Disruption of NAD(P)H:Quinone Oxidoreductase 1 Gene in Mice Leads to Radiation-Induced Myeloproliferative Disease

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Abstract

NAD(P)H:quinone oxidoreductase 1 null (NQO1−/−) mice exposed to 3 Gy of γ-radiation showed an increase in neutrophils, bone marrow hypercellularity, and enlarged lymph nodes and spleen. The spleen showed disrupted follicular structure, loss of red pulp, and granulocyte and megakaryocyte invasion. Blood and histologic analysis did not show any sign of infection in mice. These results suggested that exposure of NQO1−/− mice to γ-radiation led to myeloproliferative disease. Radiation-induced myeloproliferative disease was observed in 74% of NQO1−/− mice as compared with none in wild-type (WT) mice. NQO1−/− mice exposed to γ-radiation also showed lymphoma tissues (32%) and lung adenocarcinoma (84%). In contrast, only 11% WT mice showed lymphoma and none showed lung adenocarcinoma. Exposure of NQO1−/− mice to γ-radiation resulted in reduced apoptosis in granulocytes and lack of induction of p53, p21, and Bax. NQO1−/− mice also showed increased expression of myeloid differentiation factors CCAAT/enhancer binding protein α (C/EBPα) and Pu.1. Intriguingly, exposure of NQO1−/− mice to γ-radiation failed to induce C/EBPα and Pu.1, as was observed in WT mice. These results suggest that increased p53/apoptosis and increased Pu.1 and C/EBPα led to myeloid hyperplasia in NQO1−/− mice. The lack of induction of apoptosis and differentiation contributed to radiation-induced myeloproliferative disease in NQO1−/− mice. [Cancer Res 2008;68(19):7915–22]

Introduction

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a cytosolic protein that catalyzes the metabolism of quinones and their derivatives (1, 2). It has been shown that the two-electron reduction of quinones, catalyzed by NQO1, competes with the one-electron reduction catalyzed by cytochrome P450 and cytochrome P450 reductase. This produces a comparatively stable hydroquinone that is removed by conjugation with glutathione, UDP-glucuronic acid, etc. (1–3). The two-electron reduction of quinones does not result in the formation of free radicals (semiquinones) and highly reactive oxygen species, hence protecting cells against the adverse effects of quinones and their derivatives (1, 2). NQO1 activity is ubiquitously present in all tissue types (1, 2). NQO1 gene expression is induced in response to xenobiotics, antioxidants, oxidants, heavy metals, UV light, and ionizing radiation (1, 2, 4). Interestingly, NQO1 is part of an electrophilic and/or oxidative stress–induced cellular defense mechanism that includes the induction of more than two dozen genes (1, 2, 4). The coordinated induction of defensive genes, including NQO1, provides necessary protection for cells against free radical damage, oxidative stress, and neoplasia.

Human NQO1 gene has been localized to chromosome 16q22 (5). A cytosine to thymidine (C→T) polymorphism in exon 6 of human NQO1 gene produces a proline to serine (P187S) substitution that destabilizes and inactivates the enzyme (6, 7). The polymorphic NQO1 is rapidly degraded via ubiquitination and proteasome degradation (7). Individuals carrying both polymorphic genomic alleles are completely lacking in NQO1 activity, whereas individuals who are heterozygous with one polymorphic allele have low to intermediate NQO1 activity compared with wild-type (WT) individuals (8). Approximately 2% to 4% of human individuals are homozygous and 20% to 25% are heterozygous for this polymorphism (7–12). The frequency of NQO1 P187S is similar in Whites and African Americans, but higher in Hispanics and Asians (9–12). NQO1 P187S has been associated with greater risk of neutropenia in benzene-exposed adult Chinese workers (13) and is significantly overexpressed in radiation/chemotherapy-related (14) and de novo leukemias (15) in adults. Recently, Wiemels and colleagues (16) reported that NQO1 P187S conferred susceptibility to infant acute lymphoblastic leukemia and acute myeloid leukemia (AML) with mixed lineage leukemia translocations in a British population. More recently, Smith and colleagues (17) reported similar findings in U.S. populations.

NQO1−/− mice were generated (18). Mice deficient in NQO1 gene expression were born and developed normal, indicating that NQO1 does not play a role in mouse development. Further studies on NQO1−/− mice have revealed altered intracellular redox status and altered metabolism of carbohydrates, fatty acids, and nucleotides, and reduced accumulation of abdominal fat with age (19). In addition, studies showed that loss of NQO1 gene expression in NQO1−/− mice led to myelogenous hyperplasia of bone marrow and increased sensitivity of NQO1−/− mice to mendian-induced hepatic damage (20). NQO1−/− mice also showed benzene toxicity (21) and significantly increased sensitivity to skin carcinogenesis in response to benzo(a)pyrene (22) and dimethylbenzanthracene (23). NQO1−/− mice showed lower levels of tumor suppressor protein p53 and decreased apoptosis in bone marrow and skin (20, 24, 25).

The high frequency of P187S alleles present in spontaneous and radiation/chemotherapeutic drug–induced leukemia, combined with myeloid hyperplasia in NQO1−/− mice, raised interesting questions about the role of NQO1 in protection against de novo and radiation/chemotherapy-related leukemia. We used NQO1−/− mice to investigate the in vivo role of NQO1 in radiation-induced leukemia. A majority of NQO1−/− mice, on exposure to γ-radiation, developed myeloproliferative disease. This was evident from increased neutrophils, bone marrow hypercellularity, enlarged lymph nodes and spleen, disrupted follicular structure, loss of red

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pulp in spleen, and granulocyte and megakaryocyte invasion of spleen. NQO1-null mice exposed to γ-radiation also showed lymphoma tissues and lung adenocarcinoma. In contrast, only a few WT mice showed lymphoma and none showed lung adenocarcinoma. Further investigation revealed that exposure of NQO1−/− mice to γ-radiation resulted in reduced apoptosis in granulocytes and lack of induction of p53, p21, and Bax. In addition, NQO1−/− mice showed increased expression of myeloid differentiation factors Pu.1 and CCAAT/enhancer binding protein α (C/EBPα) as compared with WT mice. Interestingly, exposure of NQO1−/− mice to γ-radiation failed to induce C/EBPα and Pu.1, which was observed in WT mice. These results suggest that increased C/EBPα and Pu.1 led to myeloid hyperplasia, and lack of induction of apoptosis and differentiation factors contributed to radiation-induced myeloproliferative disease in NQO1−/− mice.

**Materials and Methods**

**Flow analysis of bone marrow and blood from WT and NQO1−/− mice.** Six- to nine-week-old WT and age-matched NQO1−/− mice were anesthetized using ketamine (80 mg/kg)/xylazine (16 mg/kg) mix. Blood (0.5 mL) was collected by cardiac stick in EDTA-coated tubes to avoid clotting. Mice were sacrificed by decapitation and femurs were surgically removed and cut at the ends. Bone marrow was flushed gently with cold sterile PBS. After two PBS washes, cells were resuspended in Annexin binding buffer to a concentration of 1 × 10^6/mL. Cells were then incubated with Annexin V-FITC (apoptotic marker) and myeloid lineage–specific differentiation marker Gr-1 antibody. Cells were fixed in 4% paraformaldehyde. Myeloid cells and apoptosis were measured with Coulter EPICS XL-MCL Flow Cytometer (Beckman-Coulter). In a related experiment, 100 μL of the blood were added to 1 μL of Annexin V-FITC and 2.5 μL of Gr-1 antibody (0.2 mg/mL), gently vortexed, and incubated on ice in the dark for 30 min. RBC were hemolyzed and fixed using Coulter Q-prep and analyzed using Coulter EPICS XL-MCL Flow Cytometer.

**Flow analysis of bone marrow and blood from WT and NQO1−/− mice after γ-radiation.** Six- to nine-week-old WT and NQO1−/− mice were irradiated with 3 Gy of γ-radiation (Gammacell 1000, cesium-137, Nordion International). Forty-eight hours later, mice were anesthetized and blood was collected by cardiac stick in EDTA-coated tubes to avoid clotting. Mice, after collection of blood, were sacrificed by decapitation. Femur bones were removed and bone marrow cells were flushed out with cold PBS. After two PBS washes, the cells were resuspended in Annexin binding buffer to a concentration of 1 × 10^6/mL.

One hundred microliters of blood were added to a 5-mL glass tube containing 1-mL Annexin V-FITC and 2.5-mL Gr-1 antibody (0.2 mg/mL), gently vortexed, and incubated on ice in the dark for 30 min. RBC were hemolyzed and WBC were fixed using Coulter Q-prep, and then analyzed using Coulter EPICS XL-MCL Flow Cytometer. In related experiments, the bone marrow cells were incubated with Annexin V-FITC and phycoerythrin-labeled anti-Gr-1 antibody following the procedure described above for blood cells. Assays for the determination of myeloid and apoptotic cells were essentially done using Coulter EPICS XL-MCL Flow Cytometer.

**Propidium iodide staining of bone marrow cells after γ-radiation.** Six- to nine-week-old WT and NQO1−/− mice were anesthetized with ketamine (80 mg/kg)/xylazine (16 mg/kg) mix. Mice were then irradiated with a sublethal dose of 3-Gy γ-radiation. Forty-eight hours later, mice were sacrificed. Bone marrow cells were flushed out with cold PBS and resuspended in 2 mL of cold saline. Cells were fixed by adding 5 mL of 90% cold ethanol dropwise and left for 30 min at room temperature. Each sample was stained with 1 mL of propidium iodide 50 μg/mL (Sigma Chemical Co.). One hundred milliliters of 1 mg/mL RNase were added to each sample and incubated for 30 min at 37°C. Samples were analyzed using Coulter EPICS XL-MCL Flow Cytometer (Beckman-Coulter).

**Propidium iodide staining of bone marrow cells after ex vivo γ-radiation.** Six- to nine-week-old WT and NQO1−/− mice were sacrificed and their femurs obtained. The bone were cut and marrow was flushed out gently with RPMI 10% fetal bovine serum with antibiotics. Cells were either unirradiated or irradiated with 3 Gy of γ-radiation and cultured in 24-well plates. Forty-eight hours later, cells were collected, washed with cold PBS, fixed in alcohol, and stained with propidium iodide as described above.

**Propidium iodide staining of isolated myeloid cells after γ-radiation.** WT and NQO1−/− mice were sacrificed. Bone marrow cells were flushed out with RPMI medium with 10% fetal bovine serum and antibiotics. Bone marrow cells were stained with Gr-1 antibody. Cells were sorted using Coulter Epics ALTRA Flow cytometer (Beckman-Coulter). Isolated myeloid cells were irradiated with 3 Gy of γ-radiation and then cultured in six-well plates for 48 h. Cells were washed in cold PBS, fixed in alcohol, and stained with propidium iodide. Both irradiated and nonirradiated samples were then analyzed using Coulter EPICS XL-MCL Flow Cytometer.

**Western blot analysis of bone marrow.** Six- to nine-week-old WT and NQO1−/− mice were irradiated with 3 Gy of γ-radiation. Twelve hours later, mice were sacrificed and femurs were obtained. The bones were cut on both ends. Marrow was washed with cold buffer containing 50 mMol/L Tris-Cl (pH 7.5), 150 mMol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 0.5% Triton X-100, and protease inhibitor cocktail (Roche). One hundred micrograms of each bone marrow lysate were loaded and separated on 12% polyacrylamide gels, blotted on enhanced chemiluminescence membrane, and probed with antibodies against p21, Bax (BD PharMingen), p53 (CM5 antibodies, Novacastra), NQO1 (generated in our lab), C/EBPα, Pu.1 (Santa Cruz), and actin (Sigma Chemical).

**Flow cytometry, histology, and cytogenetic analysis of mice 1 y after exposure to γ-radiation.** Seven-week-old WT and NQO1−/− mice were anesthetized using ketamine (80 mg/kg)/xylazine (16 mg/kg) mix and exposed to 0, 1, or 3 Gy of γ-radiation. Each group contained 20 mice. Mice were then fed autoclaved food and water to avoid infectious complications. One year after exposure, mice were analyzed for signs of myeloproliferation. Mice were euthanized and blood samples were collected by cardiac stick for complete blood count analysis, Wright-stained blood smear preparation, and flow cytometry analysis using phycoerythrin-labeled anti-Gr-1 antibody. Both femurs were obtained from each mouse. One femur was decalcified for histologic analysis and one was cut on both ends; bone marrow cells were flushed in ice-cold PBS for flow cytometry analysis (as mentioned above). Spleens were split into halves, one half for histology and the other half for flow cytometry analysis.

The bones were placed in 10% neutral buffered formalin (Fischer Scientific) for 24 h. After this time period, the bones were decalcified in TDB-2 Decalcifier (23% formic acid + 9% sodium citrate; Shandon Lipshaw) for 24 h. The tissues were then embedded in paraffin and cut into 4-μm sections. The sections were placed onto slides and stained with H&E (Richard-Allan Scientific). In addition to bone marrow, the lung, liver, kidneys, thymus, and lymph nodes were also obtained for histologic analysis. All tissue samples were fixed in 10% neutral-buffered formalin solution, and tissue samples were embedded in paraffin. Sections were cut and mounted on glass slides, stained with H&E, and analyzed by light microscopy.

A few of the WT and NQO1−/− male and female mice were injected i.p. with 0.2 mL of colcemid (2.0 mg/mL stock solution) 1 h before killing. Bone marrow was aspirated in a hypotonic solution (0.075 mol/L KCl) with the help of a syringe fitted with 25-gauge needle. Cell clumps were broken into single-cell suspension by mild vortexing. Cells were suspended in KCl solution for 15 to 20 min at room temperature, fixed in acetic acid/methanol (1:3 by volume), and finally dropped onto glass slides for air-dried preparations. G-banding was done following standard laboratory procedures (26). Banded chromosomes were classified following the standard nomenclature of the Committee on Standardized Genetic Nomenclature for Mice (27). An average of 15 to 20 G-banded metaphases were photographed, and complete karyotypes prepared from each animal using a Genetiscan (Perceptive System, Inc.). Additional 10 to 15 conventionally Giemsa-stained metaphase spreads from each animal were evaluated for any chromosomal or chromosome-type aberrations and for the determination of model chromosome number.
Results

Myeloid hyperplasia in NQO1−/− mice. Flow cytometry analysis of bone marrow cells showed a significant increase in myeloid cell marker Gr-1-positive cells in NQO1−/− mice compared with WT mice (Fig. 1A, left). Analysis of 15 mice in each group revealed that Gr-1-positive myeloid cells increased from 18% in WT to 30% in NQO1−/− mice (Fig. 1A, middle). The analysis also showed increase in Gr-1-positive cells in peripheral blood (Fig. 1B, left and middle). We combined myeloid cell marker Gr-1 with apoptotic marker Annexin V to determine if the loss of NQO1 had an effect on apoptosis of myeloid cells. Indeed, less apoptosis of myeloid cells was observed in NQO1−/− mouse bone marrow, as compared with WT mice (Fig. 1A, right). Further analysis also showed lower apoptosis in peripheral blood granulocytes (Fig. 1B, right). These results indicate that the loss of NQO1 in mice caused myeloid hyperplasia of the bone marrow and significant increase in blood granulocytes. The decrease in apoptosis in myeloid cells and granulocytes might have contributed to the myeloid hyperplasia in NQO1−/− mice.

Myeloid cell resistance to γ-radiation–induced apoptosis in NQO1−/− mice. We exposed WT and NQO1−/− mice to a sublethal dose of 3-Gy γ-irradiation to determine the short-term effect of radiation on myeloid cell survival and apoptosis. Forty-eight hours later, we analyzed the peripheral blood and bone marrow for γ-radiation–induced apoptosis. Phyceroerythrin-labeled anti–Gr-1 (myeloid cell marker) and FITC-labeled Annexin V (apoptosis marker) analysis revealed that exposure to γ-radiation increased apoptosis in peripheral blood granulocytes and bone marrow myeloid cells in both WT and NQO1−/− mice (Fig. 2A and B).

However, the magnitude of increase in apoptosis of blood granulocytes and bone marrow myeloid cells was significantly lower in NQO1−/− mice as compared with WT mice. γ-Irradiation more than doubled the percentage of apoptosis in myeloid cells from 20% to 43% in WT bone marrow, but only increased apoptosis in NQO1−/− myeloid cells from 14% to 18% (Fig. 2B). In a similar experiment, the propidium iodide staining also showed significantly lower apoptosis in NQO1−/− bone marrow cells as compared with WT mice (Fig. 2C, left). Apoptotic cells with condensed chromatin appear in the sub-G region after propidium iodide staining. In an active tissue like the bone marrow, apoptotic cells with condensed chromatin will be rapidly phagocytosed. Therefore, we replaced the in vivo irradiation experiment with an ex vivo irradiation. Bone marrow cells were collected and irradiated with 3-Gy γ-irradiation, cultured for 48 hours, and then stained with propidium iodide. Ex vivo experiment showed similar results as observed in the in vivo experiment (Fig. 2C, right). Lower apoptosis was observed in NQO1−/− mouse total bone marrow cells as compared with WT mouse bone marrow. To further confirm the relative resistance of NQO1−/− myeloid cells to γ-radiation–induced apoptosis, we isolated myeloid cells from WT and NQO1−/− mice using fluorescence-activated cell sorting after labeling with phyceroerythrin-labeled anti–Gr-1 antibodies. Isolated myeloid cells were irradiated with 3-Gy γ-irradiation, cultured for 48 hours, and then analyzed by propidium iodide staining. Again, NQO1−/− myeloid cells showed high resistance to γ-radiation–induced apoptosis as compared with WT myeloid cells (Fig. 2D). These results revealed that bone marrow myeloid cells deficient in NQO1 are relatively resistant to γ-radiation–induced apoptosis.
Myeloproliferative diseases in NQO1−/− mice after exposure to γ-radiation. Resistance to γ-radiation–induced apoptosis may help NQO1−/− myeloid cells to escape death after DNA damage by γ-irradiation. Thus, they may acquire mutations that may result in transformation. This could be the reason behind the observed increased incidence of radiation- or chemotherapy-related myeloid leukemia in patients with the NQO1 polymorphism. To test whether NQO1−/− mice will recapitulate the radiation-related leukemia in patients homozygous for the NQO1 polymorphism, WT and NQO1−/− mice were exposed to 0, 1, and 3 Gy of γ-radiation. Mice were housed in a clean facility and fed autoclaved food and water to avoid infection complications in case of radiation-induced immune deficiency. Mice were observed twice a week for general health and signs of disease. One year after exposure, mice were analyzed for signs of leukemia and myeloproliferation. Blood analysis and total necropsy were done for all mice. Blood samples were analyzed by complete blood count and flow cytometry for granulocytes. Wright-stained blood smears were prepared for morphologic examination of WBC. Bone marrow and spleen were analyzed by flow cytometry analysis for myeloid cells. Spleen, femur, stratum, lymph nodes, thymus, liver, lung, kidney, urinary bladder, and skin samples were collected for each mouse for histologic analysis. Histologic analysis did not reveal any sign of infection in control or γ-radiation–exposed mice. Bone marrow from a few mice in each group was also analyzed for chromosomal aberrations. Mice were diagnosed according to standardized guidelines (28). The results of blood and gross anatomy analysis are summarized in Table 1 and representative results are shown in Fig. 3A and B. The histologies of spleen and bone marrow are shown in Fig. 3C and D, respectively. Results on chromosomal aberrations are summarized in Table 2.

Histologic analysis of WT and NQO1−/− mice showed the following: Wright-stained blood smears showed a larger number of mature polymorphonuclear neutrophils, which indicate a myeloproliferative disease and not acute myeloid leukemia (Fig. 3A). The spleen and lymph nodes of irradiated NQO1−/− mice were significantly enlarged (Fig. 3B). Histologic section in the spleen on NQO1−/− mice showed loss of follicular structure (white/red pulp) due to invasion of granulocytes and megakaryocytes (Fig. 3C). Histologic section of decalcified femur from NQO1−/− mice.
showed hypercellular bone marrow (Fig. 3D). Analysis of all the mice revealed that NQO1<sup>−/−</sup> mice are significantly more susceptible to developing radiation-induced myeloproliferative disease (Table 1; \( P > 0.001 \)). About 74% of NQO1<sup>−/−</sup> mice exposed to 3-Gy \( \gamma \)-radiation developed myeloproliferative disease (Table 1). Seventy-nine percent of unirradiated and 100% of irradiated NQO1<sup>−/−</sup> mice exposed to 3-Gy \( \gamma \)-radiation developed myeloid hyperplasia. It is also noteworthy to mention that few of the radiation-exposed WT and a greater number of NQO1<sup>−/−</sup> mice also developed lymphomas (Table 1). In addition, NQO1<sup>−/−</sup> mice, but not WT mice, also developed blood unrelated tumors 1 year after \( \gamma \)-irradiation. These included lung adenomas and squamous cell

### Table 1. Frequency of myeloproliferation, lymphoma, and other malignancies

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<th>Myeloproliferation</th>
<th>Lymphoma</th>
<th>Other malignancies*</th>
<th>Total</th>
<th>Myeloid hyperplasia</th>
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<td>WT control</td>
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<td>0/20 (0)</td>
<td>0/20 (0)</td>
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<tr>
<td>NQO1&lt;sup&gt;−/−&lt;/sup&gt; control</td>
<td>0/19 (0)</td>
<td>0/19 (0)</td>
<td>3/19 (16)</td>
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<td>0/20 (0)</td>
<td>0/20 (0)</td>
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<tr>
<td>NQO1&lt;sup&gt;−/−&lt;/sup&gt; 1 Gy</td>
<td>4/18 (22)</td>
<td>3/18 (17)</td>
<td>9/18 (50)</td>
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<td>2/19 (11)</td>
<td>4/19 (21)</td>
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<tr>
<td>NQO1&lt;sup&gt;−/−&lt;/sup&gt; 3 Gy</td>
<td>14/19 (74)</td>
<td>6/19 (32)</td>
<td>16/19 (84)</td>
<td>18/19 (95)</td>
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*Most other malignancies were lung adenocarcinoma.

**Figure 3.** Blood, lymph node, spleen, and bone marrow analysis of WT and NQO1<sup>−/−</sup> mice 1 y after \( \gamma \)-irradiation. A, blood analysis. Blood were collected from WT and NQO1<sup>−/−</sup> mice 1 y after irradiation with 3-Gy \( \gamma \)-radiation. Blood smears were prepared and stained with Wright-Giemsa nuclear stain. Enlarged section of the blood smear of irradiated NQO1<sup>−/−</sup> mouse shows a large number of mature granulocytes (polymorphonuclear). B, gross anatomy. Pictures of the spleen of WT and NQO1<sup>−/−</sup> mice with and without 3-Gy \( \gamma \)-radiation showing enlarged NQO1<sup>−/−</sup> spleen after irradiation. Lymph nodes from NQO1<sup>−/−</sup> control (unirradiated) and irradiated mice are also shown. WT mice more or less showed no enlargement of lymph nodes in irradiated mice and are not shown. C, histology of spleen. Mice were irradiated with 3-Gy \( \gamma \)-radiation. One year later, spleen samples were collected, fixed in 10% buffered formalin, and embedded in paraffin. Sections were cut and stained with H&E. Spleen of NQO1<sup>−/−</sup> mice, 1 y after irradiation, shows loss of follicular structure due to invasion of granulocytes and megakaryocytes. D, histology of bone. Mice were irradiated with 3-Gy \( \gamma \)-radiation. One year later, femurs were obtained, fixed in 10% buffered formalin, decalcified, and embedded in paraffin. Sections were cut and stained with H&E. The gaps indicate adipose tissue that is lost during slide processing. The larger gaps indicate less cellular bone marrow. NQO1<sup>−/−</sup> bone marrow is hypercellular as compared with WT mice 1 y after \( \gamma \)-irradiation.
carcinomas in the head and groin area. The incidence of blood unrelated tumors was 0% for WT and 84% for NQO1−/− mice exposed to 3-Gy radiation. These data indicate that NQO1−/− mice develop myeloid hyperplasia and are highly susceptible to developing myeloproliferative disease after exposure to DNA-damaging agents like γ-radiation. Analysis of chromosomal aberrations revealed significantly higher frequency of translocations in NQO1−/− mice, as compared with WT mice (Table 2). Chromosomal aberrations were absent in control (unirradiated) WT and NQO1−/− mice (data not shown). Chromosome 5 translocations were frequent in male NQO1−/− mice exposed to γ-radiation (Table 2). Interestingly, chromosome 2 translocation in radiation-exposed female NQO1−/− mice seemed to be clonally selected because 6 of 15 cells showed translocation from chromosome 2 to chromosome X (Table 2).

Lower or lack of induction of apoptotic and myeloid cell differentiation factors in NQO1−/− mice. The above results raised intriguing questions about the mechanism of the role of NQO1 in protection against radiation-induced myeloproliferative diseases. NQO1 has been shown to protect tumor suppressor p53 against 20S proteasome degradation (29). In addition, lack of induction of p53 and reduced apoptosis were shown to contribute to benzo(a)pyrene-induced skin carcinogenesis (25). Therefore, we performed Western blot analysis to test the hypothesis that NQO1 regulates the stability of myeloid cell apoptosis and differentiation factors. Western blots of bone marrow lysates from WT and NQO1−/− mice unirradiated and irradiated with 3-Gy γ-radiation were probed with antibodies against p53 and p53 downstream genes p21 and Bax and myeloid differentiation factors Pu.1 and C/EBPα. The results are shown in Fig. 4. Bone marrow from unirradiated NQO1−/− mice showed lower levels of p53, p21, and Bax and higher levels of C/EBPα and Pu.1 as compared with WT mice (Fig. 4). Western blot analysis of the bone marrow of WT and NQO1−/− mice 12 hours after exposure to 3-Gy γ-radiation showed significant induction of p53, p21, Bax, C/EBPα, and Pu.1. Interestingly, NQO1−/− mice showed lower to lack of induction of p53, p21, Bax, C/EBPα, and Pu.1 on exposure to 3-Gy γ-radiation. Interestingly, NQO1 was induced by γ-radiation in bone marrow cells from WT mice, probably as a result of oxidative stress from the reactive oxygen species generated after exposure to ionizing irradiation (Fig. 4).

Discussion

Disruption of NQO1 gene in mice leads to myeloid cell hyperplasia as evident from the increase in myeloid cells in bone marrow and granulocytes in peripheral blood (present and previously published reports; ref. 21). Exposure of NQO1−/−

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<th>Table 2. Chromosomal aberrations</th>
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NOTE: Fifteen metaphases were analyzed in each case. Chromosomal aberrations were not observed in control (unirradiated) WT and NQO1−/− mice.
mice to γ-radiation showed myeloproliferative disease that included significant increase in blood granulocytes and bone marrow myeloid cells, enlarged lymph nodes and spleen, loss of spleen follicular structure due to invasion of granulocytes and megakaryocytes, and bone marrow hypercellularity. These results provide direct evidence that NQO1-deficient mice are highly susceptible to developing myeloproliferative disease. This conclusion is also supported by previous observations of high frequency of P187S polymorphism in NQO1 protein in AML patients after chemotherapy and radiation treatment (14, 16). NQO1 P187S protein is known to rapidly degrade, and individuals carrying homozygous polymorphism have zero and those who are heterozygous have only half of the NQO1 protein and activity (7).

Interestingly, NQO1−/− mice did not develop AML although the P187S mutant polymorphism in human NQO1 has been linked to therapy-related AML, especially after chemotherapy and radiation therapy (14). Instead, NQO1−/− mice were more susceptible to developing myeloproliferative disease after exposure to γ-radiation. However, the high incidence of myeloproliferative disease in NQO1−/− mice indicates that the NQO1−/− mouse model partially recapitulates the high incidence of therapy-related AML in patients homozygous for the null polymorphism in human NQO1 gene. It is also possible that additional factors contribute to the development of AML in the absence of NQO1, but this remains to be investigated.

Tumor suppressor p53 is associated with apoptosis (30). Increase or decrease in p53 is directly related to similar alterations in apoptosis. C/EBPα and Pu.1 are myeloid differentiation factors. C/EBPα is the main lineage determination regulator that favors myeloid versus lymphoid lineage commitment. C/EBPα knockout mice did not have any neutrophils in the blood, which indicates the essential role of C/EBPα in myeloid differentiation (31). Pu.1 knockout mice also lack blood neutrophils (32). C/EBPα and Pu.1 regulate and induce myeloid cell differentiation into mature granulocytes (33). Differentiation stops proliferation and, thus, prevents myeloproliferative disease and myeloid leukemia. Lower levels and mutations in C/EBPα and Pu.1 have been associated with AML development and prognosis (34, 35).

The present studies revealed that decreased apoptosis in myeloid cells and increased myeloid cell differentiation led to myeloid hyperplasia in unirradiated NQO1−/− mice. Lower p53 and Bax presumably led to decreased apoptosis in NQO1−/− mice. The increase in myeloid differentiation factors C/EBPα and Pu.1 most likely contributed to myeloid hyperplasia in NQO1−/− mice. In other words, increase in differentiation factors resulted in greater number of myeloid cell differentiation from its progenitors that led to myeloid hyperplasia in NQO1-null mice. Exposure to γ-radiation induced accumulation of high levels of p53/Bax and myeloid differentiation factors C/EBPα and Pu.1 in the bone marrow of WT mice. Induction of these factors resulted in the induction of apoptosis and differentiation of myeloid cells in WT bone marrow, leading to the prevention of mutagenesis and transformation. In NQO1−/− mice, γ-irradiation did not significantly induce p53/Bax and apoptosis. The lack of induction of p53/Bax and apoptosis after γ-irradiation presumably allowed cells with DNA damage to continue to proliferate. The lack of induction of C/EBPα and Pu.1 after irradiation also limited the differentiation of myeloid cells in γ-radiation–exposed NQO1−/− mice. Therefore, reduced apoptosis combined with lack of differentiation resulted in increased incidence of myeloproliferative disease in NQO1−/− mice.

One of the intriguing questions is how NQO1 regulates apoptosis/differentiation and associated factors. Recently, we and others have shown a role of NQO1 in the protection against 20S proteasomal degradation of cellular factors including p53 (29, 36). The loss of NQO1 in NQO1−/− mice leads to degradation of p53, which contributes to benzo(a)pyrene-induced skin cancer (22). Therefore, it is possible that NQO1 also protects apoptotic factor p53 and differentiation factors C/EBPα and Pu.1 against 20S proteasomal degradation in γ-radiation–exposed bone marrow from WT mice. This leads to protection by eliminating the radiation-damaged cells and promoting differentiation into myeloid cells. However, lack of NQO1 in NQO1−/− mice leads to rapid degradation of p53/C/EBPα and Pu.1. This results in significantly lower levels of these factors in radiation-exposed NQO1−/− mice, leading to myeloproliferative disease. This, however, does not explain the increase in C/EBPα and Pu.1 in unirradiated NQO1−/− mouse bone marrow. It is possible that the mechanisms that regulate expression of C/EBPα and Pu.1 are altered in NQO1−/− mice, but this remains to be determined.

Mouse chromosome 2 aberrations have been linked to the development of radiation-induced AML (37–39). Interestingly, mouse chromosome 2 contains clustered tumor suppressor gene loci and Pu.1 gene (40). In addition, loss of a whole chromosome 5 or a deletion of the long arm, del(5q), is a recurring abnormality in myelodysplastic syndromes and AML (41, 42). Intriguingly, exposure of NQO1−/− mice to 3-Gy γ-radiation induced chromosome 5 and chromosome 2 translocations. The chromosome 2 translocation seemed to be clonally selected. It is possible that chromosome 2 and chromosome 5 translocations contributed to radiation-induced myeloproliferative diseases in NQO1−/−. It is also possible that lack of induction of p53 in NQO1−/− mice in response to γ-radiation led to chromosome 2 and chromosome 5 translocations, which resulted in myeloproliferative disease, but this remains to be determined.

In summary, the results suggested that the loss of NQO1 leads to decrease in p53 protein and apoptosis and increase in differentiation factors C/EBPα and Pu.1, which lead to myeloid hyperplasia in NQO1−/− mice. In addition, lower or lack of induction of apoptotic and differentiation factors led to radiation-induced myeloproliferative disease in NQO1−/− mice. These results led to the conclusion that NQO1 acts as an endogenous factor in the protection against myeloid hyperplasia and radiation-induced myeloproliferation. This conclusion is highly significant for the 4% human individuals who are homozygous for a polymorphism P187S allele of NQO1 and lack NQO1 protein and activity. The conclusion is also significant for the 20% to 25% of individuals carrying one polymorphic NQO1 P187S allele and half of the WT NQO1 protein and activity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Disruption of NAD(P)H:Quinone Oxidoreductase 1 Gene in Mice Leads to Radiation-Induced Myeloproliferative Disease

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