Versatile activity of the master regulator of the antioxidant response in tumor initiation, progression and metastasis

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Oxidative intermediates derived from endogenous metabolism or xenobiotic stimuli are a major cause of cancer initiation. Host defense systems have evolved to cope with these oxidative stresses and prevent carcinogenesis. The basic leucine zipper transcription factor Nrf2 (Nuclear factor-erythroid derived 2-like 2, Nfe2l2) regulates the cellular defense system against oxidative stress, and governs cellular protection against chemical carcinogens. At the same time, Nrf2 enhances anti-cancer immunity in the tumor microenvironment, thereby suppressing growth and metastasis of cancer cells. These beneficial aspects of Nrf2 activities contribute to the prevention of cancer initiation, as well as its subsequent progression. On the other hand, aberrant Nrf2 activation confers malignant properties on cancer cells by enhancing proliferative ability and drug resistance. Thus, Nrf2 contributes to the prevention of carcinogenesis as well as the malignant growth of cancer cells. Increasing numbers of studies have been focusing on elucidating how these contradictory activities of Nrf2 are elicited during the multistep carcinogenic process. In this research highlight, we discuss our current understandings of Nrf2 function in cancer initiation, promotion and metastasis in a murine lung cancer model, and related topics are summarized.

Keywords: Keap1-Nrf2 system; carcinogenesis; tumor microenvironment

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Keap1-Nrf2 system

Nrf2 is a leucine zipper transcription factor, which plays a crucial role in the protective response against environmental stresses, including oxidative and electrophilic insults [1, 2]. In the steady state, Nrf2 is bound by the Keap1 (kelch-like ECH-associated protein 1)-CUL3 E3 ubiquitin ligase complex in the cytoplasm, and subjected to prompt degradation through the ubiquitin-proteasome pathway (summarized in Fig 1). Upon exposure to oxidative or electrophilic insults, reactive cysteine residues in Keap1 are covalently modified, which eliminates the Keap1-mediated degradation of Nrf2, thereafter allowing Nrf2 to accumulate in the nucleus. Nuclear accumulated Nrf2 dimerizes with small Maf protein (sMaf) and binds to a consensus DNA sequence designated as the anti-oxidant/electrophile response element (ARE/EpRE). Subsequently, a variety of antioxidant and detoxifying enzyme genes, such as NAD(P)H quinone oxidoreductase (Nqo1), heme oxygenase 1 (Ho-1), and glutamate-cysteine ligase catalytic subunit (Gclc), are
activated. These enzymes contribute to the cellular protection against oxidative and electrophilic insults. Therefore, Nrf2 has been deemed an essential factor for the oxidative stress response, as well as cancer chemoprevention.

Chemical-induced lung carcinogenesis in Nrf2-deficient mice

A series of studies have revealed that Nrf2-deficient mice are susceptible to chemically induced carcinogenesis. Perturbation of the Nrf2-regulated carcinogen detoxification system leads to cancer susceptibility in various tissues, i.e., stomach, bladder, skin and colorectal [3-6]. We addressed whether this is also the case in a chemical induced lung cancer model. To this end, we employed an established urethane-induced murine lung cancer model. Urethane (ethyl carbamate) is a prototypical carcinogen that recapitulates multi-step lung adenocarcinomagenesis [7, 8]. Urethane administration initially induces the formation of lung adenomas, which subsequently acquire activating somatic mutations in the Kras oncogene, which gives rise to adenocarcinomas [7, 8]. Urethane-induced carcinogenesis is

Figure 1. Nrf2 is bound by Keap1 in the cytoplasm and subject to proteasomal degradation during steady state condition. Upon exposure to oxidative or electrophilic insults, SH groups in reactive cysteine residues in Keap1 are chemically modified. Thereby, Keap1-mediated Nrf2 degradation is eliminated. Nuclear accumulated Nrf2 binds to consensus DNA sequence i.e., anti-oxidant/electrophile response element (ARE), and induces the expression of a variety of antioxidant and detoxification enzyme genes.
dependent on Cytochrome P450 2E1 (Cyp2e1)-mediated oxidization, which converts urethane into vinyl carbamate epoxide (VCE) \[9\]. The VCE functions as a carcinogen by binding to and inducing DNA- and protein-adduct formation (Fig 2). During detoxification, VCE is converted into 1, 2-dihydroxyethyl carbamate by microsomal epoxide hydrolase (mEH). This product is targeted for Gstp1/p2-mediated glutathione conjugation, after which the conjugate is excreted into urine \[10\]. Since both mEH and Gstp1/p2 are regulated by Nrf2, the urethane detoxification pathway appears to depend on Nrf2 activity.

As expected, Nrf2-deficient mice exhibit an increased number of tumor foci 8 weeks after urethane administration. However, after 16 weeks, Nrf2-deficient mice show a reduced number of tumors in comparison with wild-type mice (summarized in Fig 3) \[11\]. During the course of malignant progression, all of the tumors in the wild-type mice acquired activating Kras mutations \[12\], whereas Nrf2-deficient tumors were rarely associated with such mutations. These results suggest that Nrf2 plays two distinct roles during carcinogenesis. Firstly, Nrf2 prevents tumor initiation, through the induction of urethane-detoxification and cytoprotective enzymes. Secondly, Nrf2 plays a role in the malignant progression of the tumor cells, which is dependent on oncogenic Kras-signaling. Growth attenuation of the urethane-induced lung tumors in Nrf2-deficient mice supports the latter conclusion.

### Chemical-induced lung carcinogenesis in a constitutively active Nrf2 animal model

Given the enhanced cancer initiation and subsequent growth attenuation of the tumors in Nrf2-deficient mice, we next addressed how genetic activation of Nrf2 affects cancer initiation and malignant progression. For this purpose, we employed Keap1-hypomorphic mutant mice, which exhibit constitutive Nrf2 accumulation due to a systemic decrease in Keap1 expression \[13\]. We found that the Keap1-hypomorphic mice develop a lower number of urethane-induced lung tumors than the wild-type mice, indicating that global activation of Nrf2 prevents urethane-induced lung carcinogenesis \[14\]. Notably, after transplantation into nude mice, the tumor cells derived from the Keap1-hypomorphic mutant mice grew more vigorously than those from the wild-type mice, indicating that Nrf2-activated cancer cells acquire a strong cue for malignant proliferation. This enhanced proliferation ability of the urethane-induced lung cancer cells appears to be repressed by the Nrf2-dependnet antitumor immunity in the Keap1-hypomorphic mice (summarized in Fig 3).

### Nrf2 function in the tumor micro-environment

While the cellular defense activity of Nrf2 against chemical carcinogens has been well-demonstrated, Nrf2 function in the tumor microenvironment has rarely been explored. To address this issue, we examined the susceptibility of Nrf2-deficient mice to pulmonary cancer metastasis upon inoculation with the Lewis lung carcinoma (3LL) cell line. This analysis revealed that Nrf2-deficient mice reproducibly develop a higher number of pulmonary metastatic nodules compared with wild-type mice \[15\]. The lung and bone marrow of cancer-bearing Nrf2-deficient mice harbor an increased number of inflammatory cells, including myeloid-derived suppressor cells (MDSCs), a potent immunosuppressive cell type \[16\]. MDSCs attenuate the antitumor immune response through reactive oxygen species (ROS)-mediated modification of the T-cell receptor complex \[16\]. Through this process, the intracellular ROS level is a primary determinant
of the immunosuppressive activity of MDSC. Nrf2-deficient MDSCs retain elevated levels of ROS when compared with wild-type MDSCs, and thereby the Nrf2-deficient microenvironment showed a reduction in the antitumor T-cell immune response. In contrast, Keap1-hypomorphic mutant mice harboring a high-level of Nrf2 displayed enhanced resistance against pulmonary metastasis, which was accompanied by a decrease in ROS in the MDSCs. These results reveal that Nrf2-mediated regulation of the immune system redox status in the tumor microenvironment is crucial for the prevention of cancer metastasis.

**Nrf2 inducers for cancer chemoprevention**

A number of Nrf2 inducers, including phytochemicals and their synthetic derivatives, have been described. Among them, 1-[2-cyano-3,12-dioxoleana1,9(11)-dien-28-oyl](CDDO)-derivates, \textit{i.e.,} CDDO-imidazole (CDDO-Im) and CDDO-methyl (CDDO-Me or Bardoxolone), are prime candidates for clinical application. Pretreatment with CDDO-derivatives elicits tumor prevention in multiple models of carcinogenesis, presumably through prevention of tumor initiation. Given that Nrf2 regulates the antitumor immune response, Nrf2 inducers may also improve the antitumor immune environment in cancer patients. However, increased Nrf2 activity in advanced lung cancer is associated with poor prognosis in human patients, implying that Nrf2 inhibitors might serve as rational therapeutics to repress malignant progression. Therefore, the prescription of Nrf2 inducers for cancer patients must be carefully evaluated, considering the clinical stage of the tumors and the overall benefit-risk balance. For safe and efficient application of Nrf2-based therapeutics, further clinical and basic investigations are needed.

**Figure 3.** *Nrf2*-deficient mice show an increased number of microscopic tumor nodules at the early stage after urethane administration. However, in the late stage *Nrf2*-deficient mice show a reduced number of tumors in comparison with wild-type mice (left). *Keap1*-hypomorphic mice develop a lower number of urethane-induced lung tumors. Upon transplantation into immune-deficient mice, the *Keap1*-hypomorphic mouse-derived tumor grows more vigorously than those from wild-type mice, suggesting that Nrf2-activated cancer cells acquire a strong cue for high proliferation. In the context of *Keap1*-hypomorphic mice, this increased proliferation ability of Nrf2-activated cancer cells is repressed by the enhanced antitumor immunity in *Keap1*-hypomorphic mice. Reprinted with permission.
Conflicting interests

The authors have declared that no conflict of interests exists.

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Abbreviations

Nrf2: Nuclear factor-erythroid derived 2-like 2; Keap1: kelch-like ECH-associated protein 1; CDDO: 1-[2-cyano-3,12-dioxoleana -1,9(11)-dien-28-oyl.

Author contributions

T.M. wrote the manuscript

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