NRH:Quinone Oxidoreductase 2 (NQO2) Protein Competes with the 20 S Proteasome to Stabilize Transcription Factor CCAAT Enhancer-binding Protein α (C/EBPα), Leading to Protection against γ Radiation-induced Myeloproliferative Disease*

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Background: NQO2 protects against γ radiation-induced myeloproliferative disease, but the mechanism remains unknown.

Results: Radiation-induced NQO2, independent of NQO1, competes with the 20 S proteasome for interaction with C/EBPα region Ser-268 to Val-279 to stabilize C/EBPα, leading to protection against myeloproliferative disease.

Conclusion: NQO2 stabilizes C/EBPα against 20 S degradation to protect against myeloproliferative disease.

Significance: Stress-responsive NQO2 functions as an endogenous factor against myeloproliferative diseases.

NRH:quinone oxidoreductase 2 (NQO2) is a flavoprotein that protects cells against radiation and chemical-induced oxidative stress. Disruption of the NQO2 gene in mice leads to γ radiation-induced myeloproliferative diseases. In this report, we demonstrated that NQO2 protects against myeloproliferative disease, but the mechanism remains unknown.

NRH: novonic oxidoreductase 2 (NQO2) is a flavoprotein that catalyzes the reductive metabolism of quinones (1). NQO2 belongs to a two-member quinone oxidoreductase gene family that also includes NQO1 (2). NQO2 utilizes NRH as an electron donor, and its activity is inhibited by benzo(a)pyrene (1). Analysis of the crystal structure of NQO2 revealed that NQO2 contains a specific metal binding site that is not present in NQO1 (3). NQO2 and/or NQO1 are stress-inducible proteins that are known to protect tumor suppressor p53, p33(ING Ib), and p63 against 20 S proteasomal degradation (4–6).

Mice generated in our laboratory showed myeloid hyperplasia and significantly increased granulocyte levels in the peripheral blood (7). In addition, NQO2−/− mice demonstrated increased sensitivity to γ radiation-induced myeloproliferative disease and B cell lymphomas (7, 8). These studies suggest that NQO2 plays a significant role in protection against hematological disorders.

The transcription factors C/EBPα and PU.1 are known to regulate hematopoiesis (9, 10). C/EBPα is found predominantly in immature myeloid cells, whereas both lymphoid and myeloid cells express PU.1 (9, 10). Deregulation of C/EBPα has been found to be associated with myeloid transformation (11, 12). C/EBPα is known to regulate PU.1 gene expression (13).

In this report, we demonstrated that NQO2 protects myeloid differentiation factor C/EBPα against 20 S proteasomal degradation. NQO2 achieves this function by competing with 20 S proteasomes for binding with C/EBPα. NQO2 interacts with the same C/EBPα domain. NQO2−/− mice, deficient in NQO2, failed to stabilize C/EBPα. This contributed to the development of γ radiation-induced myeloproliferative disease in NQO2−/− mice.

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† The abbreviations used are: C/EBPα, CCAAT enhancer-binding protein α; Gy, gray; NRH, dihydronicotinamide riboside.
was from Invitrogen. NQO1 siRNA (catalog no. 4090) and NQO2 siRNA (catalog no. 9597) were from Ambion (Grand Island, NY). Antibodies against NQO2 (human cells) or C/EBPα and protein A/G-agarose were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). PU.1 antibody was from Cell Signaling Technology (Beverly, MA). Perfluoro-octanoic acid was from Oakwood Products (Columbia, SC). Nucleofector II and reagents were from Lonza (Cologne, Germany). Peptides were from Biosynthesis (Lewisville, TX).

Construction of Plasmids—All mouse C/EBPα truncated constructs were created by first performing a PCR to amplify the cDNA of interest using primers carrying the XbaI, EcoRI, or Xhol sites. By using XbaI (or EcoRI) and Xhol, the PCR products were digested and cloned into the pCMX vector containing a double FLAG tag, resulting in final expression constructs. To generate site-directed mutations of mouse C/EBPα cDNA, two PCRs were carried out to amplify the 5′ fragment of mouse C/EBPα cDNA (carrying the XbaI site) and the rest of the 3′ fragment of mouse C/EBPα cDNA (with site mutations and carrying the Xhol site). After blunt end ligation, the fragments that were ~1080 base pairs long were digested with XbaI and Xhol and cloned into the pCMX vector containing a double FLAG tag. This approach was applied because C/EBPα DNA sequencing was used to verify all constructs. (from Stratagene and Invitrogen) do not work. Double-strand extremely GC-rich (72%), and site-directed mutagenesis kits were from Biosynthesis (Lewisville, TX).

FLAG-C/EBPα (1–279) were created by blunt-ended cloning, and all truncated NQO2 cDNAs were digested with XbaI and reagents were from Lonza (Cologne, Germany). Pep-}

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Statistical Analysis—Microsoft Excel (2007) and Prism Graphpad (version 6) were used for statistical analysis. For statistical analysis, experiments were repeated three times, as shown in the figure legends. Differences were considered significant at $p < 0.05$.

RESULTS

NQO2 Protects C/EBP$\alpha$ against 20 S Proteasomal Degradation—WT and NQO2$^{-/-}$ mice were not irradiated or irradiated with 3 Gy of $\gamma$ radiation to determine the short-term effect of radiation on the expression of NQO2, C/EBP$\alpha$, and PU.1. The results are shown in Fig. 1A (left panel and bar graph). Bone marrow cells from non-irradiated NQO2$^{-/-}$ mice showed higher levels of C/EBP$\alpha$ and PU.1 as compared with wild-type mice, suggesting that increased C/EBP$\alpha$ and PU.1 were responsible for myeloid hyperplasia in NQO2$^{-/-}$ mice, as reported earlier (7). The mechanism of higher expression of C/EBP$\alpha$ and PU.1 in NQO2$^{-/-}$ bone marrow remains unknown. Interestingly, exposure to 3 Gy of $\gamma$ radiation increased NQO2, C/EBP$\alpha$, and PU.1 to varying levels in wild-type mice but failed to do so in NQO2$^{-/-}$ bone marrow. These results indicated that NQO2$^{-/-}$ mice, deficient in NQO2, were incapable of inducing the myeloid differentiation factors C/EBP$\alpha$ and PU.1 upon exposure to $\gamma$ radiation, as observed with wild-type mice expressing the NQO2 gene.

Next, we performed ex vivo bone marrow studies to investigate the role of protein degradation in the lack of induction of C/EBP$\alpha$ and PU.1 because of exposure to $\gamma$ radiation. Bone marrow cells from wild-type and NQO2$^{-/-}$ mice were exposed to 3 Gy of $\gamma$ radiation in the absence and presence of the proteasome inhibitor MG132 and analyzed for C/EBP$\alpha$, PU.1, and actin. MG132 treatment protected C/EBP$\alpha$ and PU.1 from $\gamma$ radiation-induced degradation (Fig. 1A, right panel and bar graph). This was evident from increased levels of C/EBP$\alpha$ in bone marrow cells from NQO2$^{-/-}$ mice exposed to $\gamma$ radiation in the presence of MG132. The results, together, suggest that exposure to $\gamma$ radiation leads to proteasomal degradation of C/EBP$\alpha$ in the absence of NQO2. In other words,
NQO2 is required for the stabilization and induction of C/EBPα in response to γ radiation.

Next, HL-60 and U937 cells were immunoblotted to compare NQO2 levels. The results showed that U937 cells express 2-fold higher levels of NQO2 as compared with HL-60 cells (Fig. 1B, left panel). These cells were used to further strengthen our observation that NQO2 controls C/EBPα. HL-60 cells transfected with the FLAG-NQO2 plasmid (Fig. 1B, center panel and bar graph) and U937 cells transfected with NQO2 siRNA (right panel and bar graph) were analyzed for alterations in NQO2 and its effect on C/EBPα and PU.1. The results revealed that overexpression of NQO2 in HL-60 cells led to the up-regulation of both C/EBPα and PU.1, whereas siRNA-mediated inhibition of NQO2 in U937 cells led to the down-regulation of both C/EBPα and PU.1. The inhibition of NQO2 followed by down-regulation of C/EBPα and Pu.1 was observed with two different NQO2 siRNA (Fig. 1B, right panel and bar graph). These results demonstrated and strengthened our earlier observation that NQO2 controls C/EBPα stability, followed by increased PU.1 expression.

We performed in vitro assays to investigate the role of the 20 S proteasome in C/EBPα degradation and the role of NQO2 protection against 20 S degradation of C/EBPα. Incubation of in vitro-translated C/EBPα with the purified 20 S proteasome showed significant degradation of C/EBPα within 1 h (Fig. 1C). Inclusion of in vitro-translated NQO2 in the incubation mixture partially protected C/EBPα. Interestingly, NQO2, in combination with its cofactor NRH in the same experiment, provided complete protection of C/EBPα against 20 S proteasomal degradation (Fig. 1C). The results collectively indicate that NQO2 stabilizes C/EBPα against 20 S proteasomal degradation.

NQO2 and 20 S Both Interact with C/EBPα but Not with PU.1—Immunoprecipitation assays were performed to investigate the role of NQO2 and 20 S interaction with C/EBPα in the inhibition of 20 S degradation of C/EBPα. Bone marrow from wild-type and NQO2−/− mice, HL-60 cells, and HL-60 cells transfected with NQO2-V5 and FLAG-C/EBPα plasmids were analyzed for interactions among C/EBPα, 20 S, and NQO2 (Fig. 2A). The results showed that NQO2 and 20 S interact with C/EBPα in mouse bone marrow (Fig. 2A, left panels), HL-60 cells (center panel), and transfected HL-60 cells (right panel). The results also showed that NQO2 and 20 S did not interact with each other (Fig. 2A). The results further indicated that C/EBPα interacted with 20 S in the absence of NQO2 in NQO2−/− mouse bone marrow. In similar experiments, as described above for C/EBPα in Fig. 2A, immunoprecipitation assays failed to show PU.1 interaction with either NQO2 or 20 S proteasomes (Fig. 2B). This confirmed that NQO2 controls PU.1 through C/EBPα, which regulates the transcription of PU.1.

Amino Acids Ser-268 to Val-279 of C/EBPα Are Required for Its Interaction with Both NQO2 and 20 S Proteasome, and Lysines 276/277 of C/EBPα Are Essentially Required for Its Interaction with NQO2—FLAG-tagged C/EBPα deletions were generated to identify protein domain(s) that interact with NQO2 and 20 S. The various deletions produced expected sizes of truncated C/EBPα bands upon transfection in HL-60 cells (Fig. 3A, left panel). Transfection and immunoprecipitation assays revealed that all deletions, except 1–162, 279–359, and Δ268–279, interacted with NQO2 and 20 S (Fig. 3A, right panel). The results, together, clearly indicate that the C/EBPα protein region amino acid 268–279 is essential for interaction with both NQO2 and 20 S. Alignment of the mouse C/EBPα protein region between amino acids 268–279 with the complementary human and rat showed conserved residues Lys-274, Lys-276, and Lys-277; Gly-269, Gly-271, and Gly-273; Ala-272 and Ala-275; Ser-278; and Val-279. Site-directed mutagenesis was used to individually mutate Lys-274, Lys-276, and Lys-277 to generate the FLAG-tagged C/EBPα K274A, K276A, and K277A mutant plasmids. Transfection and immunoprecipitation assays demonstrated that mutation of Lys-276 and Lys-277 to alanine resulted in the
loss of interaction of C/EBPα with NQO2, whereas the C/EBPα K274A mutant still interacted with NQO2 (Fig. 3B). These results suggest that Lys-276/Lys-277 of C/EBPα is required for its interaction with NQO2. Interestingly, all four C/EBPα mutants (K274A, K276A, K277A, and the double mutant K276A/K277A) still interacted with 20 S (Fig. 3B). These results indicated that mutations in individual amino acids between Ser-268 and Val-279 had no effect on C/EBPα interaction with 20 S, suggesting the requirement of all of the amino acids in the 268–279 domain for the interaction of C/EBPα with 20 S.

Histidine pull-down assays showed same results as those observed in the immunoprecipitation assays (Fig. 3C). Both NQO2 and 20 S were pulled down with C/EBPα-V5-His6, but not with C/EBPαΔ268–279-V5-His6. In related pull-down assays, 20 S, but not NQO2, was pulled down with both mutant C/EBPαK276A-V5-His6 and C/EBPαK277A-V5-His6. Ubiquitination assays results showed that C/EBPα and the mutants were all ubiquitinated to the same extent (Fig. 3D). This indicated that neither Lys-276 nor Lys-277 are the ubiquitination sites of C/EBPα.

NQO2 Deletions Fail to Interact with C/EBPα—V5-tagged deletions of NQO2 were generated to identify the domain(s) required for interaction with C/EBPα (Fig. 4A). Transfection of these mutants in HL-60 cells produced the expected size of truncated NQO2-V5 proteins (Fig. 4B, top panel). Transfection and immunoprecipitation assays revealed that all deletions failed to interact with C/EBPα (Fig. 4B). This suggests that a full-length NQO2 protein is required to interact with C/EBPα. In similar experiments, the mutations in FAD binding sites (Y104A, W105A, F106A, and Y155A), catalytic sites (F126A, F131A, and Y132A), and metal binding sites (H173A, H177A, and C222A) of NQO2 also failed to block C/EBPα protection against 20 S degradation (Fig. 4C). In related experiments, three C/EBPα peptides (Ser-268 to Val-279 (full-length), Ser-268 to Gly-273 (N-terminal half), and Lys-274 to Val-279 (C-terminal half)) were synthesized and used in in vitro assays to determine their effects on C/EBPα interaction with 20 S (Fig. 4D). All three peptides competed with C/EBPα for binding with 20 S and degradation. Interestingly, C-terminal half peptides containing basic lysine residues were significantly more efficient in competing with C/EBPα against 20 S degradation as compared with the N-terminal half containing small/neutral amino acids.

Benzo(a)pyrene Inhibits NQO2 Protection of C/EBPα against 20 S Degradation, and γ Radiation Leads to an Increase in NQO2:C/EBPα Interaction and a Decrease in 20 S:C/EBPα Interaction—We performed in vitro assays to determine the effect of benzo(a)pyrene inhibition of NQO2 on its capacity to
protect C/EBPα against 20 S degradation. Benzo(a)pyrene treatment significantly reduced NQO2 protection of C/EBPα against 20 S degradation in the presence or absence of 50 μM CYP1A1 inhibitor omeprazole (Fig. 5A, top panel). Omeprazole at 50 μM concentration inhibited 70–80% CYP1A1 activity in Hepa-1 cells and rabbit reticulocyte lysate (Fig. 5A, bottom panel). These results suggest that both NQO2 and cofactor NRH are required for protection of C/EBPα against 20 S degradation and blocking of benzo(a)pyrene inhibition. The results also demonstrate that the benzo(a)pyrene metabolism is not required for inhibition of NQO2 activity. Next, we used in vitro assays to determine direct competition between NQO2 and 20 S for C/EBPα. In vitro-translated C/EBPα and purified 20 S were mixed with increasing concentrations of NQO2 alone or NQO2 + NRH, and coimmunoprecipitation assays were performed to determine C/EBPα interactions with NQO2 and 20 S. The results indicated that both NQO2 and 20 S proteasome competed to interact with C/EBPα. Increasing NQO2 concentration in the absence of NRH provided an NQO2 concentration dependent increase in NQO2:C/EBPα interaction and a decrease in 20 S:C/EBPα that was clearly visible but not highly significant (Fig. 5B, left two panels). In related experiments, increasing the concentration of NRH reversed back the inhibitory effect of benzo(a)pyrene on NQO2 protection of C/EBPα against 20 S degradation, and increasing the NRH concentration in the presence of NQO2 led to NRH concentration-dependent, highly significant increases in NQO2:C/EBPα interaction and decreases in 20 S:C/EBPα interaction (Fig. 5B, right two panels). These results suggest that cofactor NRH, in association with NQO2, controls the interactions between NQO2: C/EBPα and 20 S:C/EBPα.

HL-60 cells, not irradiated or irradiated with γ radiation in three independent experiments, were analyzed for C/EBPα, NQO2, and 20 S interactions, and a densitometry analysis was performed (Fig. 5C). The results demonstrated a 90% increase in NQO2 interaction with C/EBPα in response to γ radiation. The results also revealed that γ radiation induced a 40% decrease in the 20 S interaction with C/EBPα (Fig. 5C). In similar experiments, WT mice were exposed to 3 Gy of γ radiation. Bone marrow cells were collected and analyzed for C/EBPα, NQO2, and 20 S interaction. A significant increase in NQO2 interaction with C/EBPα and a nearly 2-fold decrease in 20 S interaction with C/EBPα were observed (Fig. 5D). The results, together, suggested that exposure to γ radiation led to a significantly greater interaction of C/EBPα with NQO2 and reduced its interaction with 20 S proteasomes, leading to the stabilization of C/EBPα and the up-regulation of downstream gene expression.

NQO2 and NQO1 Independently Protect C/EBPα—The current report on NQO2 and a report published previously on NQO1 (14) suggest that both protect C/EBPα against 20 S proteasomal degradation during stress. Therefore, we performed in vitro and in vivo experiments to compare similarities and differences between NQO2 and NQO1 protection of C/EBPα.
In Vitro Studies—NQO1 cofactor NADH or NQO2 cofactor NRH alone were ineffective in blocking 20 S degradation of C/EBPα (Fig. 6A, top panel). However, NADH in the presence of NQO1 protected C/EBPα against 20 S degradation, and the protective effect is positively proportional to the NADH concentration from 1–10 μM. In the meantime, NRH (1–10 μM) in the presence of NQO1 was unable to provide more protection of C/EBPα than NQO1 alone (Fig. 6A, center left panel and bottom right bar graph). In a similar experiment, NRH in the presence of NQO2 protected C/EBPα against 20 S degradation, and the protective effect is positively proportional to the NRH concentration from 1–10 μM. Although NADH (1–10 μM) in the presence of NQO2 was unable to provide more protection of C/EBPα than NQO2 alone (Fig. 6A, center right panel and bottom right bar graph). In related experiments, the NQO1 inhibitor dicoumarol blocked NQO1 + NADH stabilization of C/EBPα, but NQO2 inhibitor benzo(a)pyrene was unable to do so (Fig. 6B, top panel). In a similar experiment, the NQO2 inhibitor benzo(a)pyrene abolished NQO2 + NRH protection of C/EBPα, but the NQO1 inhibitor dicoumarol was unable to do so (Fig. 6B, bottom panel). The above results collectively suggested that NQO1 + NADH and NQO2 + NRH independently protected C/EBPα against 20 S proteasomal degradation. The results also suggested that NQO1 could not complement NQO2 and vice versa because of differences in cofactor requirements.

In Vivo Studies—HL-60 cells were treated with either 10 μM NQO1 inhibitor dicoumarol or 50 μM NQO2 inhibitor benzo(a)pyrene for 24 h, not irradiated or irradiated with γ radiation, and analyzed for C/EBPα, NQO1, NQO2, and 20 S interactions. Dicoumarol treatment abrogated C/EBPα interaction with NQO1, but not with NQO2, in both control and γ radiation-exposed cells (Fig. 7A). The results also demonstrated the increase in NQO2 interaction with C/EBPα in response to γ radiation and the decrease in 20 S interaction with C/EBPα (Fig. 7A). The benzo(a)pyrene treatment in a similar experiment blocked C/EBPα interaction with NQO2 but not with NQO1 in non-irradiated and irradiated cells (Fig. 7B). Benzo(a)pyrene also increased NQO2 interaction with C/EBPα in response to γ radiation and decreased 20 S interaction with C/EBPα (Fig. 7B). The results, together, suggested that exposure to γ radiation led to a significantly greater interaction of C/EBPα with NQO1 or NQO2 and reduced its interaction with 20 S proteasomes, leading to the stabilization of C/EBPα and the up-regulation of downstream gene expression. In related experiments, HL-60 cells were transfected with either control siRNA, NQO1 siRNA, NQO2 siRNA, or a combination of NQO1 and NQO2 siRNAs, exposed to 3 Gy of γ radiation, and analyzed. The results...
revealed that siRNA-mediated inhibition of NQO1 or NQO2 both significantly reduced C/EBPα levels in HL-60 cells (Fig. 7C). Interestingly, the combined inhibition of NQO1 and NQO2 led to a complete loss of C/EBPα in γ-radiation-exposed cells (Fig. 7C). The same results were observed in bone marrow cells from wild-type, NQO1−/−, NQO2−/−, and double knockout (DKO) mice. The deletion of NQO1 or NQO2 led to a significant decrease in C/EBPα levels (Fig. 7D). In addition, the combined deletion of NQO1 and NQO2 led to a complete loss of C/EBPα in radiation-exposed cells (Fig. 7D). Collectively, the in vivo results...
FIGURE 8. Model to demonstrate NQO2 protection against stress-induced myeloproliferative diseases.

NQO2 Protection of C/EBPα

...supported *in vitro* results and led to the conclusion that NQO1 and NQO2 independently control the stability of C/EBPα against 20 S proteasomal degradation.

**DISCUSSION**

Human gene polymorphism and mouse studies have shown a direct association between NQO1 and its protection against myeloproliferative diseases, including leukemia (15–16). NQO2, structurally related to NQO1, also exhibited protection against myeloid hyperplasia (7) and myeloproliferative disease (8). This raised intriguing questions regarding the mechanism of NQO2 protection against radiation-induced myeloproliferative diseases. Disruption of C/EBPα and PU.1 is known to be associated with the development of myeloid leukemia (17). This study investigated NQO2 control of C/EBPα and PU.1 as a mechanism contributing to the protection against myeloproliferative diseases. NQO2−/− mice exposed to γ radiation showed a lack of induction of C/EBPα and PU.1. Wild-type mice, upon exposure to γ radiation, showed a significant induction of NQO2, C/EBPα, and PU.1 (this work) and the absence of myeloproliferative diseases (7, 8). Therefore, the lack of induction of C/EBPα and PU.1 contributed to myeloproliferative diseases in NQO2−/− mice. The lack of induction of C/EBPα was due to the rapid degradation by 20 S proteasomes in the absence of NQO2. NQO2 directly interacted with C/EBPα and prevented its degradation by the 20 S proteasomes. This led to the stabilization of C/EBPα, normal differentiation of myeloid progenitor cells, and protection against myeloproliferative diseases. NQO2 indirectly regulates PU.1 because PU.1 does not directly interact with NQO2 or 20 S proteasomes. C/EBPα is known to up-regulate PU.1 gene transcription (16). Therefore, NQO2-mediated stabilization or 20 S degradation of C/EBPα up- or down-regulated PU.1 transcription, respectively.

NQO2 and 20 S proteasomes competed for the same C/EBPα region between amino acids Ser-268 and Val-279. Internal deletion of this in C/EBPα resulted in the loss of interaction with both NQO2 and 20 S. The NQO2 interaction with C/EBPα was further narrowed down to two lysine residues at positions 276 and 277. Mutations of one or both of these lysine residues abolished C/EBPα interaction with NQO2 but not with 20 S. Interestingly, single mutations in the 20 S-interacting domain had no effect on CEBPα interaction with 20 S. This indicated that the complete domain was required for C/EBPα and 20 S interaction. The NQO2/20 S-interacting domain in mouse C/EBPα was highly conserved in humans and rats. Deletions in NQO2 protein had deleterious effects on NQO2 interaction with C/EBPα. This indicates that secondary/tertiary/folding structures are required for NQO2 interaction with C/EBPα. This study also revealed that NQO2 requires its cofactor NRH to protect C/EBPα against 20 S degradation. The role of NRH in NQO2 stabilization of C/EBPα requires further investigation.

A similar mechanism as that described above for NQO2 is also reported by us for NQO1 stabilization of C/EBPα against 20 S degradation (14). Therefore, we investigated similarities/differences and complementarity between NQO2 and NQO1 protection of C/EBPα. The similarities included NQO2 and NQO1 competition with 20 S proteasome for interaction with the C/EBPα domain S(268)GAGAGKAKKSV(279); the requirement of full-length NQO2 and NQO1 proteins to interact with C/EBPα; and stress-induced, increased C/EBPα interaction with NQO2 and NQO1 and decreased interaction with 20 S proteasome, leading to the stabilization of C/EBPα. The differences included the requirement of different cofactors (NADH for NQO1 and NRH for NQO2), the lack of complementarity between NQO2 and NQO1 with respect to C/EBPα stabilization because of different cofactor requirements, and the inability of the NQO1 inhibitor dicoumarol to inhibit C/EBPα:NQO2 interaction and of the NQO2 inhibitor benzo(a)pyrene to inhibit C/EBPα:NQO1 interaction. These differences led to the conclusion that the NQO2 + NRH and NQO1 + NADH stabilization of C/EBPα against 20 S degradation are independent of each other. This is supported by the observation that combined siRNA inhibition of NQO1 and NQO2 in HL-60 cells and double NQO1 and NQO2 deletion in mice both demonstrated complete degradation of C/EBPα by 20 S proteasomes. Individual deletions of NQO1 and NQO2 reduced, but did not eliminate, C/EBPα stabilization.
NQO2 Protection of C/EBPα

The expression of the NQO1 and NQO2 genes is coordinately activated in response to radiation and chemicals and is critical in the protection against myeloproliferative diseases (7, 8, 18). The induction of NQO2 enhances interaction with C/EBPα and reduces 20 S interaction with C/EBPα, leading to the protection and stabilization of C/EBPα. NQO2-mediated protection of C/EBPα allows the accumulation of C/EBPα and downstream PU.1 that is required for differentiation of myeloid cells. NQO2+/− mice and, possibly, human individuals lacking or deficient in NQO2 fail to accumulate C/EBPα, which leads to myeloproliferative diseases. It is noteworthy that human NQO2 gene promoter polymorphism is known to lead to lower expression of NQO2 in a significant portion of the population (19, 20).

A hypothetical model of NQO2 protection of C/EBPα against 20 S degradation is shown in Fig. 8. Under normal conditions, both 20 S and NQO2 interact with C/EBPα and control the physiological levels of C/EBPα, PU.1, and myeloid differentiation. Exposure to γ radiation leads to increased NQO2 levels and increased NQO2 interaction with C/EBPα. Increased NQO2 interaction with C/EBPα and decreased interaction of C/EBPα with 20 S lead to the stabilization of C/EBPα, increased expression of PU.1, and differentiation of myeloid cells and protection against γ radiation. On the contrary, the loss of NQO2 leads to increased 20 S interaction with C/EBPα, degradation of C/EBPα, down-regulation of PU.1, lack of myeloid cell differentiation, and myeloproliferative diseases, including leukemia/lymphoma.

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