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Perspective

Targeting Transcription Factors for Cancer Prevention—the Case of Nrf2

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NF-E2-related factor 2 (Nrf2) is one of several transcription factors that are altered in human cancer and that modulate susceptibility to carcinogenesis in mouse models (1–3). Nrf2 up-regulates transcription of a number of oxidant and electrophile detoxication genes through an antioxidant response element present in their transcriptional promoters. In this issue of the journal, Khor et al. (4) follow up their own and others' previous findings that Nrf2 protects against dextran sodium sulfate–induced colitis, inflammation, and aberrant crypt foci with a study now showing that tumor incidence, multiplicity, size, and stage of progression are increased in Nrf2-deficient mice in an azoxymethane-dextran sodium sulfate colon carcinogenesis model. Expressions of the proinflammatory genes cyclooxygenase-2 (*COX-2*) and 5-lipoxygenase (*5-LOX*) and their products prostaglandin E_2 and leukotriene B_4 were increased in tumors induced in the knockout mice compared with wild-type tumors and with normal mucosa. Induction of prototypic protective enzymes UDP-glucuronosyl transferase 1A and NAD(P)H:quinone oxidoreductase 1 following azoxymethane-dextran sodium sulfate failed to occur in Nrf2-deficient mice. Although the association between chronic inflammation and cancer has been noted for several cancer sites including liver, pancreas, stomach, bladder, and colon, the molecular basis for this association is not well understood. Khor et al. now implicate activation of pro-oxidant enzymes such as COX-2 and 5-LOX and lowered expression of antioxidant enzymes such as NAD(P)H:quinone oxidoreductase 1 as likely mediators of the inflammation-associated colon tumor promotion and progression in Nrf2-deficient mice. This suggests that activation of Nrf2 or Nrf2 signaling might be a promising strategy for prevention of inflammation-associated cancer.

A number of oncogenic transcription factors such as activator protein 1 (AP-1), nuclear factor κB (NF-κB), and signal transducer and activator of transcription (STAT)-3/STAT5 are overactivated in human cancer and thus may present promising targets for cancer prevention (see Tables 1–3 for partial listings; refs. 5–11). Mouse skin carcinogenesis is inhibited by targeting AP-1 in a dominant negative jun (K14-TAM67)–expressing mouse. This targeting is effective whether carcinogenesis is induced by 7,12-dimethylbenz(*a*)anthracene/12-*O*-tetradecanoylphorbol-13-acetate, UVB, or human papilloma virus oncogenes (11–13). Also in this issue of the journal, Shen et al. present the first *in vivo* extension of AP-1 targeting by TAM67 beyond skin carcinogenesis to now address mammary carcinogenesis (14). These investigators show the effectiveness of targeting AP-1 transactivation for attenuation of ER-negative mammary carcinogenesis in a trigenic mouse model. In this model, induced expression of the AP-1 inhibitor dominant negative jun produced a 44% decrease in tumor incidence and a substantial increase in tumor-free survival in MMTV-ErbB2 mice. AP-1 blockade also attenuated the development of premalignant lesions (hyperplasia and mammary intraepithelial neoplasia, which is similar to human ductal carcinoma *in situ*) and invasive mammary tumors, showing that blockade of AP-1 suppresses early and late stages of carcinogenesis. AP-1 is thereby implicated as a critical transducer of erbB2 and a rational target for the development of drugs to prevent ER-negative breast cancer. The importance of AP-1–dependent gene expression is further underscored by the observation that the mammary tumors and ductal carcinoma *in situ* that do develop have lost expression of the TAM67 transgene. Although the transgene is intact, revealing the mechanism by which expression is down-regulated calls for further studies. This observation suggests that combination chemoprevention to achieve AP-1 blockade and at the same time prevent inactivation of the AP-1 blocking agent may be indicated.

The mechanisms by which AP-1 blockade acts to prevent carcinogenesis in the skin differ somewhat from those in mammary glands. In mouse skin, tumorigenesis is ~80% suppressed by TAM67 without inhibition of cell proliferation or tumor promoter–induced hyperproliferation (11). In contrast, in cell lines derived from erbB2 transgenic mammary tumors, the induction of (previously uninduced) TAM67 is accompanied by inhibition of cell proliferation. This and other observations of Shen et al. implicate cell cycle mediators as operative targets of TAM67 for breast, whereas genes associated with inflammation and invasion may be important targets in the case of skin carcinogenesis (11, 12).

Although a few tumor-suppressing transcription factors such as p53 and Rb are known to be underactivated (Table 1) in human cancer, much less is known about the possibility of activating or stabilizing tumor-suppressing transcription factors. Despite decades of effort directed to exploiting p53 for cancer treatment or prevention, progress has been minimal. Perhaps targeting a transcription factor such as Nrf2 that is less fundamental than is p53 to cell proliferation and cell survival may prove more tractable. Nrf2, in contrast to p53, is an important modifier of susceptibility, but no cancer-prone phenotypes are observed in the absence of carcinogen challenge to Nrf2-deficient mice. Several hundred genes are transcriptionally controlled by Nrf2 through the antioxidant response element (1). The corresponding enzymatic gene products enhance glutathione biosynthesis, increase production of reducing equivalents, facilitate oxidant

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Table 1. Transcription factors show altered activity during cancer progression in humans (6, 9)

Factor	Cancers
Oncogenic transcription factors overactivated during human cancer progression	
AP-1	Breast, colon, lung
NF-κB	Pancreas, breast, prostate
STAT3	Colon
β-Catenin	Lung
c-Myc	Colon
Suppressor transcription factors underactivated during human cancer progression	
p53	Colon, breast, prostate
Rb	Liver

Table 3. Transcription factors can be targeted by drugs or nutritional interventions (5, 9)

Molecular target	Intervention
AP-1	Curcumin, gingerol, EGCG
NF-κB	Curcumin, sulforaphane, NSAIDs
ER	Tamoxifen, raloxifene
HIF-1	Gingerol
STAT3	Curcubatacin-Q
p53	Resveratrol, CP31398
Nrf2	Sulforaphane, oltipraz, triterpenoids

Abbreviations: EGCG, epigallocatechin-3-gallate; NSAIDs, nonsteroidal anti-inflammatory drugs; ER, estrogen receptor; HIF-1, hypoxia-inducible factor 1.

detoxication and electrophile conjugation, and elevate many subunits of the proteasome, thereby restricting the levels of oxygen radicals and, indirectly, nitrogen radicals, and consequently preventing overactivation of oncogenic events. These radical species are known to activate oncogenic transcription factors such as AP-1 and NF-κB (15) that have been shown in mouse models to be required for carcinogenesis in the skin and/or other cancer sites (11–13).

Nrf2 contrasts with AP-1, which drives both early and late stages of carcinogenesis and responds to blockade with attenuation of both early and later stages (4, 14). A puzzling feature of Nrf2 is its apparent operation as a double-edged sword. Nrf2 is overactivated in human non–small cell lung, head and neck, and breast cancers, often as a result of alterations in its negative regulator, Keap1 (2, 3). This overactivation may contribute to chemotherapeutic drug export, drug resistance, and, consequently, tumor cell survival. Yet because Nrf2 deficiency in mice confers increased susceptibility to chemical carcinogenesis, Nrf2 loss, not gain, may contribute to tumorigenesis. Up-regulation of Nrf2 activity in mice and humans by various chemopreventive agents suggests that basal Nrf2 activity is insufficient to protect against carcinogenesis. Nrf2 seems to function as a suppressor of tumorigenesis but not of tumor survival. Whether it can function as a suppressor of tumor progression (adenoma to adenocarcinoma) not only in mice but also in humans is not clear.

Table 2. Mice with genetically inactivated transcription factors validate molecular targets for prevention

Inactivated transcription factor	Resistant to skin carcinogenesis	Resistant to colon carcinogenesis
AP-1 (11)	+	?
STAT3 (7)	+	?
NF-κB (8)	–	+
	Sensitive to skin carcinogenesis	**Sensitive to colon carcinogenesis**
Nrf2 (4, 10)	+	+

If COX-2 and 5-LOX elevation or NAD(P)H:quinone oxidoreductase 1 and UDP-glucuronosyl transferase 1A down-regulation are operative mediators of the Nrf2 deficiency, then blocking COX/LOX or activating NAD(P)H:quinone oxidoreductase 1/heme oxygenase 1 may counteract the oncogenic activity of Nrf2 deficiency in double knockouts, double transgenics, or knockout/transgenic models. More likely, if the implicated inflammation/carcinogenesis prevention activities of Nrf2 are not restricted to the regulation of a small number of genes such as those measured by Khor et al. (4), activating Nrf2 itself may prove to be a better strategy, particularly in light of the difficulties with COX-2 inhibitors. A number of chemopreventive food factors including curcumin/turmeric, isothiocyanates/sulforaphane/broccoli, dithiolethiones, and triterpenoids have been shown to stimulate Nrf2 expression (9). Recent studies indicate that not only is sensitivity to chemical carcinogenesis increased but also the chemopreventive efficacy of these food factors is lost in Nrf2-deficient mice (16).

Does Nrf2 indirectly suppress transcription of COX-2 and 5-LOX? Cross talk between COX-2, Nrf2, and apoptosis pathways is beginning to be revealed (17). What about inducible nitric oxide synthase, is it also associated with inflammation and reactive oxygen and nitrogen stress? How does deficiency of Nrf2 work to increase expression of these genes? When Nrf2 directly activates transcription of NAD(P)H:quinone oxidoreductase 1 and UDP-glucuronosyl transferase 1A, do these enzymes suppress steps upstream or downstream of redox-sensitive transcription factors AP-1 or NF-κB or others implicated in inflammation-associated carcinogenesis such as STAT3/STAT5 or β-catenin? It would be interesting to measure levels of activated AP-1 and NF-κB in the azoxymethane-dextran sodium sulfate–treated colon and tumors as a function of Nrf2 deficiency.

It would be interesting to know just how early in the carcinogenesis process the altered Nrf2-dependent gene expression is seen. Can Nrf2-regulated antioxidant genes be implicated in blocking early stages before colon polyp (adenoma) formation? Blocking dextran sodium sulfate–induced aberrant crypt

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foci was reported, but there is some uncertainty about whether aberrant crypt foci constitute an obligatory pre-polyp stage. If Nrf2 is important for early-stage colon carcinogenesis, this would suggest that it might serve not only as a molecular target but also as a useful biomarker for identifying individuals likely to respond to a Nrf2-targeted intervention to prevent polyp recurrence.

In conclusion, the findings of Khor et al. (4) and others—increased carcinogen-induced colon tumor yield and decreased downstream protective gene expression in Nrf2-null mice—implicate Nrf2 as a promising molecular target for cancer prevention. Targeting tumor suppressors such as Nrf2, however, presents challenges that are quite different from those involved in targeting an oncogenic transcription factor such as AP-1. It seems to be more difficult to up-regulate a beneficial target than it is to suppress a harmful target. Oncogenic factors frequently can be targeted directly (18), whereas tumor suppressors require indirect targeting or stabilization.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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