Myeloid Lineage–Specific Deletion of Antioxidant System Enhances Tumor Metastasis

Keiichiro Hiramoto1,2, Hironori Satoh1, Takafumi Suzuki1, Takashi Moriguchi1, Jingbo Pi3, Tooru Shimosegawa4, and Masayuki Yamamoto1

Abstract
Oxidative stress accelerates the pathogenesis of a number of chronic diseases including cancer growth and its metastasis. Transcription factor NF-E2–related factor-2 (Nrf2), which regulates the cellular defense system against oxidative stress, elicits essential protection against chemical-induced carcinogenic insults. We recently demonstrate that the systemic deletion of Nrf2 leads to an increased susceptibility to cancer metastasis, which is associated with aberrant reactive oxygen species (ROS) accumulation in myeloid-derived suppressor cells (MDSC). However, it remains elusive whether cellular antioxidant defense system in the myeloid lineage cells plays indispensable roles for metastatic cancer progression. We herein found that myeloid lineage–specific Nrf2-deficient mice exhibited an increased susceptibility to pulmonary metastasis of the mouse Lewis lung carcinoma cells, and ROS level was more highly elevated in MDSCs of cancer-bearing Nrf2-deficient mice. Similarly, myeloid lineage–specific deletion of selenium-cysteine-tRNA gene (Tesp), which is essential for synthesis of antioxidant selenoenzymes, resulted in increased number of metastatic nodules along with ROS accumulation in MDSCs of cancer-bearing mice. These results thus indicate that the antioxidant systems directed by Nrf2 and selenoenzymes contribute to the clearance of ROS in MDSCs, efficiently preventing cancer cell metastasis. Consistent with this notion, a synthetic triterpenoid 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole (CDDO-Im), a potent Nrf2 inducer, attenuated the ROS production in MDSCs, and thereby reduced metastatic nodules. Taken together, this study provides compelling lines of evidence that Nrf2 inducer retains therapeutic efficacy against cancer cell metastasis. Cancer Prev Res; 7(8); 835–44. ©2014 AACR.

Introduction
Cancer cell metastasis is one of the major health burdens, which often leads to cancer-related death in humans. It has been well known that cellular oxidative stress accelerates the pathogenesis of a number of chronic diseases, including cancer growth and its metastasis to multiple organs (1–3). Although the importance of oxidative stress in cancer metastasis is well recognized, it remains uncertain how oxidative stress provokes metastasis or how metastasis therapy is attained through targeting oxidative stresses.

Transcription factor NF-E2–related factor-2 (Nrf2) plays an important role in cellular defense against toxic electrophiles and oxidative stresses (4–6). Target genes of Nrf2 include NAD(P)H quinone oxidoreductase 1 (Nqo1), glutamate-cysteine ligase catalytic subunit (Gclc), and heme oxygenase-1 (Ho-1). Disruption of Nrf2 enhances susceptibility of mice to chemically induced carcinogenesis in stomach (7), bladder (8), colon (9), and upper aerodigestive tract (10). The cancer preventive activity of Nrf2 seems to be mainly enhanced by detoxification and/or excretion of carcinogens.

We recently demonstrate that host Nrf2 exerts antimetastatic activity against inoculated cancer cells (11). Germline deletion of Nrf2 increases susceptibility to experimental lung metastasis of mouse 3LL (Lewis lung carcinoma) cells (11). In the cancer-bearing Nrf2-deficient mice, accumulation of reactive oxygen species (ROS) was observed in the cells that coexpress Mac1 (or CD11b) and Gr1, which is known as myeloid-derived suppressor cells (MDSC; ref. 12). MDSCs have been known to be a heterogeneous population of myeloid precursors composed of macrophages, dendritic cells, and granulocytes (13) and suppress antitumor immune system through influencing T-cell activation (14). Thus, we speculate that Nrf2 in myeloid lineage cells may exert antimetastatic activity by maintaining the cellular redox balance.
For therapeutic use to prevent onsets of various diseases, substantial attention has been paid to identify and develop Nrf2-inducing chemicals. Because Nrf2 stability is tightly regulated by Kelch-like ECH-associated protein 1 (Keap1; refs. 15, 16), many types of chemicals that inactivate Keap1, thereby induce Nrf2, have been identified (reviewed in ref. 17). Of the chemicals, synthetic triterpenoid 1-[2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oyl] (CDDO) derivatives show potent inducer activity of Nrf2 (18–20). Although CDDO derivatives are reported to suppress incidence of cancer metastasis (21, 22), there remains uncertainty as to how Nrf2 contributes to the suppression of cancer cell metastasis.

Selenoenzymes, another major antioxidant system, function cooperatively with the Keap1–Nrf2 system. Selenoenzymes, such as glutathione peroxidase (GPx) and thioredoxin reductases (TrxR), reduce hydrogen and lipids peroxide, and contribute to the control of cellular redox homeostasis (23–25). Selenoenzymes contain selenium in the form of selenocysteine, an amino acid that is indispensable for catalytic activity of selenoenzymes (26). The selenocysteine-tRNA acts as an adaptor for selenocysteine and for translational insertion of the amino acid into selenoenzymes. Selenoenzymes are important for maintaining cellular redox balance in various tissues, including myeloid lineage cells, erythrocytes, and hepatocytes (27, 28). Indeed, dietary supplementation with high selenium reduces incidence of metastasis in murine lung cancer models (29). Hence, these observations suggest that antioxidant systems, such as Nrf2-battery genes and selenoenzymes, may be important for the suppression of metastasis.

To address this hypothesis, we generated myeloid lineage cell-specific knockout mice of Nrf2 gene (N-MKO: Nrf2-Myeloid knockout) or selenocysteine-tRNA (Trsp) gene (T-MKO: Trsp-Myeloid knockout). These two types of antioxidant system-deficient mice were inoculated in the metastatic 3LL lung cancer cells. We herein demonstrate that the antioxidant systems directed by Nrf2 and selenoenzymes in myeloid lineage cells are crucial for maintenance of redox balance in MDSCs and eventual prevention of cancer cell metastasis.

Materials and Methods

Cell line

3LL cell line (30) was a kind gift from the Institute of Development, Aging and Cancer at Tohoku University (Sendai, Japan). Cells were regularly tested for Mycoplasma contamination using the e-Myclo Mycoplasma PCR Detection Kit (iNtRON Biotechnology). No cell authentication was done by the authors. The cell culture medium was RPMI-1640 (Wako) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and penicillin–streptomycin (10 U and 0.1 mg/mL, respectively).

Inoculation of cancer cells

In the metastasis model experiments, 3LL cells (1 × 10⁶ in 100-μL PBS) were inoculated into the left thigh muscle. Weights of lungs and primary tumors were measured 21 days after inoculation. Numbers of lung surface nodules were counted after fixation with Tellyesniczky’s solution (31).

Experimental animals and pharmacologic induction of Nrf2

Nrf2<sup>F/F</sup> (32) and Trsp<sup>F/F</sup> mice (27) on C57Bl6/J genetic background were as described previously. Deletion of the floxed Nrf2 or Trsp gene in myeloid lineage cells was performed by crossing these floxed animals with heterozygous animals expressing Cre recombinase under regulation of the lysozyme M (LysM) locus (33). Nrf2<sup>F/F</sup>:LysM-Cre (N-MKO) mice and Trsp<sup>F/F</sup>:LysM-Cre (T-MKO) mice were used in this study. For CDDO-Im treatment study, animals were orally administrated CDDO-Im (30-μmol/kg body weight; CDDO-Im was a kind gift from Mochida Pharmaceuticals Co., Ltd.) or vehicle consisting of 10% dimethyl sulfoxide (DMSO), 10% Cremophor-EL, and 80% PBS by gavage. All animal experiments were carried out with the approval of the Tohoku University Animal Care Committee.

Histologic analysis

Lung tissues from the mice were fixed with Tellyesniczky’s solution (31) and embedded into paraffin. Five-micrometer thick sections were stained with hematoxylin and eosin (H&E) using the standard techniques.

FACS analysis

Single-cell suspensions from cancer-bearing mouse lungs were prepared as described previously (34). Analyses of lung cells, splenocytes, and bone marrow cells were performed using FACS Caliber (BD Biosciences). For the separation of MDSCs, each cell suspension was incubated with allophycocyanin (APC)-conjugated anti-Mac1 and phycoerythrin (PE)-conjugated anti-Gr1 monoclonal antibodies (eBioscience). For quantification of ROS levels, each cell suspension was incubated with 5-μmol/L 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA; Invitrogen) at 37°C for 30 minutes (35). FACS analysis was performed using FlowJo (TOMY Digital Biology) software.

Isolation of thioglycollate-elicted peritoneal macrophages

Macrophages were isolated by lavage from mice that had received a 2-mL intraperitoneal injection of 4% thioglycollate broth 4 days before. The cells were transferred onto a 10-cm dish at a density of 5 × 10⁵ cells/mL for immunoblot analyses or RNA extraction. The macrophages were maintained in RPMI-1640 medium containing 10% FBS and penicillin–streptomycin.

Preparation of plastic-adherent splenocytes and immunofluorescent analysis

Whole spleen cells were dispersed and incubated on a 10-cm dish with RPMI-1640 medium containing 10% FBS and penicillin–streptomycin (10 U/0.1 mg/mL) for 2 hours. Nonadherent cells were removed and then the dish
was washed twice with PBS. Adherent cells were subjected to the experiments as splenic myeloid cells. An anti-mouse Mac1 monoclonal antibody (clone M1/70; eBioscience) was used for the detection of splenic myeloid cells.

**Immunoblot analysis**

Peritoneal macrophages were collected and lysed in sodium dodecyl sulfate (SDS) sample buffer and stored at −80°C (36). The samples were subjected to immunoblot analysis using anti-Nrf2 (clone 103; ref. 37) and anti-α-tubulin (Sigma) antibodies.

**Quantitative PCR analyses**

Total RNA was extracted from peritoneal macrophages, plastic-adherent splenocytes, and liver tissues using ISOGEN (Nippon Gene). First-strand cDNA was synthesized from total RNA using ReverTra Ace qPCR RT Master Mix (TOYOBO). Real-time PCR was performed using an ABI PRISM 7300 sequence detector system (Applied Biosystems) and THUNDERBIRD Probe qPCR Mix (TOYOBO). The primer sequences for Nrf2, Nqo1, and Gclc were described previously (38).

**Statistical analyses**

All the data are presented as mean ± SD. Statistical differences were determined using the Student t test or the Mann–Whitney U test. P < 0.05 were considered statistically significant.

**Results**

**Myeloid lineage–specific Nrf2-deficient mice are susceptible to tumor cell metastasis from distant primary tumor**

To ascertain whether the increased susceptibility of tumor cell metastasis in Nrf2−/− mice is attributable to the Nrf2 deficiency in myeloid lineage cells, we crossed Nrf2F/F mice with LysM-Cre mice (Fig. 1A) to generate myeloid lineage–specific Nrf2-deficient mice (N-MKO). Through this crossbreeding, Nrf2 protein, which is normally accumulated by proteasome inhibitor MG132 in the control mice, was abrogated in peritoneal macrophages of N-MKO mice (Fig. 1B). We found that Nrf2F/F mice showed slightly lower level of Nrf2 than wild-type (WT) mice did, and this may be due to residual Neo cassette. Therefore, to conduct reasonable comparison under this hypomorphic expression, we have exploited Nrf2F/F mice as a control throughout this study. We also examined mRNA expression level of Nrf2 in peritoneal macrophages of N-MKO mice and found that the level also markedly decreased (Supplementary Fig. S1A). The upregulation of Nqo1, a typical Nrf2 target gene, in response to diethyl maleate (DEM), a well-known Nrf2 inducer, was observed in the peritoneal macrophages from WT and Nrf2F/F mice, whereas the inducible Nqo1 expression was significantly diminished in those from N-MKO mice (Supplementary Fig. S1B). These results thus demonstrate that the transcript and protein levels of Nrf2 are significantly knocked out in N-MKO mice, and thereby the inducible Nqo1 expression is diminished in the macrophages.

We reported previously antimetastatic activity of Nrf2 through the analyses of systemic Nrf2 knockout mice. However, there remains uncertainty as to in which cell lineage Nrf2 deficiency actually contributes to the activity. To address this issue, we inoculated 3LL cells into left thigh muscle of Nrf2F/F and N-MKO mice and developed primary tumors in the tissue. Mice were euthanized 3 weeks after the inoculation, and lung metastatic nodules were...
macroscopically and histologically examined (Fig. 1C). N-MKO mice exhibited a higher number of lung surface nodules (12 ± 9.1; n = 11) than did Nrf2F/F mice (3 ± 5.7; n = 11; P = 0.0344; Fig. 1D; Table 1). In contrast, lung weight and primary tumor weight of thigh muscle were not significantly different between Nrf2F/F and N-MKO mice (Supplementary Fig. S1C and Table 1). These results thus indicate that Nrf2 activity in myeloid lineage cells exerts the antimetastasis activity in the host lung tissue.

**Nrf2 is important for regulating intercellular ROS level of MDSCs**

It has been shown that the MDSC population expands in many types of cancer patients and cancer model animals, and in mice. MDSCs are characterized by the coexpression of two myeloid lineage differentiation antigens, Mac1 and Gr1. Using fluorescence-conjugated antibodies and FACS, we examined the percentages of MDSCs (Mac1−Gr1+) in multiple tissues of cancer-bearing Nrf2F/F and N-MKO mice. The lung cancer metastasis evoked expansion of MDSC fraction in the lung and spleen to the same extent in both Nrf2F/F and N-MKO mice. Meanwhile, the MDSC population in the bone marrow was not significantly changed upon lung metastasis in both genotypes of mice (Fig. 2A and B).

We also analyzed intracellular ROS levels in MDSCs using a fluorescence marker H2DCFDA. In this metastatic lung cancer model mice, ROS levels of MDSCs in the lung, spleen, and bone marrow of Nrf2F/F and N-MKO mice were both elevated in comparison with tumor-free control mice of each genotype (Fig. 2C and D). Consistent with the increased metastasis susceptibility, the tumor-bearing N-MKO mice showed a higher ROS accumulation in the MDSCs of all the tissues in comparison with the tumor-bearing Nrf2F/F control mice (Fig. 2C and D). These data thus indicate that Nrf2 activity is important for regulating intracellular ROS level in MDSCs and the suppression of metastasis.

**Myeloid-specific deletion of selenoenzymes leads to higher susceptibility to metastasis and ROS accumulation in MDSCs**

Although metastatic tumor nodules were increased on the surface of N-MKO mouse lungs, it remains unclear whether ROS accumulation in MDSCs underlies this phenomenon. To explore this issue, we used myeloid lineage–specific Trsp-deficient mice (T-MKO). Because selenocysteine-tRNA is indispensable for synthesizing selenoenzymes, such as GPx and TrxR, and maintaining cellular redox homeostasis, T-MKO mouse will serve as an important model of ROS-accumulated myeloid lineage cells.

T-MKO mice showed larger number of surface nodules (5 ± 9.36; n = 13) than TrspF/F mice did (3 ± 3.33; n = 13; P = 0.0365; Fig. 3A and B; Table 1). Lung weight and primary tumor weight on thigh were not significantly different between T-MKO and TrspF/F mice (Supplementary Fig. S2 and Table 1). In the metastatic lung cancer models, the MDSC population in lung and spleen were both increased in T-MKO and TrspF/F mice, whereas in contrast, bone marrows contained comparable number of MDSCs irrespective of the 3LL cell inoculation (Fig. 3C). Importantly, the cancer cell inoculation provoked remarkable ROS accumulation in MDSCs from lung, spleen, and bone marrow of T-MKO mice compared with those of control TrspF/F mice (Fig. 3D). Taken together, the increase in intracellular ROS levels in MDSCs upon deletion of either Nrf2 or Trsp is well correlated with the enhancement of metastatic progression.

### Table 1. Numbers of metastatic lung surface nodules and weights of lung and primary tumor by inoculation of 3LL cells

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Number of lung surface tumors (median ± SD)</th>
<th>Weight, g (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lung</td>
<td>Primary tumor</td>
</tr>
<tr>
<td>Nrf2F/F (n = 11)</td>
<td>—</td>
<td>3 ± 5.70</td>
<td>0.153 ± 0.029</td>
</tr>
<tr>
<td>N-MKO (n = 11)</td>
<td>—</td>
<td>12 ± 9.10a</td>
<td>0.162 ± 0.021</td>
</tr>
<tr>
<td>TrspF/F (n = 13)</td>
<td>—</td>
<td>3 ± 3.33</td>
<td>0.159 ± 0.021</td>
</tr>
<tr>
<td>T-MKO (n = 13)</td>
<td>—</td>
<td>5 ± 9.36b</td>
<td>0.148 ± 0.029</td>
</tr>
<tr>
<td>Nrf2F/F</td>
<td>Vehicle (n = 11)</td>
<td>9 ± 5.59</td>
<td>0.148 ± 0.021</td>
</tr>
<tr>
<td></td>
<td>CDDO-Im (n = 10)</td>
<td>2.5 ± 3.02c</td>
<td>0.150 ± 0.028</td>
</tr>
<tr>
<td>N-MKO</td>
<td>Vehicle (n = 11)</td>
<td>12 ± 13.13c</td>
<td>0.167 ± 0.026</td>
</tr>
<tr>
<td></td>
<td>CDDO-Im (n = 13)</td>
<td>10 ± 14.12c</td>
<td>0.153 ± 0.030</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with Nrf2F/F control mice.

*aP < 0.05 compared with Nrf2F/F control mice.

*bP < 0.05 compared with TrspF/F mice.

*cP < 0.05 compared with vehicle-treated Nrf2F/F mice.
Suppression of ROS accumulation in MDSCs and attenuation of lung metastasis by CDDO-Im treatment

We surmised that Nrf2 activation in the host microenvironment may exert preventive efficacy against cancer cell metastasis. To address this possibility, we used CDDO-Im, a potent Nrf2-activator. As shown in Fig. 4A, Nrf2/F/F and N-MKO mice were administrated CDDO-Im (30–μmol/kg body weight) or vehicle three times per week (Fig. 4A, black arrows) from 2 days before cancer cells inoculation (open arrow). CDDO-Im–treated Nrf2/F/F mice showed a significant decrease in the number of lung surface nodules (2.5 ± 3.0; n = 10) compared with vehicle-treated Nrf2/F/F mice (9 ± 5.6; n = 11; P = 0.0260; Fig. 4B; Table 1). Meanwhile, there was not significant difference in the number of surface nodules between vehicle-treated N-MKO (12 ± 13.1; n = 11) and CDDO-Im–treated N-MKO mice (10 ± 14.1; n = 13; P = 0.4996; Fig. 4B; Table 1). These observations clearly indicate that CDDO-Im treatment has efficacy for prevention of cancer cell metastasis and this efficacy depends on Nrf2 expression in myeloid lineage cells.

Both Nrf2/F/F and N-MKO mice did not display significant difference in the primary tumor size, lung weight, and primary tumor weight (Supplementary Fig. S3A–S3C and Table 1), regardless of CDDO-Im treatment, indicating that growth of the primary tumor in thigh tissue is independent of the Nrf2 activity in this model.

Next, we examined whether CDDO-Im treatment reduces the ROS accumulation in MDSCs from tumor-bearing mice. CDDO-Im treatment significantly reduced the ROS levels in MDSCs of Nrf2/F/F mice, whereas the MDSCs of N-MKO...
mice did not show apparent decrease in ROS levels even after CDDO-Im treatment (Fig. 4C). These data indicate that CDDO-Im–induced Nrf2 activity decreases the intracellular ROS levels in MDSCs. On the contrary, CDDO-Im treatment did not affect the populations of MDSCs in the lung, spleen, and bone marrow in each group, indicating that CDDO-Im treatment did not have any cytotoxic effect within the doses in our experiments (Supplementary Fig. S3D).

We next examined whether CDDO-Im treatment induces Nrf2 activity in myeloid lineage cells from Nrf2<sup>F/F</sup> and N-MKO mice. For this purpose, splenic myeloid lineage cells were collected as splenic adherent cells; majority of the adherent cells (89.87%) expressed Mac1 immunoreactivity (Supplementary Fig. S4), indicating that major cell type of the splenic adherent cells is myeloid lineage cell. The CDDO-Im treatment induced the expressions of both Nqo1 and Gclc genes in splenic myeloid cells from Nrf2<sup>F/F</sup> mice, but not the cells from N-MKO mice (Fig. 4D). The induction of these Nrf2 target genes in splenic myeloid cells from Nrf2<sup>F/F</sup> mice were found to persist for at least 48 hours after treatment (Fig. 4D). These results indicate that Nrf2 activity is maintained at high level throughout the metastasis experiments with our CDDO-Im treatment protocol.

Collectively, these results demonstrate that CDDO-Im induces the expression of Nrf2 target genes in myeloid lineage cells, and thereby decreases the ROS accumulation in MDSCs. Thus, we conclude that Nrf2-mediated antioxidant system in myeloid lineage cells takes principal responsibility for the prevention of cancer cell metastasis.

Discussion

This study demonstrates that the ROS levels in myeloid lineage cells are regulated by Nrf2 and selenoenzymes, and this regulation is critical for susceptibility of host animals for the lung cancer cell metastasis. The myeloid lineage–specific Nrf2-deficient mice provide a useful assay system to verify the participation of Nrf2 in myeloid lineage cells into the suppression of cancer cell metastasis. In addition to the Nrf2 pathway, myeloid lineage–specific selenoenzymes-deficient mice display increased ROS production in MDSCs and high-level susceptibility to metastasis of cancer cells compared with the control mice. In contrast, administration of a potent Nrf2 activator, CDDO-Im, reduced intracellular ROS levels in MDSCs and repressed lung metastasis of cancer cells in an Nrf2-dependent manner in myeloid lineage cells. These results thus indicate that the antioxidant systems regulated by both the Keap1–Nrf2 pathway and selenoenzymes play indispensable roles for maintaining redox balance in MDSCs and prevention of cancer cell metastasis (summarized in Fig. 5).

ROS accumulation has been suggested to participate in the MDSC-mediated immune suppression (reviewed in ref. 39). For instance, MDSCs with low level of ROS production (gp91null-mice) lack ability of inducing T-cell tolerance, suggesting that ROS production takes fundamental responsibility for MDSC-mediated CD8<sup>+</sup> T-cell tolerance (40). The immunosuppressive activity of myeloid lineage cells is abrogated by the treatment with ROS scavenger, catalase (41). Showing very good agreement with these preceding observations, this study demonstrates that myeloid lineage–specific deficiency of antioxidant systems leads to aberrant ROS accumulation and resultant increase in lung metastasis. These observations further support the notion that ROS levels in myeloid cells are one of the critical determinants of the progression of cancer cell metastasis.

Although precise mechanisms underlying how ROS enhance metastasis remain unclear, several hypotheses have been proposed (reviewed in ref. 42). The present study showed that myeloid lineage–specific Nrf2- or Trsp-deficient mice did not show significant changes in the CD8 single-positive and CD4 single-positive T-cell populations despite significant increase in the lung metastasis (Supplementary Fig. S5), whereas the systemic Nrf2-null mice with cancer...
cell metastasis showed decreased in CD8⁺ T-cell population in our previous study (11). One plausible explanation for this discrepancy is that antioxidant system in T cells may be maintained to support the T-cell proliferation and/or survival in N-MKO and T-MKO mice even after cancer metastasis.

Alternatively, alterations in T-cell characters may give rise to the enhancement of cancer metastasis in the N-MKO and T-MKO mice. In this regard, one of the most likely mechanisms is the ROS-mediated modification of T-cell receptor (TCR)–CD8 complex. In combination with production of nitric oxide (NO) in cancer microenvironment, ROS accumulation in MDSCs seems to introduce peroxynitrite (ONOO⁻) modifications of the TCR–CD8 complex on the cell surface of CD8⁺ T cells. This aberrant modification disrupts the interaction between the TCR–CD8 complex and MHC class I molecules on antigen-presenting cells. Thereby, CD8⁺ T cell–mediated anticancer immunity is compromised eventually (40, 43).

Search for novel Nrf2–inducing drugs has been dramatically accelerated in hope of identifying therapeutics against various types of diseases. Those Nrf2 inducers should also work for cancer chemoprevention, given that pharmacologic induction of Nrf2 is effective for the prevention of...
NRF2 activators may lead to cancer progression. However, it turns out that pharmacologic induction can attain only a weak level activation of NRF2 and may not be harmful for cancer cell development. In addition, our current study unequivocally provides emphatic answer that CDDO-Im administration reduces cancer metastasis. Thus, the therapeutic benefit by activating host Nrf2 exceeds the exacerbation risk by activating Nrf2 in cancer cells. We surmise that Nrf2-mediated reinforcement of cancer immunity generates robust cancer-resistant environment, which makes a countercharge against metastatic cancer cells.

In summary, we provide promising lines of evidence that the antioxidant activity in myeloid lineage cells contributes to the suppression of ROS accumulation and subsequent prevention of metastatic cancer progression. Furthermore, we have proved that Nrf2-activator drugs give rise to therapeutic benefits rather than adverse side effects. Taking all these observations into account, we argue that the Keap1–Nrf2 system in myeloid lineage cells could serve as an attractive therapeutic target for anti-metastatic treatment.

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

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Conception and design: K. Hiramoto, H. Satoh, T. Suzuki, M. Yamamoto
Development of methodology: K. Hiramoto, H. Satoh, M. Yamamoto
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Hiramoto, H. Satoh, T. Suzuki, M. Yamamoto
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Hiramoto, H. Satoh, T. Moriguchi, M. Yamamoto
Writing, review, and/or revision of the manuscript: K. Hiramoto, H. Satoh, T. Suzuki, T. Shimosegawa, M. Yamamoto
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Hiramoto, H. Satoh, T. Suzuki, T. Shimosegawa, M. Yamamoto
Study supervision: T. Suzuki, T. Shimosegawa, M. Yamamoto

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