Brief report

Nfe2l3 (Nrf3) deficiency predisposes mice to T-cell lymphoblastic lymphoma

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We have previously generated mice deficient for Nfe2l3 (NF-E2 p45 related factor 3 or Nrf3), a member of the cap ‘n’ collar family of basic-leucine zipper transcription factors. To examine whether Nrf3 is involved in chemical-induced carcinogenesis, we exposed the mice to benzo[a]pyrene (B[a]P), a carcinogen found in cigarette smoke. Contrary to wild-type mice, Nrf3-null animals are highly susceptible to B[a]P, exhibiting significantly increased mortality. Pathology analysis of affected tissue sections revealed a high incidence of T-cell lymphoblastic lymphoma in B[a]P-treated Nrf3−/− mice. Lymphoblastic lymphoma occasionally metastasized into the lung as demonstrated by perivascular malignant lymphocytic infiltration. Together, our studies show that the absence of Nrf3 predisposes mice to lymphoma development, suggesting a protective role of this transcription factor in hematopoietic malignancies. Our data demonstrate the first in vivo function of Nrf3 and its link to tumor development. Nrf3-deficient mice may serve as a preclinical mouse model to study carcinogen-induced lymphomagenesis. (Blood. 2011;117(6):2005-2008)

Introduction

The polycyclic hydrocarbon benzo[a]pyrene (B[a]P) is an environmental pollutant, a major component of cigarette smoke, and a well-characterized rodent and human carcinogen.1 For instance, B[a]P can induce lymphoma in different genetically modified mouse models.2,3 Biotransformation of B[a]P is a requisite for its detoxification and excretion. The first step is catalyzed by cytochrome P450-dependent mono-oxygenases (phase I), and their products are subsequently coupled to endogenous metabolites (phase II).4 However, certain reactive intermediates interact covalently with DNA to form adducts that ultimately result in mutagenicity and/or carcinogenicity. It has been reported that absence of the cap ‘n’ collar (CNC) factor Nrf2 renders mice more susceptible to tumorigenesis caused by B[a]P5,6 probably because of an incapacity to detoxify the carcinogen. These data provided a link between CNC factor-mediated induction of phase II and antioxidant enzymes and the susceptibility to carcinogens.

The CNC family includes p45 NFE2, NRF1, NRF2, NRF3, BACH1, and BACH2 proteins and can form heterodimers with small MAF proteins. We and others previously identified NRF3 as an endoplasmic reticulum-associated protein that is Asn-glycosylated.7,8 We showed that Nrf3 gene expression is induced by butylated hydroxytoluene in the lung of mice.9 Recently, Pepe et al showed a role for Nrf3 in smooth muscle cell differentiation.10 We have generated mice lacking a functional Nrf3 and found that these mice do not show any obvious abnormalities under nonchallenging conditions.11 Thus, to investigate whether the mice deficient for Nrf3 are tumor-prone, we challenged the mice with the carcinogen B[a]P. Our studies revealed a novel role for Nrf3 in the protection of mice against carcinogen-induced lymphomagenesis.

Methods

Animals and treatments

Male wild-type and Nrf3-deficient mice11 (129S6/SvEvTac, 8 weeks old) were treated weekly for 4 consecutive weeks by gavage (150 μL) with B[a]P (Sigma-Aldrich) at a dose of 100 mg per kilogram of body weight dispersed into corn oil as vehicle. Mice were weighed and monitored weekly and killed 30 weeks after the first administration of B[a]P or earlier if they showed signs of distress. On necropsy, tissues were excised, weighed, and stored for further analysis. Procedures involving animals and their care were conducted according to McGill University guidelines, which are set by the Canadian Council on Animal Care. Mice were kept at 22°C with equal periods of darkness. Water and food were available ad libitum.

Analysis of clonality by Southern blotting

Genomic DNA isolation and Southern blotting were performed as previously described.11 Details are provided in supplemental data (available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

Histology and immunohistochemistry

Mouse tissue processing, staining of hematoxylin and eosin slides, and immunohistochemistry were performed according to standard procedures. Details are provided in Supplemental data. All sections were examined by a board-certified veterinary pathologist (M.P.).

Statistical analysis

Statistical analysis was performed using GraphPad Prism Version 4.0a for Macintosh (GraphPad Software). Kaplan-Meier test was used to calculate the survival curves, and the log-rank test was used for evaluation of significance. A P value of less than .05 was considered statistically different.


The online version of this article contains a data supplement.
Results and discussion

High sensitivity of Nrf3-deficient mice to B[a]P exposure

Although elevated NRF3 transcript levels have been found in many different types of human cancers,12-20 no strong evidence exists of a link between NRF3 and tumor development. Here, we examined whether the mice deficient for the Nrf3 gene are susceptible to exposure to the carcinogen B[a]P. We treated wild-type and Nrf3-deficient mice weekly for 4 consecutive weeks with B[a]P (100 mg/kg) and monitored the mice for tumor formation and survival until death at week 30. We did not observe any change with respect to body weight of mice on B[a]P exposure (data not shown), corroborating previously reported results.6 Only one of 16 (6%) B[a]P-treated wild-type mice died before week 30 (Figure 1A). In contrast, 6 of 19 (32%) Nrf3-deficient mice died early, starting from the 15th week after B[a]P treatment (Figure 1B). This clearly indicated a high sensitivity of Nrf3-null mice to carcinogen exposure.

High incidence of T-cell lymphoblastic lymphomas in B[a]P-treated Nrf3-null mice

Approximately one-third of Nrf3-deficient mice treated with B[a]P died before the end of the treatment with signs of respiratory distress. On necropsy, we found the thymus of these mice to be enlarged in most cases (67%, 4 of 6; Figure 2A), reducing space and compressing the lungs caudally against the diaphragm, which corroborates with the breathing difficulties exhibited by these mice.21 Occasionally, we detected tumors in the mucosa of the forestomach of mice, which are probably the result of B[a]P treatment.6 But the number of these tumors in wild-type and Nrf3-null mice was not significantly different (data not shown). Histopathologic examination of hematoxylin and eosin-stained tissue sections revealed that all Nrf3−/− mice that died prematurely developed lymphoma originating mostly from the thymus and few from the spleen. The malignant lymphocytes invaded and effaced the normal thymic and splenic architecture (Figure 2B). In addition, we found the presence of metastatic lymphoma in the lungs of a majority of these animals. We also observed splenomegaly in approximately 50% of the Nrf3−/− mice developing lymphoma (Figure 2A). Immunohistochemistry analysis of the affected tissues with immature lymphoid cell (terminal deoxynucleotidyl transferase), T-cell specific (CD3) and B-cell specific (CD45) markers revealed that the most common type of lymphoma (67%, 4 of 6) found in Nrf3−/− mice was of T-cell origin (Figure 2C). Because clonal population is a hallmark of malignancy, we assessed by Southern blot the rearrangement of T-cell receptor, an important event in T-cell ontogeny.22,23 Accordingly, we observed a rearrangement at the joining region Jβ2 of the T-cell receptor (TCR) β locus in the tissues of mice developing T-cell lymphoblastic lymphoma (supplemental Figure 1). The second type of lymphoma observed in Nrf3−/− mice does not originate from the thymus. This lymphoma, derived in the spleen from an immature lineage with terminal deoxynucleotidyl transferase-positive cells, is characterized by a probable leukemic phase visible on the lung tissue sections (supplemental Figure 2). In contrast to Nrf3-null mice, only one wild-type mouse of 16 (6%) had a distinct type of lymphoma observed late at the time of death (week 30; Figure 2D). This different type of lymphoma was characterized as a splenic marginal zone lymphoma as indicated by both hematoxylin and eosin staining and positive immunostaining of the spleen using an anti-immunoglobulin κ antibody. Clonal origin of this lymphoma was confirmed by an immunoglobulin H rearrangement present in spleen, lung, and thymus of the mouse (data not shown). Together, our results clearly showed a distinct spectrum of lymphoma subtypes and a significant increase in the incidence of lymphoma in the carcinogen-treated Nrf3−/− mice (32%) compared with their wild-type counterparts (6%) (Figure 2D). This result strongly suggests that Nrf3 protects mice from B[a]P-induced lymphoma formation, in particular T-cell lymphoblastic lymphoma. In accordance with these data, we had previously shown that NRF3 transcripts are highly expressed in the thymus.21 In addition, we did not find any compensatory regulation of the Nrf3 homologs NRF1
and Nrf2 at the transcript level in the thymus of Nrf3−/− mice compared with wild-type animals (data not shown).

Similar to the effect described in the present paper, other mouse models have been described as highly susceptible to B[a]P-induced lymphomagenesis, including mice deficient for the genes Msh2 (mutS homolog 2)3 or XPA (xeroderma pigmentosum complementation group A).2 Interestingly, both MSH2 and XPA proteins are involved in the mechanism of DNA repair; and according to the present data, one could speculate that the Nrf3 gene might also be involved in this process.

Of interest, a series of laboratories have observed increased levels of human NRF3 transcripts in Hodgkin lymphoma, in non-Hodgkin cell lineages as well as in mantle cell lymphoma specimen using gene chip arrays.13-17,20 Nevertheless, the functionality and modulation of NRF3 protein in these cells have not been demonstrated. Considering that NRF3 acts as a tumor suppressor gene, one cannot exclude that these human lymphoma cells harbor a nonfunctional NRF3.

In humans, T-cell lymphoblastic lymphoma is a rare but aggressive form of non-Hodgkin lymphoma mostly affecting children, adolescents, and young adult males.24 Various genetic aberrations have been described for T-cell lymphoblastic lymphoma. In most cases, these translocations juxtapose promoter and enhancer elements of T-cell receptor genes located at chromosome 7 to transcription factors involved in T-cell differentiation.25 Interestingly, the NRF3 locus maps to this region,11 and further experiments will be required to determine whether the Nrf3 gene is affected in the translocations causing different lymphoblastic lymphomas.26-28

In conclusion, our studies demonstrate that the absence of Nrf3 renders mice more susceptible to lymphomagenesis, particularly of T-cell origin, in response to chemical carcinogenesis. Our results suggest that the Nrf3 deficiency can predispose to development of hematologic malignancies. Additional studies at the molecular level will be needed to confirm the close link between lymphomagenesis and NRF3 function in humans. We finally hypothesize that NRF3 is a tumor suppressor gene whose function is deregulated by loss and/or mutation in lymphoma and its inactivation may contribute to lymphomagenesis.

Acknowledgments

The authors thank Zaynab Nouhi and Anna Derjuga for outstanding mouse husbandry support, helpful discussions, and critical reading of the manuscript; Jadwiga Gasiorek for critical reading of the manuscript; Dr Monica Justice for helpful discussions; and Julie Hinsinger, Micheline Fortin, and Méline Narlis of the histopathology facility (Institute for Research in Immunology and Cancer, Montreal, QC) for the great help with immunohistochemistry.

This work was supported by the Canadian Institute of Health Research (grant MOP-97932; V.B.). G.C. holds a postdoctoral fellowship of the Fonds de la recherche en santé du Québec.
Authorship

Contribution: G.C. helped in the design and performed the majority of the experiments, analyzed the data, and wrote the manuscript; M.P. provided histopathology expertise; and V.B. designed and supervised the research and edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


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