NRH:Quinone Oxidoreductase 2-Deficient Mice Are Highly Susceptible to Radiation-Induced B-Cell Lymphomas

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Abstract

Purpose: NRH:quinone oxidoreductase 2 (NQO2) is known to protect against myelogenous hyperplasia. However, the role of NQO2 in prevention of hematologic malignancies remains unknown. Present studies investigated in vivo role of NQO2 in prevention of myeloproliferative disease and lymphomas.

Experimental Design: Wild-type and NQO2-null mice were exposed to 0, 1, and 3 Gy γ-radiation. One year later, the mice were analyzed for the development of myeloproliferative disease and lymphomas. Immunohistochemistry analysis determined the B- and T-cell origin of lymphomas. The mice were also sacrificed at 6 and 48 h after radiation exposure and bone marrow was collected and analyzed for p53, Bax, and B-cell apoptosis. Bone marrow cells were cultured and the rate of degradation of p53 was analyzed.

Results: Seventy-two percent NQO2-null mice showed development of B-cell lymphomas in multiple tissues compared with 11% in wild-type mice exposed to 3 Gy γ-radiation. In contrast, only 22% NQO2-null mice showed myeloproliferation compared with none in wild-type mice. Further analysis revealed that bone marrow from NQO2-null mice contained lower levels of p53 compared with wild-type mice due to rapid degradation of p53. In addition, the exposure to radiation resulted in lower induction of p53 and Bax and decreased B-cell apoptosis in NQO2-null mice.

Conclusion: NQO2-null mice are highly susceptible to develop radiation-induced B-cell lymphomas. The lack of significant induction of p53 and Bax and decrease in B-cell apoptosis presumably contributed to the development of lymphomas. NQO2 functions as endogenous factor in prevention against radiation-induced B-cell lymphomas.

Quinone oxidoreductases [NRH:quinone oxidoreductase 2 (NQO2) and NAD(P)H:quinone oxidoreductase 1 (NQO1)] are cytosolic proteins that catalyze metabolic detoxification and/or activation of quinones and its derivatives (1, 2). Although overlapping substrate specificities have been observed for NQO2 and NQO1, such as for CB1954 activation, significant differences exist in relative affinities for the various substrates (3, 4). The cofactor requirement for protein activity is very selective, pointing out dihydronicotinamide riboside (NRH) for NQO2 and NAD(P)H for NQO1 as an electron donor (3, 5). Although NQO2 is resistant to typical activity inhibitors of NQO1, such as dicoumarol, cibacron blue, and phenindone, NQO2 is inhibited by flavones such as quercetin (3). Benzo(a)pyrene is another known inhibitor of NQO2 (5). Analysis of the crystal structure of NQO2 revealed that NQO2 contains a specific metal binding site, which is not present in NQO1 (6). Cellular studies have shown a role of NQO2 in metabolic activation of CB1954 leading to cytotoxicity and cell death (4).

The physiologic functions of NQO2 and NQO1 proteins are emerging from recent studies. A major role of NQO2 and NQO1 has been shown in protection against 20S proteasomal degradation of tumor suppressor p53 during radiation and chemical stress (7, 8). 20S proteasomes degrade p53 under normal conditions to maintain a base level of p53 inside cells. NQO2 and NQO1 both are stress-responsive proteins and are induced during chemical or radiation stress (9). Induction of NQO2 and NQO1 provides protection to p53 by reducing or eliminating 20S degradation of p53 (9). This leads to cellular protection and promotes cell survival. NQO2 and NQO1 expression go down with fading stress. This brings the level of p53 back to normal. It is expected that NQO2 and NQO1 protection against 20S proteasomal degradation is not restricted to tumor suppressor p53 and is extended to other factors that regulate growth, survival, proliferation, and differentiation of cells (7). A recent report of NQO1 protection of skin tumor suppressor p34 [ING1b] against 20S degradation is yet another example (10). In other words, NQO2 and NQO1 protection of factors against 20S proteasomal degradation is a general mechanism that stabilizes and provides increased amount of...
NQO1-null mice on exposure to gamma-radiation developed B-cell lymphomas. The lack of significant induction of p53 and Bax and decrease in B-cell apoptosis contributed to the development of lymphomas in NQO2-/- mice. These results suggest that NQO2 functions as endogenous factor in prevention against radiation-induced B-cell lymphomas. A 29-bp insertion polymorphism in human NQO2 gene promoter is known. Human individuals carrying the NQO2 gene promoter polymorphism express significantly lower levels of NQO2 and might be susceptible to the development of radiation-induced B-cell lymphomas. Future studies are required to determine this. The studies also suggest that NQO2 could be explored as a potential target to develop natural or synthetic drugs that activate NQO2 gene expression and protect against lymphoma development especially in individuals carrying the NQO2 gene promoter polymorphism.

Factors for cell growth and survival by inhibiting 20S degradation of factors.

NQO2-null and NQO1-null mice were generated (11, 12). The mice deficient in NQO2 and NQO1 gene expression were born and developed normal, indicating that NQO2 and NQO1 do not play a role in mouse development. However, the loss of NQO2 in NQO2-null and NQO1 in NQO1-null mice led to myelogenous hyperplasia of bone marrow and B-cell deficiency/decreased immunity (11, 13, 14). NQO2-null and NQO1-null mice also showed significantly increased sensitivity to skin carcinogenesis in response to benzo(a)pyrene and dimethylbenzanthracene (15–17). Both NQO2-null and NQO1-null mice showed lower levels of tumor suppressor p53 and decreased apoptosis in bone marrow that contributed to myelogenous hyperplasia of bone marrow (13, 15, 18). Lack of induction of p53 and decreased apoptosis also contributed to chemical-induced skin carcinogenesis (15, 18). Interestingly, NQO2-null and NQO1-null mice showed opposite responses to menadione-induced hepatic damage (11, 12). NQO2-null mice showed resistance to menadione-induced hepatic damage (11). In contrast, NQO1-null mice showed increased sensitivity to menadione-induced hepatic damage. In other words, NQO2 metabolically activated and NQO1 detoxified menadione.

Recently, we used NQO1-null mice to investigate in vivo role of NQO1 in radiation-induced leukemia (19). A majority of NQO1-null mice on exposure to gamma-radiation developed myeloproliferative disease. This was evident from increased neutrophils, bone marrow hypercellularity, enlarged lymph nodes and spleen, disrupted follicular structure, loss of red pulp in spleen, and granulocyte and megakaryocyte invasion of spleen. Most of the NQO1-null mice exposed to gamma-radiation showed myeloproliferative disease similar to myeloid leukemia. A few mice also showed tissue lymphoma and lung adenocarcinoma. In contrast, only a few wild-type mice showed lymphoma and none showed lung adenocarcinoma. NQO2-null mice like NQO1-null mice show myeloid hyperplasia (11). However, the role of NQO2 in prevention of myeloproliferative diseases remains unknown.

Human NQO2 gene is precisely localized to chromosome 6p25 and its gene locus is highly polymorphic (20). A recent report has identified mutation in the first intron of NQO2 gene associated with decreased expression of NQO2 gene and clozapine-induced agranulocytosis in clozapine-treated schizophrenic patients (21). A 29-bp promoter polymorphism associated with differential expression of NQO2 gene is reported (22, 23). Human NQO2 gene promoter with the 29-bp insertion expressed significantly lower amount of NQO2 protein (22, 23). Insertion of 29-bp in human NQO2 gene promoter generated SP3 binding site that repressed NQO2 gene expression (23). However, an association between NQO2 polymorphism and leukemia remains unknown.

In this report, we investigated the in vivo role of NQO2 in myeloproliferation and hematologic malignancies. Wild-type along with NQO2-null mice were exposed to gamma-radiation and analyzed for myeloproliferative diseases. Interestingly, a majority of mice showed B-cell lymphoma in multiple tissues. Further analysis revealed that the loss of NQO2 led to destabilization of tumor suppressor p53 and development of B-cell lymphomas. Cytogenetic analysis showed increased chromosomal aberrations in radiation-treated NQO2-null mice compared with radiation-treated wild-type mice.

Materials and Methods

NQO2-null mice and gamma-radiation exposure. NQO2-null mice were generated in our laboratory. The mice deficient in NQO2 were born and developed normal (11). The mice were housed in polycarbonate cages in the animal facility maintained with a 12-h light/dark cycle, a temperature of 24 ± 2°C, a relative humidity of 55 ± 10%, and a negative atmospheric pressure. The mice were fed with standard rodent chow and acidified water ad libitum. The studies were approved by the Institutional Animal Care and Use Committee. Animals received humane care throughout the experiment according to the American Association of Laboratory Animal Care guidelines for animal welfare.

Wild-type and NQO2-null mice (7-9 weeks old) were irradiated with 0, 1, and 3 Gy gamma-radiation (Gammacell 1000: cesium 137; Nordion International). Each group contained 20 mice. Each group also contained half male and half female mice. Mice were fed autoclaved food and water to avoid infectious complications.

Flow cytometry, histology, and immunohistochemistry analysis. Wild-type and NQO2-null mice, 1 year after gamma-radiation exposure, were analyzed for signs of myeloproliferation and hematologic malignancies. 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. Mice after collection of blood were sacrificed by decapitation. Both femurs were collected from each mouse. One femur was decalcified for histology analysis. The other femur was flushed in ice-cold PBS for flow cytometry analysis. After two PBS washes, the cells were resuspended in the Annexin V binding buffer to a concentration of 1 × 10⁶ cells/mL. Blood or bone marrow (100 μL) was added to a 75 μL glass tube containing 1 μL Annexin V-FITC and 2.5 μL B-cell-specific CD19 or granulocyte specific 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 (Beckman-Coulter).

In addition to blood and bone marrow, several tissues including spleen, thymus, lymph nodes, salivary glands, liver, lung, and kidney
were also collected from radiation unexposed and exposed mice. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with H&E. The tissue sections were evaluated for lesions by Prof. Roberto Barrios, an expert pathologist at M. D. Anderson Cancer Center, with knowledge of human and mouse pathology. The tissue sections were also analyzed by immunohistochemistry for the presence of B or T cells in lymphomas of the various tissues. Antibody B220 probed B cells and antibody CD3 probed for T cells. Immunohistochemical analysis was done by procedures as described previously (24). Briefly described, the formalin-fixed tissues were embedded in paraffin and cut into 4 μm sections. Serial sections of tissues were probed with anti-B220 (1:200 dilution) and anti-CD3 antibody (1:250 dilution). The immunocomplex was visualized using ABC staining kit (Vector Laboratories) in accordance with the manufacturer’s instructions. The slides were counterstained with Mayer’s hematoxylin and mounted with Paraplast solution (Sigma).  

**Western analysis of the bone marrow.** The wild-type, NQO1-null, and NQO2-null mice were irradiated with 0, 1, and 3 Gy γ-irradiation. After 6 and 48 h, mice were sacrificed. Femurs were obtained. NQO1-null mice were included in this study because it was reported previously that a majority of NQO1-null mice on exposure to γ-radiation develop myeloproliferative disease (19) and to compare NQO1-null and NQO2-null bone marrow response to radiation. The bones were cut on both ends. Marrow was flushed with cold buffer containing 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.5% Triton X-100, and protease inhibitor cocktail (Roche). Each bone marrow lysate (100 μg) was

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

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Myeloproliferation</th>
<th>Other malignancies*</th>
<th>Myeloid hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Wild-type control</td>
<td>0/20 (0)</td>
<td>0/20 (0)</td>
<td>0/20 (0)</td>
</tr>
<tr>
<td>2 NQO2-null control</td>
<td>5/20 (25)</td>
<td>0/20 (0)</td>
<td>0/20 (0)</td>
</tr>
<tr>
<td>3 Wild-type 1 Gy</td>
<td>P &lt; 0.0281</td>
<td>P &lt; 0.9999</td>
<td>P &lt; 0.9999</td>
</tr>
<tr>
<td>4 NQO2-null 1 Gy</td>
<td>0/20 (0)</td>
<td>0/20 (0)</td>
<td>0/20 (0)</td>
</tr>
<tr>
<td>5 Wild-type 3 Gy</td>
<td>8/19 (42)</td>
<td>5/19 (26)</td>
<td>6/19 (32)</td>
</tr>
<tr>
<td>6 NQO2-null 3 Gy</td>
<td>P &lt; 0.0002</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>7 Wild-type control</td>
<td>13/18 (72)</td>
<td>4/18 (22)</td>
<td>3/18 (17)</td>
</tr>
<tr>
<td>8 NQO2-null control</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0205</td>
</tr>
</tbody>
</table>

NOTE: Numbers in parentheses show the percentage of mice with lymphoma, myeloproliferation, other malignancies, and myeloid hyperplasia. The experiments were set up in four groups and each group contained 5 animals. P values for wild-type and NQO2-null control are shown in line 3. P values for wild-type 1 Gy versus NQO2-null 1 Gy are shown in line 6. P values for wild-type 3 Gy versus NQO2-null are shown in line 9. *Most other malignancies were lung adenocarcinoma.
loaded and separated on 12% polyacrylamide gels, blotted on the enhanced chemiluminescence membrane, and probed with polyclonal antibodies against p53 (1:100 dilution; CM5 antibodies), Bax (1:100 dilution; BD Pharmingen), NQO1 (1:500 dilution; generated in our laboratory), NQO2 (1:500 dilution; Santa Cruz Biotechnology), and actin (Sigma).

Degradation of p53 in wild-type and bone marrow cells. The wild-type and NQO2-null mice were sacrificed and bone marrow cells were

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**Fig. 2.** Histology of tissues. Wild-type and NQO2-null mice (7-9 week-old) were exposed to 0, 1, and 3 Gy γ-radiation. One year after, mice were sacrificed and bone marrow and other tissues were collected by procedures as described in Materials and Methods. The tissues were fixed in 10% buffered formalin, decalcified, and embedded in paraffin. Sections were cut and stained with H&E.
removed and cultured by previously described procedures (25). Bone marrow cells were treated with 20 μmol/L MG132 for 6 h. Cells were washed twice with medium and treated with 30 μg/mL cycloheximide for different time points (1 and 2 h). The cells were washed with ice-cold PBS, lysed, and analyzed by Western blotting and probing with p53 antibody.

Cytogenetic preparations and analysis. The wild-type and NQO2-null male and female mice were injected intraperitoneally with 0.2 mL colcemid (2.0 mg/mL stock solution) 1 h before sacrificing the mice. Bone marrow was aspirated in a hypotonic solution (0.075 mol/L KCl) with the help of a syringe fitted with a 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, v/v), and finally dropped onto glass slides for air-dried preparations. G-binding was done by standard procedures. Banded chromosomes were classified following the standard nomenclature of the Committee on Standardized Genetic Nomenclature for mice. An average of 15 to 20 G-banded metaphases was photographed and complete karyotypes were prepared from each animal using a Genetiscan (Perceptive System). An additional 15 conventionally Giemsa-stained metaphase spreads from each animal were evaluated for chromatid or chromosome-type aberrations and for the determination of model chromosome number.

Statistical analysis. The statistical analysis (one-way ANOVA) was done by ANOVA followed by post hoc Tukey using the software StatsDirect. Twenty mice used in Fig. 1 and Table 1 were divided into four groups each group containing 5 mice for statistical analysis. Experiments were repeated in Fig. 4 three to five times as indicated in figure legend and used for statistical analysis. Differences were considered significant at P < 0.05.

Results

Blood and histologic analysis of wild-type and NQO2-null mice unirradiated and irradiated with γ-radiation did not show any sign of infection. Blood analysis showed myeloid cell hyperplasia in NQO2-null mice as reported earlier (11). Exposure of wild-type and NQO2-null mice with γ-radiation did not lead to a significant increase in myeloid cells in a majority of NQO2-null mice. However, exposure of wild-type and NQO2-null mice to a single dose of 3 Gy γ-radiation led to the loss of >80% of lymphoid cells in both strains of mice 48 h after γ-radiation exposure (data not shown).

One year after exposure of mice to 3 Gy γ-radiation led to enlarged spleen and lymph nodes in NQO2-null mice compared with wild-type mice (Fig. 1A). The unirradiated and irradiated wild-type mice showed normal spleen and lymph nodes same as shown for unirradiated normal NQO2-null mice in Fig. 1A. Interestingly, 25% of 1-year-old NQO2-null mice deficient in NQO2 showed multiple tissue lymphomas (P > 0.0281; Fig. 1B; Table 1). Age-matched wild-type mice in the same experiment failed to show tissue lymphomas. Exposure of mice to 1 and 3 Gy γ-radiation led to the development of multiple tissue lymphomas in 42% (P < 0.0002) and 72% (P < 0.0001) NQO2-null mice, respectively (Fig. 1B; Table 1). In the same experiment, exposure of wild-type mice to 1 Gy γ-radiation did not show lymphoma of tissues. However, 11% wild-type mice exposed to 3 Gy γ-radiation showed multiple tissue lymphomas (Fig. 1B). Histologic analysis showed lymphomas in all the tissues analyzed in the present studies (Fig. 2). The histology of bone marrow, lymph node, spleen, and thymus lymphomas from NQO2-null mice exposed to 3 Gy γ-radiation is shown in Fig. 2A. The lymphomas of salivary glands, liver, kidney, and lungs are shown in Fig. 2B. Immunohistochemistry analysis of lymphoma sections with B-cell-specific
antibody showed that lymphomas were of B-cell origin (Fig. 3). The data are shown only for spleen and lymph nodes. However, lymphomas from all the tissues showed B-cell positive staining. The sections were also probed with T-cell-specific antibody CD3 (data not shown). The results showed presence of only few T cells that were the same between control and lymphoma tissues.

The analysis of wild-type and NQO2-null mice exposed to γ-radiation also showed myeloproliferation in 26% (P < 0.0001) of NQO2-null mice exposed to 1 Gy γ-radiation and 22% (P < 0.0001) of NQO2-null mice exposed to 3 Gy γ-radiation (Table 1). Thirty-two percent (P < 0.0001) NQO2-null mice exposed to 1 Gy and 17% (P < 0.0205) exposed to 3 Gy γ-radiation also showed other malignancies, most of which were lung adenocarcinomas (Table 1).

Wild-type and NQO2-null mice were exposed to 0, 1, and 3 Gy γ-radiation. Forty-eight hours after radiation exposure, the mice were sacrificed and bone marrow was collected and analyzed for CD19+ B cells and B-cell apoptosis. This experiment was repeated three times. Representative results. The bands intensities were determined by densitometry and p53/actin and Bax/actin data calculated and plotted in bar diagram with statistical analysis. A Western analysis of rate of degradation of p53. Bone marrow cells were cultured for 12 h. Cell culture was pretreated with MG132 for 6 h. This was followed by treatment with MG132 + cycloheximide for time intervals. The cells were lysed and analyzed by SDS-PAGE and Western blotting. The blot was probed with p53 antibody followed by actin antibody. This experiment was repeated five times. Representative results.

Fig. 4. Tumor suppressor p53 and Bax and B-cell apoptosis in wild-type and NQO2-null bone marrow with or without γ-radiation. A and B Western analysis of NQO1, NQO2, and factors. Wild-type, NQO1-null, and NQO2-null mice (7-9 week-old) were irradiated with 0, 1, and 3 Gy γ-radiation. After 6 and 48 h, mice were euthanized and femurs were surgically removed. Bone marrow was flushed and lysed with cold buffer containing 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.5% Triton X-100, and protease inhibitor cocktail (Roche). Each bone marrow lyase (100 µg) was loaded and separated on 12% polyacrylamide gels, blotted on the enhanced chemiluminescence membrane, and probed with antibodies against NQO1, NQO2, p53, Bax, and actin. These experiments were repeated three times. Representative result. C, B-cell apoptosis. Wild-type and NQO2-null mice (7-9 week-old) were unirradiated or irradiated with 3 Gy γ-radiation. Forty-eight hours after irradiation, mice were sacrificed and bone marrow was collected and analyzed for CD19+ B cells and B-cell apoptosis. This experiment was repeated three times. Representative results. The bands intensities were determined by densitometry and p53/actin and Bax/actin data calculated and plotted in bar diagram with statistical analysis.
48 h after irradiation. SDS-PAGE and Western blotting analyzed protein level of p53, Bax, and actin. The results are shown in Fig. 4B. Both NQO1-null and NQO2-null mice bone marrow showed lower levels of tumor suppressor p53 compared with wild-type mice. p53 showed first decrease at 6 h and then increase at 48 h in wild-type and NQO1-null strains of mice exposed to γ-radiation compared with control mice. NQO2-null mice in the same experiment showed a slight increase in p53 at 6 h then a substantial increase at 48 h after irradiation. However, the magnitude of increase at 48 h was significantly higher in wild-type mice (P < 0.0001) than NQO1-null and NQO2-null mice (P < 0.01). Proapoptotic protein Bax showed decreased levels in NQO1-null mice. However, Bax levels were higher in NQO2-null mice. Bax levels increased in response to radiation. However, the three strains of mice showed differential induction of Bax in response to exposure to radiation. Bax increased at 6 h after radiation exposure and remained elevated at 48 h after irradiation in wild-type mice. On contrary, Bax increased in NQO2-null mice bone marrow at 6 h but decreased at 48 h after irradiation. Bax levels increased with time in NQO1-null mice but remained significantly lower compared with wild-type mice. In similar experiments, bone marrow from wild-type and NQO2-null mice was analyzed for B cells and apoptosis in B cells (Fig. 4C). The results showed increased B cells in NQO2-null mice bone marrow. This was same as reported earlier (14). Wild-type and NQO2-null mice both showed increased apoptosis of B cells in γ-radiation irradiated mice. However, the B-cell apoptosis was lower in NQO2-null mice compared with wild-type mice (Fig. 4C, right). In a related experiment, as expected, bone marrow cells from NQO2-null mice showed lower levels of p53 compared with wild-type mice (Fig. 4D, compare lane 1). The cells were treated with proteasome inhibitor MG132 to determine if the decrease in p53 was due to degradation of p53 in NQO2-deficient cells. The exposure of bone marrow cells to MG132 resulted in stabilization of tumor suppressor p53 within the cells (Fig. 4D). In the same experiment, the cells treated with MG132 were treated with cycloheximide to follow the rate of degradation of p53 in wild-type and NQO2-null bone marrow cells. In the presence of cycloheximide, p53 protein degraded at faster rate in absence of NQO2 (Fig. 4D).

Analysis of chromosomal aberrations revealed significantly higher frequency of translocations in NQO2-null mice compared with wild-type mice (Table 2). Chromosomal aberrations were absent in control (unirradiated) wild-type and NQO2-null mice (data not shown). Chromosome translocations were frequent in male as well as female NQO2-null mice exposed to γ-radiation (Table 2). Interestingly, chromosome 2 translocation in radiation-exposed male NQO2-null mice appeared clonally selected because 4 of 15 cells showed translocation from chromosome 2 to unknown chromosome (Table 2).

Discussion

Disruption of NQO1 and NQO2 is known to lead to myeloid hyperplasia of bone marrow (11, 12). A majority of NQO1-null mice deficient in first form of quinone oxidoreductase NQO1 on exposure to γ-radiation developed myeloproliferative disease (19). This was evident from increased neutrophils, bone marrow hypercellularity, enlarged lymph nodes and spleen, disrupted follicular structure, loss of red pulp in spleen, and granulocyte and megakaryocyte invasion of spleen (19). In contrast, only a few wild-type mice showed γ-radiation-induced myeloproliferation. These studies established that NQO1 protected individuals against myeloproliferative diseases. However, the role of NQO2 in protection against myeloproliferative and related hematologic diseases remains unknown. We used wild-type mice expressing NQO2 and NQO2-null mice deficient in second form of quinone oxidoreductase NQO2 to determine the in vivo role of NQO2 in hematologic abnormalities and diseases.

We have shown that a single dose of γ-radiation resulted in enlarged lymph nodes and spleen and produced multiple tissue lymphomas in NQO2-null mice. Lymphomas were detected in bone marrow, spleen, lymph nodes, thymus, salivary glands, kidney, and lungs. Further analysis revealed that lymphomas were of B-cell origin. These results show that NQO2 is an endogenous factor in protection against B-cell lymphoma development. Some of the NQO2-null mice showed radiation-induced myeloproliferation as observed earlier in the majority of NQO1-null mice. NQO2 and NQO1 both are highly homologous proteins with overlapping substrate affinities yet show differences in bone marrow response to radiation. NQO2 predominately protects against lymphomas, whereas NQO1 predominately provided protection against myeloproliferative diseases (present report; ref. 19). Therefore, NQO2 and NQO1 combined play significant role in protection against lymphoma and leukemia.

Table 2. Chromosomal aberrations

<table>
<thead>
<tr>
<th>1</th>
<th>Wild-type male control</th>
<th>40, XY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>NQO2-null male control</td>
<td>40, XY</td>
</tr>
<tr>
<td>3</td>
<td>Wild-type male 3 Gy</td>
<td>t(2;?) (one cell); 13q+ (one cell)</td>
</tr>
<tr>
<td>4</td>
<td>NQO2-null male 3 Gy</td>
<td>t(2;?) (four cells); +nce of fragment (one cell)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>6</td>
<td>Wild-type female 3 Gy</td>
<td>9q+ (one cell); break in chromosome 11 (one cell)</td>
</tr>
<tr>
<td>7</td>
<td>NQO2-null female 3 Gy</td>
<td>t(2;?) (one cell); t(4;?) (one cell); br 6q (one cell); 13q- (one cell); 39 XX, -13 (one cell)</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>$P &lt; 0.01$</td>
</tr>
</tbody>
</table>

NOTE: Chromosomal aberrations were not observed in control (unirradiated) wild-type and NQO2+/− mice. $P$ values were calculated based on the number of cells carrying aberrations. $P$ values for wild-type male 3 Gy versus NQO2-null male 3 Gy are shown in lane 5. $P$ values for wild-type female 3 Gy versus NQO2-null female 3 Gy are shown in lane 8. Fifteen metaphases were analyzed in each case.
Previous reports have shown that p53+/− mice expressing lower levels of p53 are extremely susceptible to radiation-induced tumorigenesis, mostly lymphomas and sarcomas (26). Interestingly, p53 levels decreased in NQO2-null mice because of rapid degradation of p53 in the absence of NQO2. Irradiation of mice also failed to sufficiently induce p53 in NQO2-null mice compared with wild-type mice. These results suggested that lower levels and lack of significant induction of p53 contributed to the development of lymphomas in NQO2-null mice. NQO1-null mice also show lower p53 in bone marrow and lack of induction of p53 in response to exposure to γ-radiation. Therefore, it is evident from the present studies that both NQO2 and NQO1 regulate stability of p53 in bone marrow. It might be noteworthy that NQO2 and NQO1 protection of p53 is not limited to bone marrow. It was reported earlier that both NQO2 and NQO1 stabilize p53 in other tissues including skin tissue (7). Lack of induction of p53 in skin contributed to benzo(a)pyrene-induced skin tumors in NQO1-null mice (18). The studies have also shown that NQO2 and NQO1 stabilize p53 by direct interaction of p53 and protecting p53 against 20S proteasomal degradation in skin (7). Interestingly, exposure to γ-radiation increased NQO1, yet it did not provide increased stability of p53 in NQO2-null mice. It is possible that the increase in NQO1 was not sufficient compared with the loss of NQO2. This means stabilization of p53 occurred but was not visible. It is also possible that simultaneous increase in both NQO1 and NQO2 is required to stabilize p53 and remains to be determined. In addition to p53, the lower induction of Bax in bone marrow and decrease in B-cell apoptosis also contributed to γ-radiation-induced B-cell lymphomas in NQO2-null mice.

The results further suggested that additional mechanisms contribute to radiation-induced lymphomas development in NQO2-null mice. This is because lower p53 and lack of induction are observed in both NQO2-null and NQO1-null mice, yet NQO2-null and not NQO1-null mice predominately develop multiple tissue lymphomas in response to exposure to radiation. On contrary, NQO1-null mice predominately show myeloproliferative disease (19). Therefore, it is possible that NQO2 and NQO1 also regulate distinct factor(s) involved in protection against lymphomas and myeloproliferative disease, respectively.

Chromosomal aberrations were observed in both wild-type and NQO2-null mice exposed to radiation. However, the magnitude of aberrations was significantly higher in NQO2-deficient NQO2-null mice compared with wild-type mice. Intriguingly, exposure of NQO2-null mice to 3 Gy γ-radiation-induced chromosome 2 and 4 translocations. The chromosome 2 translocation appeared clonally selected. Interestingly, mouse chromosome 2 contains clustered tumor suppressor gene loci (27). It is possible that chromosome 2 translocations contributed to radiation-induced lymphomas in NQO2-null. It is also possible that lack of induction of p53 in NQO2-null mice in response to γ-radiation led to chromosome 2 translocations, which resulted in multiple tissues lymphomas and remains to be determined.

In summary, the results suggested that the loss of NQO2 leads to increased susceptibility to γ-radiation-induced multiple tissues B-cell lymphoma development. The lack of significant induction of p53 and Bax and B-cell apoptosis presumably contributed to radiation-induced lymphomas in NQO2-null mice. The results led to the conclusion that NQO2 acts as an endogenous factor in protection against radiation-induced multiple-tissue B-cell lymphomas. This conclusion is highly significant for human individuals who are polymorphic for NQO2 promoter expressing lower amounts of NQO2 gene (23).

References

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