthermore, when the role of mutated κB1 in pHO1 mutARE construct was examined (construct E), we additionally showed a similar raised basal response compared with wild-type pH01 construct, albeit with slightly less overall luciferase activity (Fig. 3B). As a positive control for the activation of the ARE by Nrf2, we used the antioxidant sulforaphane, a potent inducer of phase II detoxification enzymes, to maximally induce luciferase activity of HO-1 promoter (Fig. 3B). We also showed that no effect was observed when using HO-1 siRNA in this system (Fig. 3B). All these results suggest that the κB site located at −2200 upstream of the transcription start site may be responsible for the reduced expression of HO-1 in AML cells that we observe.

**In vivo recruitment of p65 and p50 to the HO-1 promoter.** Chromatin immunoprecipitation (ChIP) assay is a powerful technique to determine in vivo binding of transcription factors to chromatin in intact cells. To determine the in vivo relevance of p50 and p65 in negatively regulating basal HO-1 expression, we evaluated the recruitment of p50 and p65 to the HO-1 promoter on cells untreated or treated with BAY 11-7082. We also analyzed recruitment of Nrf2 to these sites, as we have shown that Nrf2 is involved in the regulation of...
Figure 4. Inhibiting HO-1 plus NF-κB in combination induces cell death in AML cells. A, AML and control cells were treated with increasing doses of ZnPP IX or CuPP IX (1–25 μmol/L) as indicated, either alone or combined with 10 μmol/L BAY 11–7082 for 24 h, and then cell number was assessed. B, AML and control cells treated as indicated with 10 μmol/L ZnPP IX alone or in combination with 10 μmol/L BAY 11–7082 for up to 72 h. C, AML cells were treated with BAY 11–7082 and ZnPP IX for 24 h and then examined by flow cytometry for apoptosis by the amount of cells positive for Annexin V–FITC/PI; the percentage of cells in each quadrant was indicated. D, AML and control cells transfected with 30 nmol/L of control or HO-1 siRNA and incubated for 24 h (protein) or 48 h (cell viability). Values indicate means ± SEM (n = 3). * P < 0.05.
HO-1. ChIP assays of untreated and BAY 11-7082–treated THP-1 cells were performed with the corresponding antibodies followed by PCR with specific primers amplifying three regions: ARE, κB1, and κB2 (Fig. 3C). ChIP analyses revealed that recruitment of p50 was markedly enhanced to the κB1 site on the HO-1 promoter in untreated THP-1 cells (Fig. 3C), recruitment that was inhibited by the presence of BAY 11-7082. ChIP analyses also revealed that Nrf2 is recruited to the ARE under basal conditions and is not changed by BAY 11-7082 treatment. The results for p65 were a little more ambiguous in that recruitment was observed but at a much reduced level; p65 recruitment was also BAY 11-7082 inhibitable. Figure 3D shows the relative expression levels of p50, p65, and Nrf2 at the ARE, κB1, and κB2 sites in these assays using real-time PCR and further supports the ChIP findings. What is interesting here is that we observed p50 NF-κB1 and p65 RelA binding at the ARE site (Fig. 3C and D), suggesting some molecular interplay between p50, p65, ARE, and Nrf2. This clearly needs further investigation.

**Inhibiting HO-1 and NF-κB in combination induces apoptosis in AML cells.** To resolve whether inhibiting NF-κB in combination with HO-1 inhibition can induce cell death in AML cells, AML blasts, THP-1, and CD34+ HSC were treated with increasing concentrations of ZnPP IX (an HO-1 inhibitor) or its control compound CuPP IX. This was performed in combination with 10 μmol/L BAY 11-7082 inhibition for 24 hours. Alone, increasing concentrations of ZnPP IX or CuPP IX had little or no effect on AML cell death; however, in combination with NF-κB inhibition, ZnPP IX but not CuPP IX induced cell death in AML cells but not in CD34+ cells (Fig. 4A). At the highest concentration of ZnPP IX, cell death was also observed in primary cells, suggesting that this compound is toxic to primary cells at this higher concentration. To determine if the effects of ZnPP IX were time dependent, we analyzed its effect over a 72-hour time period at the suboptimal concentration of 10 μmol/L on AML blasts, AML cell lines, and nonmalignant control cells. All AML blast samples tested were susceptible to cell death from the treatment of ZnPP IX plus BAY 11-7082 in combination but not with either inhibitor separately (Fig. 4B). The mode of cell death of primary cells was apoptotic, as determined by Annexin V and propidium iodide (PI) staining (Fig. 4C). To confirm the results observed with ZnPP IX, we also used HO-1 siRNA knockdown in AML blasts, THP-1, and primary monocytes. Figure 4D shows HO-1 siRNA-targeted knockdown in these cell lines.

**Figure 5.** ROS regulate the apoptotic potential of AML cells. A, AML and nonmalignant control cells were transfected with HO-1 siRNA for 24 h and then treated with NF-κB inhibition (10 μmol/L BAY 11-7082) for 4 h followed by washing with PBS and incubation for 15 min with 10 μmol/L of H$_2$DCFDA. Cells were then assessed for H$_2$DCFDA oxidation using flow cytometry, B, AML and nonmalignant control cells were transfected with HO-1 siRNA and then treated with both 10 μmol/L BAY 11-7082 and 10 mmol/L NAC for 24 h following examination for apoptosis by Annexin V–FITC/PI fluorescence-activated cell sorting analyses. The % of total cells in each quadrant is shown. C, results as in B for three different experiments are shown (means ± SEM). *, statistical significance of $P < 0.05$ exists between the different treatment groups.
cells in the presence of BAY 11-7082 and shows that BAY 11-7082 in combination with HO-1 targeted knockdown-induced cell death of AML cells but not of the noncancerous control cells. Thus, blockade of HO-1 activity in combination with NF-κB inhibition is sufficient to cause cytotoxicity of AML cells, where NF-κB inhibition alone is not.

**ROS play a key role in regulating the apoptotic potential of AML.** Because it has been shown that HO-1 helps regulate the redox equilibrium in human cells (32), and that ROS are increased in response to NF-κB inhibition (33), the role of ROS was examined as an inducer of apoptosis in AML cells on both NF-κB and HO-1 inhibition. We analyzed the production of ROS in monocytes and AML cells using H2DCFDA (a ROS-sensitive fluorescent dye) in response to NF-κB and HO-1 inhibition. H2DCFDA oxidation occurred in response to NF-κB inhibition and was further increased when we blocked HO-1 expression using HO-1 siRNA (Fig. 5A). The antioxidant N-acetylcysteine (NAC), which has been used to quench ROS activity within the cellular environment, blocked the apoptotic responses seen in NF-κB-inhibited and HO-1–inhibited THP-1 and primary AML cells (Fig. 5B and C). No such ROS effect was observed in human monocytes (Fig. 5). This suggest that ROS are the principal effectors of apoptosis in AML cells in response to NF-κB and HO-1 inhibition.

**Discussion**

The role of HO-1 in cancer biology is far from understood. In this study, we showed that AML has low HO-1 expression when compared with nonmalignant control cells. Interestingly, expression of HO-1 is usually increased in solid tumors compared with surrounding healthy tissues (13). In addition, primary chronic myeloid leukemia (CML) cells express HO-1 in a constitutive manner, and BCR/ABL fusion protein was found to upregulate HO-1 production in CML cells (14). Further work showed that targeting HO-1 activity plus imatinib-induced growth arrest in CML patient cells and imatinib-resistant CML cells were killed by blocking HO-1 activity. In this study, we show that AML samples become susceptible to apoptotic processes in the presence of combined NF-κB and HO-1 expression inhibition.

It is now becoming recognized that many transcription factors control the expression of the HO-1 gene. Among the transcription factors, NF-κB, Nrf2, and AP-1 families are arguably the most important, and among the best studied, regulators of the cellular stress response in vertebrates (21–23). Moreover, other transcription factors, including heat shock factors, hypoxia-inducible factor-1, signal transducers and activators of transcription proteins, CCAAT/enhancer binding protein factors, and USF family members (26), are known to regulate HO-1 transcription under specific circumstances and may work in conjunction with Nrf2, AP-1, and NF-κB (26). In addition, the HO-1 gene is transcribed under homeostatic conditions, and this activity is modulated in a tissue-dependent manner during development and differentiation and by stress-independent stimuli, such as growth factors (34). Thus, one explanation for the difference of HO-1 levels in cancers cells known to harbor high levels of NF-κB activity is that HO-1 is regulated by many different, even contrasting, factors. Combined with the local environment “niche” in which AML blasts are produced, these facets provide unique settings to control the regulation of gene expression in AML cells.

The signaling pathway from HO-1 to downstream apoptosis is poorly understood in cancer cells, although there are several publications now showing HO-1 to be antiapoptotic in several cell types (32). The mechanism by which HO-1 protects against apoptosis is thought to be through several different effectors pathways, including the regulation of redox-active iron (35), heme catabolism into the gas CO that acts through the activation of the p38 mitogen-activated protein kinase signaling (36), and finally by increasing bilirubin levels, thought to provide potent scavenging potential (37). In this study, we show that HO-1 protects AML cells from apoptosis through the inhibition formation of ROS. However, though what pathway this occurs is still unknown and warrants further investigation because this could be a possible target for AML therapy.

To date, several studies have indicated that NF-κB can control the induced expression of HO-1 (23–25). The κB site in the HO-1 promoter, which is thought to control this induced expression, was first described by Lavrovsky and colleagues (23). One of the main arguments against HO-1 expression being controlled by this κB site at −160 is that this site is not conserved in mammals (26). The κB site set at −2200 is conserved in mammals, suggesting that this is an important site in regulating HO-1 expression. However, one important point to be addressed is what makes p50 and p65 actively inhibit HO-1 expression in AML cells but not in normal cells, although both proteins are active in each cell type. One possible explanation to this is that we and other groups have shown that there is a difference in NF-κB activity levels between normal and AML cells under unstimulated basal conditions. Moreover, under activated conditions, other transcription factors, such as Nrf2 and AP-1, may function to override any suppressive NF-κB activity there is on the HO-1 gene in normal cells.

The concept that NF-κB suppresses transcription opposes its classic role in stimulating the transcription of target genes. Nonetheless, several recent studies have suggested that NF-κB may downregulate cellular responses to specific stimuli. For example, TNF-mediated c-Jun NH2-terminal kinase signaling is enhanced in NF-κB knockout mice (38, 39). Furthermore, NF-κB has been shown to have opposing effects on IFN-induced gene expression (40). Here, we have shown that NF-κB suppresses basal HO-1 expression in human AML cells but not in normal nonmalignant cells. Interestingly, mutating the κB1 site but not κB2 or Nrf2 sites causes an increase in HO-1 promoter activity. Moreover, examination of the in vivo recruitment of the κB1 site shows that both p50 and p65 subunits bind to κB1 site in AML cells but not in noncancerous control cells. We have also shown that inhibition of IkBα phosphorylation using BAY 11-7082 increases HO-1 expression in AML cells but not in nonmalignant control cells. To negate any nonspecific effects of BAY

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3 S.A. Rushworth and D.J. MacEwan, unpublished data.
11-7082, which include inducing cell death in NF-κB-independent manner in Ewing’s sarcoma (41), we also showed that siRNA-targeted knockdown of either p50 or p65 shows that HO-1 protein increases to levels comparable with those seen in control nonmalignant cells. Taken together, these results show that basal HO-1 expression is under the control of NF-κB subunits p50 and p65 in AML. Interestingly, we also observed that p50 (and to a lesser extent p65) could bind to the ARE site at −4100 (Fig. 3). How p50 interacts with the ARE is unknown; however, our future studies will determine the interplay between p50, Nrf2, and ARE. This is not the first time that another transcription factor has been associated with Nrf2 and the ARE-binding site; for example, a recent study showed that p53 can suppress Nrf2-dependent transcription by displacing Nrf2 bound to the promoter of ARE-dependent genes (42). These results suggest a complexity in the transcription of HO-1, which needs further investigation especially because HO-1 is increasingly becoming a likely target for new types of cancer therapy.

The findings we report here in this study give further support to the hypothesis that HO-1 and its transcriptional machinery have a significant role in AML; however, this role is only revealed when you inhibit NF-κB. In summary, we have shown that in AML-derived cells (but not their nonmalignant counterparts) HO-1 is suppressed by constitutive NF-κB activation. Furthermore, the NF-κB subunits p50/NF-κB1 and p65 seem to be responsible for this suppression of HO-1 by binding to only one of the κB sites in the HO-1 promoter. Why this response occurs only in these cancerous cells is unclear; however, we have shown previously that HO-1 underlies resistance of AML cells to apoptotic stimuli. Subsequently, we hypothesize that high basal activity of NF-κB suppresses normal apoptotic processes in AML cells; however, if we inhibit this basal NF-κB activity, HO-1 is subsequently induced and therefore provides a second form of defense against anticancerous agents. Taken together, these findings suggest that anticancer therapy needs to target both NF-κB and HO-1 to efficiently combat AML.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Correction: Online Publication Dates for Cancer Research April 15, 2010 Articles

The following articles in the April 15, 2010 issue of Cancer Research were published with an online publication date of April 6, 2010 listed, but were actually published online on April 13, 2010:


Dudka AA, Sweet SMM, Heath JK. Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification. Cancer Res 2010;70:3391–401. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3033.

NF-κB–Inhibited Acute Myeloid Leukemia Cells Are Rescued from Apoptosis by Heme Oxygenase-1 Induction

Stuart A. Rushworth, Kristian M. Bowles, Prahlad Raninga, et al.

Cancer Res 2010;70:2973-2983. Published OnlineFirst March 23, 2010.

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