

Cytoprotection “gone astray”: Nrf2 and its role in cancer

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Abstract: Nrf2 has gained great attention with respect to its pivotal role in cell and tissue protection. Primarily defending cells against metabolic, xenobiotic and oxidative stress, Nrf2 is essential for maintaining tissue integrity. Owing to these functions, Nrf2 is regarded as a promising drug target in the chemoprevention of diseases, including cancer. However, much evidence has accumulated that the beneficial role of Nrf2 in cancer prevention essentially depends on the tight control of its activity. In fact, the deregulation of Nrf2 is a critical determinant in oncogenesis and found in many types of cancer. Therefore, amplified Nrf2 activity has profound effects on the phenotype of tumor cells, including radio/chemoresistance, apoptosis protection, invasiveness, antisenescence, autophagy deficiency, and angiogenicity. The deregulation of Nrf2 can result from various epigenetic and genetic alterations directly affecting Nrf2 control or from the complex interplay of Nrf2 with numerous oncogenic signaling pathways. Additionally, alterations of the cellular environment, eg, during inflammation, contribute to Nrf2 deregulation and its persistent activation. Therefore, the status of Nrf2 as anti- or protumorigenic is defined by many different modalities. A better understanding of these modalities is essential for the safe use of Nrf2 as an activation target for chemoprevention on the one hand and as an inhibition target in cancer therapy on the other. The present review mainly addresses the conditions that promote the oncogenic function of Nrf2 and the resulting consequences providing the rationale for using Nrf2 as a target structure in cancer therapy.

Keywords: xenobiotic and oxidative stress, tumorigenesis, cancer therapy, transcription factor, carcinogen

Introduction

The integrity and function of mammalian cells is under the tight control of numerous homeostatic signaling pathways that provide protection against endogenous and environmental hazards. However, the deregulation and amplification of these pathways can cause devastating diseases, as particularly seen in cancer, where cytoprotective mechanisms are often inadequately engaged, giving rise to highly stress-tolerant cells that escape homeostatic control.

One prominent example is the oxidative and xenobiotic stress-response pathway, under the control of Nrf2, which has an essential role in cytoprotection.¹⁻⁴ Based on this role, in particular by preventing deoxyribonucleic acid (DNA) damage and mutagenic events, Nrf2 was regarded for a long time as a tumor inhibitor, thus providing the rationale of tumor-prevention strategies using Nrf2 activators.⁵⁻⁸ However, this view had to be modified when it was recognized that deregulated Nrf2 activity can also favor a malignant phenotype and promote carcinogenesis.^{6,9-12} Ample evidence

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has accumulated that Nrf2 either acts itself as a proto-oncogene or that Nrf2 supports the transforming potential of other proto-oncogenes. For example, the Nrf2-dependent upregulation of antioxidative and antiapoptotic pathways essentially contributes to the forced proliferation and survival of kRas-transformed cells.¹³ Other Nrf2 effects that add to a malignant phenotype include an altered metabolism and an enhanced regulated protein turnover by the ubiquitin–proteasome system.

Therefore, under homeostatic conditions, Nrf2-dependent cytoprotective effects avoid excessive cellular damage and dysfunction, but when they lose their tight control, these effects substantially add to the malignant phenotype of cancer cells. Accordingly, enhanced Nrf2 activity has been found in a great number of solid and hematologic tumors resulting either from mutational events, epigenetic effects, or constitutive stress adaptation. This condition of deregulated Nrf2 provides several growth advantages to cancer cells, including antisenescence, proliferation, and antiapoptosis, as well as a profound resistance to drugs and radiotherapy. Therefore, Nrf2 has become of great interest with regard to its use in therapy and the diagnosis of malignant diseases.

The present review primarily discusses the most recent aspects regarding the role of Nrf2 in the initiation and development of cancer. Other findings beyond this issue have been the subject of many excellent reviews on the dual role of Nrf2 in tumorigenesis, published recently.^{5–12}

Oxidative and xenobiotic stress

During evolution, cells became exposed to an oxygen-enriched (aerobic) atmosphere, and thereby acquired essential growth advantages. In particular, an oxidative metabolism along with a maximum of energy yield has favored the development of complex (higher) organisms, but many hazards also arose from oxygen. Deriving from molecular oxygen, various reactive molecules and free radicals (eg, superoxide, hydrogen peroxide, hydroxyl radical, hydroxyl ion, and nitric oxide) can form, which are collectively designated as reactive oxygen species (ROS).^{14,15} Besides environmental conditions, eg, heat and irradiation, ROS are also produced biologically in aerobic cells. In these cells, most ROS are generated as by-products during mitochondrial electron transport. ROS are also produced as intermediates of enzymatic oxidation reactions, having an important role as intra- and intercellular messengers.¹⁶ In addition, ROS – mainly released by phagocytes – are

effective in the cellular defense against microbes, thus having a pivotal role in innate immunity.¹⁷

Owing to their deleterious effects on cell components (lipids, proteins, and DNA) ROS need to be efficiently neutralized in aerobic cells, and a balance between generation and removal of ROS is an essential prerequisite for cellular survival. This balance is maintained by a number of tightly regulated defense mechanisms, a failure of which leads to a condition termed oxidative stress.¹⁸ If left uncompensated, oxidative stress impacts on cellular and tissue integrity, particularly through accumulating DNA damage, giving rise to severe pathologies,¹⁹ including cancer.^{20,21} Representing the major protection system in higher organisms, Nrf2 drives ROS detoxification^{22,23} through inducing expression of many antioxidative enzymes, such as glutamate–cysteine ligase catalytic/modifier subunit (GCLC/M) and glutathione *S*-reductase for glutathione synthesis and recovery, heme oxygenase (HO)-1, peroxiredoxin-1/4, superoxide dismutases, thioredoxin or thioredoxin reductase 1, and aldo-keto reductases (AKRs).^{24,25}

Another environmental hazard for cells emerges from xenobiotics, collectively designating natural or synthetic compounds foreign to the organism's metabolism and biochemistry.²⁶ Xenobiotics include drugs, food additives, environmental pollutants, and fungal or microbial toxins making up hundreds of thousands of compounds affecting cellular integrity,²⁷ and the exposure to these foreign compounds is known as xenobiotic stress. To cope with this stress condition, all major groups of organisms possess particular metabolic pathways to detoxify potentially hazardous compounds.²⁶ However, intermediates of xenobiotic metabolism may become toxic as well, and sometimes give rise to ROS formation.²⁸

Detoxication of these xenobiotic compounds includes three phases.²⁹ In phase I, xenobiotics are oxidized by enzymes like nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1 (NQO1), reduced by enzymes like alcohol dehydrogenase, aldehyde dehydrogenases (ALDHs) and AKRs or hydroxylated by cytochrome P450 monooxygenases.³⁰ In phase II, the phase I products are conjugated to hydrophilic compounds, such as glucuronic acid (by uridine 5'-diphospho-glucuronosyltransferases [UGTs]), sulfate (by sulfotransferases), or glutathione (by glutathione *S*-transferases [GSTs]), thereby generating water-soluble products that can be easily eliminated from the body.³¹ In phase III, these products are exported from cells by ABC/ MRP or ABC/MDR transporters. Notably, many phase I, II, and III enzymes are target genes of Nrf2.³² These include

NQO1, AKR1B1, ALDH3A1, GSTA1, GSTN1, UGT1A1, ABCB6, or ABCC15.^{24,25,32,33}

The CNC-bZIP protein family and control of Nrf2 signaling

Together with two closely related proteins – Nrf1 and Nrf3 – as well as with NF-E2 and Bach-1, and Bach-2, Nrf2 belongs to a family of transcription factors termed CNC-bZIP proteins.³⁴ Quite unique to Nrf2 is the Neh2 domain near to the amino-terminal end, which serves as interaction domain with the Nrf2 inhibitory protein Keap1.³⁵ This interaction depends on the low-affinity binding of a DLG and the high-affinity binding of an ETGE motif. Under homeostatic conditions, Nrf2 is kept at low levels when bound via DLG and ETGE to a homodimer of Keap1 through the DC domain of either one of the Keap1 subunits, leading to Cullin3/Rbx1-catalyzed polyubiquitination and subsequent proteasomal degradation of Nrf2 (Figure 1A).^{36,37} During conditions of oxidative/electrophilic or xenobiotic stress, the Cullin3/Rbx1-dependent polyubiquitination of Nrf2 under the assistance of Keap1 is blocked, thereby leading to the accumulation of Nrf2 protein and its subsequent translocation to the nucleus (Figure 1B).³⁶ The derepression of Nrf2 in this way depends on the dissociation of the Nrf2 polyubiquitination site from Cullin3/Rbx1 without releasing Nrf2 from the protein complex (according to the hinge-latch model, in which the ETGE motif remains bound to Keap1), hence saturating Keap1 and competing with free Nrf2 that instead can enter the nucleus (Figure 2).^{38,39} An alternative mode of induction occurs through the release of Cullin3 from Keap1, thereby directly impeding the polyubiquitination of Nrf2.⁴⁰ During Nrf2 activation, Keap1 itself can be Lys63 polyubiquitinated,⁴¹ which does not target the protein to the proteasome (in contrast to the Lys48 polyubiquitination of Nrf2), but instead seems to destabilize its Cullin3 interaction. Recently, the ubiquitin-specific protease-15 deubiquitinase was identified to deubiquitinate Keap1,⁴² thereby restoring Cullin3 recruitment and accordingly Nrf2 suppression. This ubiquitination/deubiquitination of Keap1 may function as an additional Nrf2-regulatory mechanism.

Along with its release from Keap1-dependent suppression, Nrf2 undergoes certain posttranslational modifications⁴³ that support its nuclear translocation and binding to the antioxidant response element (ARE)/electrophile response element (EpRE) serving as a recognition motif in the target gene promoters: 1) PKC-dependent phosphorylation on Ser-40,⁴⁴ 2) phosphorylation through the MAPK/PERK

signaling pathway in response to endoplasmic reticulum/unfolded protein stress⁴⁵ or by casein kinase 2,⁴⁶ and 3) CBP/p300- or hMOF (males absent on the first)-mediated acetylation of lysyl residues within the Neh1 domain of Nrf2,^{47,48} facilitating binding to the ARE/EpRE sites.⁴⁹ The activation of Nrf2 is further promoted by additional signal transduction pathways, eg, ERK, JNK, or PI3K/Akt.^{38,50,51} Negative control of Nrf2 is exerted by (for example) p38/MAPK, GSK3B, or PTEN.^{52–54}

For DNA binding, Nrf2 further needs to heterodimerize with other bZIP proteins, eg, with c-Jun or with one of seven Maf proteins.^{55,56} These proteins – in particular the three small Maf proteins Maf-F, -G, and -K (themselves lacking transactivation activity) – associate with extended ARE/EpRE motifs at the promoters, and are essential for binding of Nrf2 to the core ARE/EpRE motif and thereby for Nrf2-dependent transactivation.⁵⁷

The interaction of Nrf2 with Maf proteins is also under the control of several signal-transduction pathways, eg, ERK, JNK, p38/MAPK, and PI3K/Akt,^{58–60} and requires additional cofactors, eg, JDP2.⁶¹ In contrast to Maf proteins, the two proteins⁶² Bach-1 and Bach-2 act rather as transcriptional repressors of Nrf2.^{63–65}

The major principle of Nrf2 activation is the redox-dependent alteration of the Keap1 conformation conferred by the sulfhydryl groups of more than 20 intrinsic cysteine residues. ROS and electrophilic compounds lead to disulfide formation and cysteine conjugates, respectively, similarly affecting the function of Keap1 as an E3-ligase receptor for Nrf2.^{66–68} There is a restriction of cysteine residues within the Keap1 protein, eg, Cys251, Cys273, and Cys288, with respect to the access of electrophilic compounds defining the activity of Nrf2-inducing substances like *tert*-butylhydroquinone (tBHQ), diethyl maleate, sulforaphane, 15-deoxy- Δ 12,14-prostaglandin J2, 4-hydroxynonena, bardoxolone methyl (CDDO), curcumin, resveratrol, and many more.^{69,70} Meanwhile, hundreds of natural product-derived compounds have been established as inducers of the Nrf2/ARE pathway, including polyphenols, quinones, organosulfur compounds, polyenes, flavonoids, chalcones, and terpenoids. These agents have been successfully tested in experimental prevention studies with inflammatory bowel and neurodegenerative diseases, diabetes, and metabolic disorders, as well as lung, heart, and kidney diseases.⁷¹ All these effectors of the Nrf2/ARE pathway are summarized in a comprehensive review by Kumar et al.⁷²

Representing an alternative mechanism of Nrf2 control, a ternary complex of Keap1 and Nrf2 is tethered to the outer

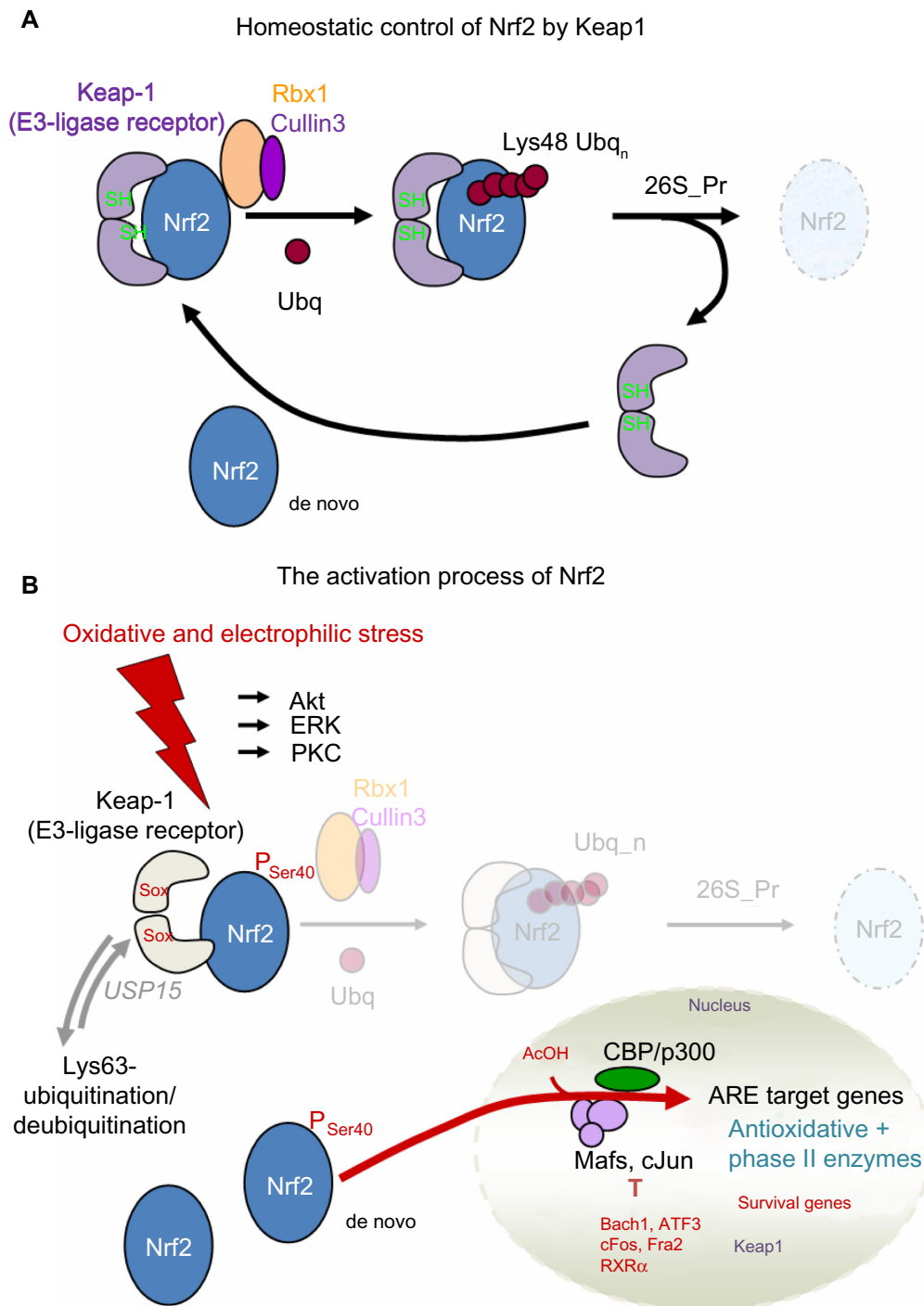


Figure 1 (A and B) Principle of controlling Nrf2 by Keap1 and its activation.

Notes: (A) Under homeostatic conditions, Nrf2 is kept at low levels through its Keap1-dependent targeting to Cullin3/Rbx1-mediated Lys-48 polyubiquitination and subsequent proteasomal degradation. (B) During exposure to stress activators, the interaction between Nrf2 and Keap1 is weakened, thereby impairing Nrf2 polyubiquitination. While free Nrf2 then can enter the nucleus and drive antioxidant response element (ARE)-dependent gene expression along with certain modifications (eg. Ser-40 phosphorylation, acetylation), Keap-1 can be subject to Lys-63 polyubiquitination that leads to the release of Nrf2. Ubiquitin-specific protease (USP)-15 deubiquitinase reverses Keap1 polyubiquitination.

mitochondrial membrane through PGAM5,⁷³ a member of the phosphoglycerate mutase family. Thereby, PGAM5 builds up a molecular framework that provides a direct link between mitochondrial functions and Nrf2-dependent antioxidant gene expression. Intriguingly, Keap1 has been reported

to interfere also with the Bcl-xL apoptosis inhibitor in a PGAM5-dependent fashion.⁷⁴ Another condition of Keap1 deactivation is its interaction with phospho-p62 accumulating during autophagy deficiency.⁷⁵ Consequently, Keap1 fails to control Nrf2 stabilization if bound to phospho-p62,

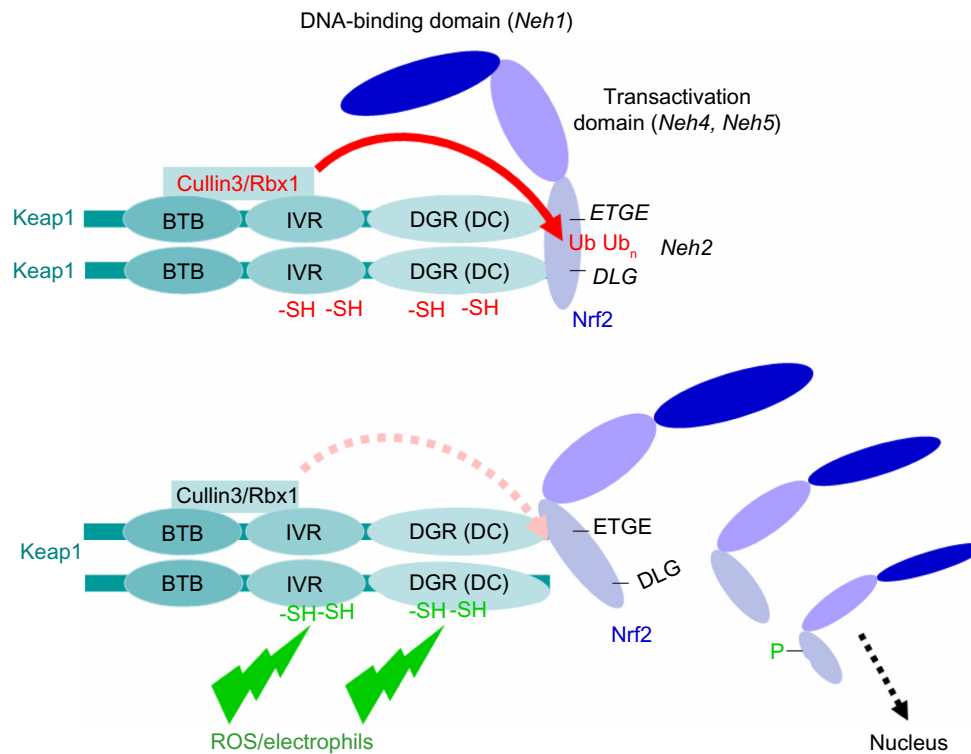


Figure 2 Interaction of Nrf2 and Keap1 according to the hinge-latch model.

Notes: Nrf2 tightly binds to homodimeric Keap1 through interaction of the ETGE and DLG motifs within the Neh2 domain and either one of the double-glycine repeat (DGR) sites of Keap1. This configuration facilitates polyubiquitination of Nrf2 by Cullin3/Rbx1. Upon sulfhydryl modifications of certain cysteine residues of Keap1, the DLG motif of Nrf2 is released from the interaction with the DGR site. This blocks polyubiquitination of Nrf2, which however remains bound to the Keap1 dimer through the ETGE/DGR interaction. Thereby, Keap1 is saturated with Nrf2 leaving yet-unbound Nrf2 free and capable of entering the nucleus.

Abbreviations: DNA, deoxyribonucleic acid; BTB, bric-a-brac; IVR, intervening region.

and autophagy-deficient cells show up with enhanced Nrf2 activation.⁷⁶ A similar disrupting effect on Keap1-mediated control of Nrf2 has been reported for the cyclin inhibitor p21/Waf1.⁷⁷ Therefore, the prosurvival effects described for p21 may at least partially rely on increased Nrf2 activation along with antioxidative protection. Besides Keap1, the E3 ligase receptor and F-box protein β TrCP is able to control the stability of the Nrf2 protein when phosphorylated in the Neh6 domain by GSK3B.^{78,79}

The tumor suppressor p53 is also capable of preventing Nrf2 activation,⁸⁰ an effect involving destabilization of the Nrf2 protein and regarded as another mode of action by which p53 controls apoptosis.⁸¹ This is restricted to cytotoxic stress conditions, because p53 also positively affects Nrf2, eg, through its target gene *p21/Waf1*. Hence, the modulation of Nrf2 by p53 is exerted in two ways, differentially affecting basal and stress-induced Nrf2 activity. Moreover, certain mutations of p53 even result in decreased Nrf2 activation and thereby in the amplification of oxidative stress,⁸² pointing to the complexity of the cross talk between p53 and Nrf2.⁸¹

Furthermore, negative regulation of Nrf2 is exerted by the GSK3B/Src-Fyn tyrosine-kinase pathway.^{54,83} Upon

Fyn-dependent phosphorylation, Nrf2 is redirected to the cytoplasm, where it is bound to Keap1, itself being a target gene of Nrf2.⁸⁴ Nrf2 activation is also impaired by the NF- κ B subunit p65/RelA, which coimports Keap1 into the nucleus,⁸⁵ and by E-cadherin, which blocks nuclear translocation of Nrf2 in a β -catenin-dependent fashion.⁸⁶ Other pathways negatively interfering with Nrf2 include those initiated by TFG β 1⁸⁷ through ATF3,⁸⁸ by nuclear hormone receptors, eg, estrogen receptor alpha (ER α), estrogen related receptor beta (ERR β), glucocorticoid receptor (GR),⁸⁹ and under certain conditions also retinoid receptors (RXR α /RAR α).⁹⁰⁻⁹³ Since the complex regulation of Nrf2 activation and the structural basis thereof have been the subject of numerous excellent reviews found in the recent literature,^{9-11,94,95} this issue is not addressed in greater detail here.

The antitumorigenic role of Nrf2

Before the protumorigenic effects exerted by Nrf2 are outlined in detail, some key aspects of its tumor-inhibiting potential should be briefly highlighted here. Over the years, a plethora of studies demonstrated that Nrf2 exerts tumor-preventive activity through its cytoprotective effects. These

protect cells from DNA damage and the deterioration of proteins, carbohydrates, and lipids if exposed to oxidative and xenobiotic stress.^{5,7,8} Accordingly, an impaired function of Nrf2 accounts for many stress-induced pathological disorders,⁹⁶ which could be most impressively seen in per-se viable Nrf2-knockout mice.⁹⁷ For instance, greater tissue damage in the lung, kidney, brain, liver, eye, or heart was seen in Nrf2-knockout mice subjected to acute insults like cigarette smoke, hyperoxia, ischemic reperfusion, portal vein embolization, and chemical toxins or when being subject of aging.^{96,98–102} Similarly, mice treated with the mutagen azoxymethane and/or the colitis-inducing chaotropic agent dextran sulfate sodium became much more sensitive to colonic inflammation and colon cancer formation if the Nrf2 gene had been ablated.^{103–105} Likewise, nitrosamine-induced carcinogenesis in the bladder, ultraviolet-induced skin carcinogenesis, or aflatoxin-induced formation of human hepatocellular carcinomas (HCCs) was greatly enhanced in Nrf2-deficient mice when compared with Nrf2-proficient mice.^{106–110} Accordingly, the cancer-preventive effects of Nrf2-inducing agents like oltipraz or CDDO-imidazole were abrogated in such mouse models if Nrf2-dependent gene expression had been shut down.^{111,112} Other chemoprevention strategies, eg, by curcumin or sulforaphane, were also affected by Nrf2 deficiency in mouse models for such organ sites as the lung, kidney, breast, liver, small intestine, stomach, and skin.^{96,111,113–116} For instance, development of colon tumors in APC^{Min} mice was efficiently prevented by sulforaphane, but this effect was abrogated in Nrf2-deficient mice. Moreover, mice expressing an aberrant APC protein were per se more sensitive to colon-tumor formation if the Nrf2 gene had been ablated.¹¹⁷ These observations strongly indicate that Nrf2 also protects from deleterious effects initiated by certain genetic alterations, eg, of the *APC* gene, presumably through the inhibition of further, ROS-dependent genetic lesions and by interfering with metabolic alterations that could favor tumorigenesis. Under certain conditions, Nrf2 can suppress metastasis formation, eg, in pulmonary cancer in mice, which was shown to be related to control of the redox balance in inflammatory cells, including myeloid-derived suppressor cells.¹¹⁸

Additional evidence for the view that Nrf2 exerts antitumor effects, eg, in hormone-sensitive tumors, was provided by the observation that Nrf2 interferes with nuclear hormone receptors, eg, in prostate cancer. A recent study showed that Nrf2 and the related CNC-bZIP protein Nrf1 differentially modulate AR transactivation in prostate cancer.¹¹⁹ While an N-terminally truncated variant of Nrf1 (p65-Nrf1) promoted

the expression of androgen-regulated genes and thereby facilitated recurrent growth of castration-resistant prostate cancer cells in the presence of residual trace amounts of androgens, Nrf2 inhibited this effect of Nrf1. Besides a role as transdominant repressor of Nrf2,¹²⁰ p65-Nrf1 is obviously able directly to form a transactivation complex with the AR, and Nrf2 dampens the formation of p65-Nrf1.¹¹⁹

Altogether, there seems to be no doubt that Nrf2 can efficiently protect from cancer initiation/development, mainly by mitigating genotoxic insults that emerge from exposure to carcinogens and extrinsically or intrinsically generated ROS. This issue has been addressed by numerous, sometimes enthusiastic, articles and reviews published recently.^{7,121–123} Indeed, as long as the cytoprotective action of Nrf2 is kept under tight control, it could serve as a target in cancer prevention. Based on this idea, many efforts have been undertaken to establish conditions of increased Nrf2 activity and thereby to avoid ROS or carcinogen-induced tissue damage and hence pathologies like cancer. In fact, most of these studies were first carried out in cell-culture settings and then in animal studies – mainly in Nrf2-proficient versus -deficient mice – by using established disease models. It was therefore not surprising that as long as the investigators made use of timely Nrf2 activation close to the damaging and disease-causing insult, a favorable response was always quite well (and mostly) affected by Nrf2 knockout. This included not only cancer but also other severe pathologies, such as diabetes, obesity, pulmonary and cardiovascular diseases, or neurodegenerative disorders.^{29,70,124–128}

Considering Nrf2 in this way as an inducible and cancer-preventive target, several population-based clinical trials have been conducted or are under way, particularly in the People's Republic of China.¹²⁹ For example, CDDO, sulforaphane, and oltipraz applications have been shown to be well tolerated in humans, resulting in elevated levels of cytoprotective enzymes.^{130,131} Moreover, aflatoxin intoxication as a risk factor for HCC was decreased by oltipraz during a 12-week study with a high-risk Chinese population.¹³² Other trials, however, eg, with CDDO or bardoxolone, have been unsuccessful or resulted in severe side effects.¹³³ Even though most of these trials still await evaluation, the rather positive view regarding Nrf2-based prevention strategies has begun to change. Since the oncogenic activity of Nrf2 has been clearly demonstrated, concerns regarding the safe use of Nrf2 activators, eg, in long-term administration, are increasing. Therefore, more efforts are needed to better understand the modalities by which Nrf2 can be used as a therapeutic target. For example, the use of the recently established conditional

Nrf2-knockout mouse,¹³⁴ allowing ablation of the gene in a cell/tissue-specific and even better in a temporarily controlled fashion (if crossed with the appropriate CreER[T] system), would bring detailed insights into the dual role of Nrf2 in carcinogenesis and by what cellular context this dualism is defined.

The protumorigenic role of Nrf2

Molecular mechanisms of Nrf2 amplification in cancer

It is well established that a great number of tumors frequently exhibit enhanced Nrf2 activity, which may essentially contribute to a malignant phenotype. Nrf2 amplifications range from a frequency of 20%–25% in some tumor types up to 80%–95% in lung, breast, ovarian, endometrial, pancreatic, colorectal, or prostate cancer.^{6,9–11,95,135,136} This amplified Nrf2 activation manifests in increased nuclear accumulation of Nrf2,¹³⁷ and even more in its Ser-40 phosphorylated form,¹³⁸ and by greater expression levels of phase II and antioxidative enzymes, as well as a wide range of other Nrf2-regulated genes. Besides a few exceptions, such as acute myeloid leukemia and myelodysplastic syndrome,¹³⁹ the presence of activated Nrf2 in tumors generally correlates with a poor clinical outcome, as reported for (for example) pancreatic, cervical,

or lung cancer, and indicates poor responsiveness to chemo- and/or radiotherapy. However, the exact modalities by which Nrf2 is deregulated and thereby becomes protumorigenic are quite complex and only partially understood.^{9,10}

By conferring protection from oxidative stress or carcinogen-induced damage of cellular components – in particular, DNA, thereby avoiding mutational events – Nrf2 primarily acts in a tumor-preventive fashion. However, Nrf2 may become protumorigenic if persistently activated. This can result from certain genetic and epigenetic alterations affecting the Nrf2/Keap1 pathway, from other oncogenic pathways, or from long-term exposure of epithelial cells to persistent oxidative stress. For example, the latter occurs during chronic inflammation (Figure 3), and represents a condition favoring the early onset of malignant transformation. In fact, precursor lesions of tumors already exhibit elevated Nrf2 activities with considerable frequency,^{140–143} presumably reflecting a critical stage when Nrf2 is part of the cellular stress adaptation and exerts pro- as well as antitumorigenic effects. At that point, additional events could then direct Nrf2 to act as an oncogene.

It is well established that certain somatic genetic alterations affecting the *KEAP1* gene (frequencies of up to 30% in gallbladder, ovarian, or lung cancer) or the Nrf2 gene itself

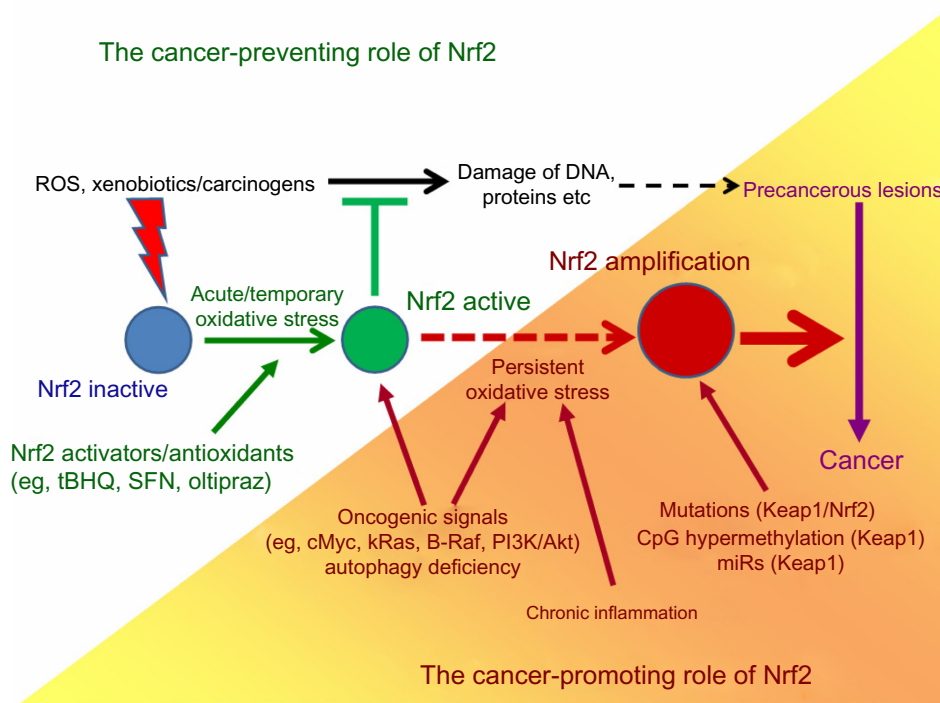


Figure 3 The dual role of Nrf2 in cancer.

Notes: As long as it is kept under homeostatic control, Nrf2 activation protects cellular components like DNA from damaging insults arising from acute/temporary oxidative and xenobiotic stress. In this way, Nrf2 prevents tumor development. When deregulated by 1) epigenetic and genetic alterations affecting the Keap1–Nrf2 pathway, 2) persistent stress conditions, and/or 3) oncogenic pathways, Nrf2 activation facilitates the growth and survival of transformed cells, thus promoting tumorigenesis.

Abbreviations: ROS, reactive oxygen species; DNA, deoxyribonucleic acid; tBHQ, *tert*-butylhydroquinone; SFN, sulforaphane.

(less frequent) directly impact on Nrf2 signaling.^{95,135,144,145} The most common genetic alterations affect Keap1 in its DC domain, and here mainly within the DLG motif, thereby disrupting Nrf2 binding. In lung cancer, these mutations are found with the greatest frequency. Other tumor entities exhibit mutations in the intervening region (IVR) domain (eg, in prostate cancer) and sometimes in the bric-a-brac (BTB) domain of the *KEAP1* gene (eg, in liver and ovary cancer). These mutations impair the interaction of Keap1 with Cullin3 and its homodimerization, thereby affecting polyubiquitination and binding of Nrf2, respectively.^{95,135} Many of the mutations affect the reactive cysteine residues residing in the IVR and DC domains of Keap1, while others affect additional amino acids, eg, serine at position 104 in the BTB domain, essential for Keap1 homodimerization. Another set of cancer-derived mutations that have been identified include those of glycine (positions 186, 423), arginine (positions 320 or 470), and other amino acids within the BTB, IVR, and DC domains. As shown for lung cancer, these mutations do not impair polyubiquitination of Nrf2, but instead suppress its degradation.¹⁴⁶ The functional significance and underlying mechanisms of these so-called superbinder mutations still need to be elucidated.

In contrast to the rather widespread mutations in the *KEAP1* gene, gain-of-function-like mutations in the Nrf2 gene are restricted to the DLG and ETGE motifs within the Neh2 domain.¹⁴⁷ These mutations are found, eg, in skin, larynx, lung, and squamous esophageal cancers,^{148,149} but overall are much less frequently found in tumors than *KEAP1* mutations.

Moreover, a number of epigenetic alterations affecting Keap1 expression have been described that can lead to disinhibition of Nrf2 in cancer cells as well.^{95,135,144} In several tumor entities, disruption of Keap1 expression due to CpG DNA hypermethylation has been reported. These modifications are found in 51% of breast, 20% of colorectal, and 12% of lung cancers, accounting for decreased levels of Keap1 and conversely enhanced Nrf2 activation.^{94,150} Accordingly, 5'-aza-2'-deoxycytidine (5-Aza-dC) treatment could restore Keap1 expression and the control of Nrf2 activity in a number of tumor cell lines.^{151,152} Interestingly, a recent study revealed that Keap1-promoter hypermethylation is more frequent in preinvasive breast neoplasms than in the breast cancers.¹⁴¹ Furthermore, Nrf2 was identified as a methylation target. During the development of prostate cancer in TRAMP (transgenic adenocarcinoma of the mouse prostate) mice, a modified expression of Nrf2 based on hypermethylation was demonstrated,¹⁵³ and treatment with 5-Aza-dC restores the expression of Nrf2 and its target genes. Another study showed

that curcumin, a DNA hypomethylating agent, reverses the methylation status of the Nrf2 promoter and leads to reexpression of Nrf2/NQO1.¹⁵⁴ A DNA methylation-dependent alteration of the inhibitory feedback loop between the integrin adaptor protein p66Shc and Nrf2 has been demonstrated in lung cancer, accounting for tumoral Nrf2 upregulation.¹⁵⁵

Also a number of micro-ribonucleic acids (miRNAs) have been reported to attenuate Keap1 expression. miR141 and miR200a, both member of the miR200 family, suppress Keap1 and are overexpressed in ovarian and breast cancers, respectively.^{156,157} Downregulation of Nrf2-suppressing miRNAs has been described in cancer cells as well. Recent studies identified decreased targeting of Nrf2 by miR28 in breast cancer,¹⁵⁸ and four miRNAs are downregulated in esophageal squamous cell carcinomas (miR-507, -634, -450a, and -129-5p),¹⁵⁹ the reexpression of which negatively regulate Nrf2 and its oncogenic pathways.

Besides these genetic and epigenetic effects that have been mainly found in solid-tumor entities, metabolic conditions marked by fumarate hydratase deficiency lead to cysteine succinylation of Keap1, hampering its Nrf2 suppressive activity.^{160,161} Nrf2 activation may be enhanced also in hematologic tumors and/or deregulated in solid tumors through its cross talk with different tumor-associated pathways and proto-oncogenes. This is outlined in the next section.

Cross talk of Nrf2 with oncogenic and tumor-suppressor pathways

Numerous signaling pathways have been shown to cooperate intimately with Nrf2. These pathways are either oncogenic per se and assisted by Nrf2 to induce a transformed phenotype (eg, the kRas or PI3K/Akt pathway), or are Nrf2-dependently modulated to favor malignant transformation (eg, the ubiquitin/proteasome pathway, metabolic pathways). Therefore, inhibition of Nrf2 would either dampen the oncogenic potential of certain pathways or even switch off cellular functions that are essential for cancer cell survival. Moreover, tumor-suppressor pathways can positively and negatively cooperate with Nrf2 (eg, p53), depending on the duration of oxidative and genotoxic stress. Following are several pathways that have been reported to be part of the oncogenic activity of Nrf2.

Nrf2 and kRas, BRaf, and cMyc oncogenes

A number of studies reported a close association of kRas transformation and an increased activity of Nrf2 along with accelerated cell growth and increased cellular survival. In a pancreatic cancer mouse model,¹³ it was shown that oncogenic kRas activates expression of a series of antioxidant

and metabolic genes via Nrf2 that are collectively used as the reducing power for ROS detoxification.¹⁶² During kRas-dependent lung carcinogenesis in mice, Nrf2 seems to have two roles: a preventive one during tumor initiation and a promoting one during tumor progression and metastasis formation.^{163,164} In non-small-cell lung cancer (NSCLC), Nrf2 has been shown to be a downstream mediator of both EGFR and Ras signaling, making Nrf2 an important molecular target for the treatment of NSCLC with mutations in the *EGFR* and *KRAS* genes.¹⁶⁵ BRAf mutations or mutated H-Ras autonomously driving activation of the ERK pathway similarly enhance Nrf2-dependent cytoprotection.^{13,166} The involvement of Nrf2 in oncogene-dependent pathways confers protection from ROS and provides reducing power for biosynthesis in proliferating cells. Interestingly and in striking contrast, Nrf2-dependent oxidative stress response is suppressed in H-Ras-transformed mesenchymal stem cells and breast cancer cells through the Ras/Raf/Erk pathway, thus indicating that oncogenic Ras and Nrf2 may also negatively cooperate in certain types of tumors.¹⁶⁷

Like kRas, the cMyc oncogene has been linked to Nrf2-dependent cytoprotection. In a transgenic mouse model, cMyc has been shown to directly increase Nrf2 gene expression,¹³ and a recent study in *Drosophila* revealed Myc-dependent activation of Nrf2 through an increased expression and phosphorylation of p62 as well as activation of PERK.¹⁶⁸ In contrast, cMyc has been reported to interfere negatively with Nrf2 transactivation, causing suppression of phase II enzyme expression.¹⁶⁹

Nrf2 and the PI3K/Akt pathway, PTEN, and mTOR

Since activation of Nrf2 is linked to the PI3K/Akt pathway there is a clear association in cancers that frequently are subject to amplification of PI3K/Akt. By inducing cell proliferation, apoptosis, migration, and differentiation, the PI3K/Akt signaling pathway is a major determinant in cancer development and malignant progression.¹⁷⁰ In multiple studies with cancer and/or epithelial cell lines, it was shown that Nrf2-mediated defense mechanisms against intracellular ROS are induced by the activated PI3K/Akt signaling pathway.^{171–173} Accordingly, functional loss of PTEN, an event occurring frequently in cancer, can lead to enhanced Nrf2 activation along with greater cytoprotection.^{53,174} Moreover, PTEN deficiency might affect the GSK3 β -dependent regulation of Fyn¹⁷⁵ or the recruitment of Nrf2 to β TrCP,¹⁷⁶ thereby preventing Keap1-independent control of Nrf2 activation.

Beyond that, Nrf2 acts not only downstream of Akt but also synergizes with Akt for providing growth advantages

to tumor cells. In an experimental study with breast cancer cells, the transcriptional coactivator AIB1 was able to activate Akt and Nrf2 concurrently. Activated AIB1 results in an enhanced oncogenic effect of Akt through an upregulation of antiapoptotic Bcl-2 as well as an Nrf2-mediated increase of antioxidative enzymes, both leading to enhanced growth and chemoresistance.¹⁷⁷

Besides the reports on mTOR controlling Nrf2 activation, eg, through modulation of the Keap1 sequestration by the autophagy protein p62/SQSTM1,¹⁷⁸ an upregulation of the mTOR pathway in response to an amplified Nrf2 activation has been demonstrated. As one underlying mechanism, an indirect induction of the small G-protein RagD by activated Nrf2 has been proposed.¹⁷⁹ Consequently, tumors exhibiting enhanced Nrf2 activity may serve as an indicator for sensitivity against mTOR inhibitors.

Nrf2 and estrogen/ER

There are different pathways by which estrogen (E2) contributes to cancer development. Besides DNA–E2 adduct formation leading to DNA mutations that are critical for cancer initiation, it is known that oxidative estrogen metabolites generate ROS,¹⁸⁰ and the induction of Nrf2-dependent detoxifying enzymes, eg, NQO1, is regarded as an important mechanism to remove these toxic agents.⁹¹ Through ER α and ER β , estrogen differentially affects Nrf2 activation, and hence it has been shown that estrogen inhibits Nrf2 activity, resulting in augmented genotoxicity. This inhibition can result from physical interactions between ER α and Nrf2,¹⁸¹ from competitive binding of ER α and the histone deacetylase SIRT1 to the promoter of Nrf2-inducible genes like *NQO1*,⁹¹ or from interference with p300 recruitment leading to histone acetylation of Nrf2 target genes.¹⁸² Moreover, estrogen-dependent decrease of miR-200a in breast cancer cells has been reported, resulting in increased cytoplasmic Keap1 levels and consequently in reduced Nrf2 expression.¹⁵⁶ Likewise, modulation of the miR-200 family by ERR α ¹⁸³ may affect the Keap/Nrf2 pathway.

In contrast to ER α -mediated Nrf2 repression, ER β -dependent Nrf2 induction has been reported. Equol, a specific activator of ER β , leads to an induction of the PI3K/Akt pathway in endothelial cells, which in turn causes a translocation of activated Nrf2 into the nucleus.¹⁸⁴ Similar results were published for breast epithelial cells, and estrogen has been recently shown to induce Nrf2 activity in BRCA1-deficient breast cancer cells through activating the PI3K/Akt pathway.¹⁸⁵ Moreover, estrogen-bound ER β forms a complex with Nrf2, and leads subsequently to an

upregulation of ARE-dependent Nrf-2 target genes in MCF7 and MDA-MB-231 breast cancer cells.¹⁸⁶

Nrf2 and NF- κ B

The cross talk with NF- κ B as another important tumor-driving pathway certainly has a crucial role in the shaping of the Nrf2 function toward a protumorigenic one.¹⁸⁷ On the one hand, both pathways negatively interfere with each other.¹⁸⁸ Therefore, activated NF- κ B competes with Nrf2 for CBP coactivators and leads to histone deacetylase 3 binding to the ARE/EpRE and thereby to Nrf2 inactivation.¹⁸⁹ Induction of NF- κ B target genes, such as *COX2*, can also lead to Nrf2 suppression.¹⁹⁰ Therefore, anti-inflammatory agents, such as resveratrol,^{191,192} curcumin,¹⁹³ shogaol derivatives,¹⁹⁴ or ethyl 3',4',5'-trimethoxythionocinnamate,¹⁹⁵ may suppress NF- κ B signaling pathways and activate Nrf2 at the same time. Nrf2 also averts cellular damage of inflammation-exposed cells, thereby indirectly preventing sustained NF- κ B activation.¹⁸⁸ On the other hand, under conditions of NF- κ B-driven immune responses, Nrf2 may be activated indirectly by NF- κ B as a result of the oxidative stress during inflammation. Moreover, a direct inducing effect of NF- κ B on Nrf2 expression has been recently reported in acute myeloid leukemia cells,¹⁹⁶ and several common target genes of NF- κ B and Nrf2, such as *IL8*, *HO1*, *GCLC*, and *G α 2*, have been described.¹⁸⁸ Evidence also exists that Nrf2 may trigger the activation of NF- κ B. This results from increased proteasome activity, which can potentiate degradation of the NF- κ B inhibitor I κ B α .¹⁹⁷

Nrf2 and the apoptosis regulators Bcl-2 and Bcl-xL

Both *Bcl-2* and *Bcl-xL* represent ARE-controlled target genes of Nrf2.^{198,199} Accordingly, Nrf2 activation leads to an increased expression of these apoptosis-regulating proteins. Through the upregulation of Bcl-2 and Bcl-xL, amplified Nrf2 activity would therefore directly confer apoptosis protection to tumor cells, a condition essentially adding to the oncogenic effect of Nrf2 and its impact on a malignant phenotype. In addition to the direct transcriptional control by Nrf2, the fate of these two apoptosis regulators has been shown to be influenced by their interaction with Keap1 affecting their protein stability.⁷⁴ However, the physiological significance of this effect is not clear yet.

Nrf2 and p53

The tumor-suppressor gene *p53*, which substantially defines the cellular fate by regulating both pro- and antiapoptotic responses, is another cross-talk partner of Nrf2.^{188,200}

When kept at homeostatic low protein levels, p53 controls ROS production and the cellular redox status²⁰¹ by modulating antioxidative and metabolic gene expression, eg, *GLS2* and *C12orf5*.^{202,203} In this way, p53 may exert chemoprotective or tumor-suppressive effects in collaboration with Nrf2.²⁰⁰ Moreover, p53 is stabilized through Nrf2 target genes like *NQO1*,²⁰⁴ and in turn p53 has been shown to support Nrf2 activity,⁸² thus pointing to a positive-feedback mechanism between both pathways. By contrast, p53 can also be suppressed through Nrf2-dependent induction of *MDM2*, representing an ARE-regulated target gene.²⁰⁵ Therefore, the interplay between p53 and Nrf2 is of a complex nature and subject to subtle fine tuning that might be severely impaired in cancer.

Nrf2 and Notch

By controlling cell fate and cell renewal, the Notch pathway has a pivotal role in development and tissue homeostasis. Even though it exerts tumor-suppressive activities, the hyperactivation of Notch signaling has been implicated as oncogenic in several types of cancer.²⁰⁶ Recently, activation of the Notch pathway was reported to control the Nrf2 promoter, leading to increased Nrf2 expression.²⁰⁷ In turn, Nrf2 cooperates with the Notch pathway through inducing the expression of the soluble Notch ligand JAG1.²⁰⁸ Therefore, the interplay between Nrf2 and Notch may represent a condition turning both Nrf2 and Notch protumorigenic, eg, with respect to self-renewal of cancer stem cells.²⁰⁹

Nrf2 and caveolin

Recent studies have demonstrated that direct interaction with the scaffold protein caveolin 1 reduces the cytoprotective effects of Nrf2.^{210,211} Moreover, in colon cancer cells, loss of caveolin 1 contributes to a transformed phenotype in an Nrf2-dependent fashion.²¹² When restoring caveolin 1 expression, a p53-dependent senescence pathway is initiated, due to the suppressed oxidative stress response.²¹² Caveolin 1 has been shown also to modulate TFG β 1-dependent signaling in cancer cells, eg, related to epithelial–mesenchymal transition (EMT),²¹³ providing a possible link between oncogenic TFG β 1 and Nrf2 activation.

Nrf2 and autophagy

Representing the basic degradation machinery of cell components and organelles, selective autophagy is an integral part of the cellular stress response and cell-fate decision.²¹⁴ Selective autophagic cargos, such as ubiquitinated organelles, assemble together with the scaffold protein p62/SQSTM1

phosphorylated in an mTORC1-dependent manner. Intriguingly, phospho-p62 interferes with the Keap1/Nrf2 pathway,¹⁷⁸ and the accumulation of phospho-p62 leads to the persistent activation of Nrf2 in autophagy-deficient cells.^{75,215} Consequently, autophagy deficiency in cancer cells is accompanied by the cytoprotective and growth-supportive effects of Nrf2 that add, eg, to the growth of HCCs.^{76,216} The role of the Keap1/Nrf2 pathway in the control of selective autophagy is underscored by the fact that *p62* is a target gene of Nrf2.²¹⁷ Therefore, Nrf2 may compensate for the deficiency of selective autophagy on the one hand and for the resulting cytotoxicity on the other hand. Accordingly, in other tumor entities, an increase of selective autophagy has been demonstrated that may be linked to the enhanced Nrf2 activity resulting from other amplification mechanisms.^{218,219} By contrast, Nrf2 has been shown to block autophagy induction by temozolomide in glioma or by mitoquinone in breast cancer cells,^{220,221} and a recent study demonstrated that tBHQ-mediated induction of autophagy is required for full activation of Nrf2 in hepatocytes.²²² These findings point to a complex interrelation between autophagy and cellular redox control.

Nrf2 as modulator of HIF1 α and angiogenesis

Experimental evidence strongly suggests that Nrf2 also controls the fate of HIF1 α and thereby contributes to angiogenesis.^{223,224} By stabilizing HIF1 α through its impact on mitochondrial O₂ consumption and ROS formation, Nrf2 promotes VEGF-dependent microvessel formation in tumors, eg, in colon cancer or glioblastoma. Accordingly, Nrf2 inhibition suppresses tumor growth in vitro and in vivo, involving reduced tumor vascularization and invasiveness as a direct result of HIF1 α destabilization and downregulation.^{225,226}

Nrf2 as modulator of the ubiquitin/ proteasome pathway

The huge battery of Nrf2 target genes includes a cluster of more than 20 genes encoding for proteasomal subunits of both the 20S core and the 19S regulatory particle.^{227–229} Accordingly, Nrf2 activation confers increased proteasomal activity, probably reflecting the need for renewal of the protein-degradation machinery in cells subjected to ROS-dependent protein damage.^{230,231} The resulting gain of proteasome activity further enables cells to drive signaling pathways crucial for proliferation and survival more efficiently.^{232–235} Therefore, in particular, tumor cells acquire substantial growth advantages from this condition, as recently

reported for colon and pancreatic cancer.^{138,197,236} Proteasome inhibitors (PIs) used for the therapy of certain types of tumors, eg, multiple myeloma, are likewise able to induce Nrf2^{237,238} and thereby a compensatory mechanism by de novo synthesis of proteasomal proteins.²³⁹ Therefore, enhanced Nrf2 activation must be regarded as one condition accounting for the failure of PI-based therapies in solid tumors.²⁴⁰ Recent studies have demonstrated that TCF11/Nrf1 has an essential role in maintaining proteasomal homeostasis also, adding to the oxidative stress-associated effects of Nrf2.²⁴¹

Nrf2 as modulator of EMT and TFG β 1 signaling

EMT is a transdifferentiation process by which epithelial/ carcinoma cells can acquire an invasive and metastatic phenotype. Moreover, EMT-associated alterations have been discussed in the context of adaptation to oxidative stress. While inducing effects of ROS on EMT and a role of TFG β 1 through interfering with Nrf2 have been shown recently,^{87,242,243} the effects of Nrf2 on EMT and TFG β 1 signaling are a matter of considerable debate. On the one hand, several reports indicate that Nrf2 prohibits the fibrotic reaction through TFG β 1, an effect that was attributed to a direct inhibition of Smad3 and thereby of EMT-related gene expression,^{244,245} and Nrf2 has been shown to decrease the motility of hepatoma cells.²⁴⁶ On the other hand, Nrf2 is able to promote a motile and invasive phenotype of glioma, gallbladder carcinoma, or esophageal squamous carcinoma cells,^{247,248} eg, through induction of matrix metalloproteinase (MMP)-2/9 expression, and favors tumor invasiveness and metastasis formation in mice.²⁴⁹ Given an involvement in EMT and EMT-associated alterations, Nrf2 would have a particularly great impact on early events in malignant transformation that precede its later effects on tumor progression (eg, chemoresistance) or tumor angiogenesis.^{223,225}

Nrf2 and metabolic reprogramming

Another hallmark of cancer is an altered metabolism (metabolic reprogramming),²⁵⁰ ensuring glucose as a source for biomass production along with cell proliferation and tumor growth. Recent data have indicated that Nrf2 cooperates with the PI3K/Akt pathway in the metabolic reprogramming of cancer cells.^{66,251,252}

A number of key enzymes of the glycolysis (eg, phosphofructokinase), pentose phosphate pathway (PPP; eg, glucose-6-phosphate dehydrogenase [G6PD], phosphogluconate dehydrogenase, transketolase, transaldolase [TALDO]-1) and lipogenesis (eg, malic enzyme

[ME]-1, isocitrate dehydrogenase 1) have been shown to be upregulated by Nrf2. Accordingly, the amplified activity of Nrf2 has been associated with oncogenic metabolism with, eg, high levels of lactate or inosine monophosphate and low levels of fructose-6-phosphate, sedoheptulose-7 phosphate, or pyruvate. Some (eg, *TALDO1*, *ME1*) of these genes are direct ARE-regulated targets of Nrf2. Moreover, the cellular repertoire of glycolytic, PPP, or tricarboxylic acid cycle enzymes, as well as of electron transport-chain components, can change and result in a shift toward anabolic/catabolic pathways by epigenetic effects through Nrf2-inducible miRNAs, eg, miR-29a/b, miR-320a, and miR-143. Similarly, through its inducing effect on HDAC4, Nrf2 leads to downregulation of miR1 and miR206, thereby increasing the expression of PPP-associated genes (eg, *G6PD*, *TALDO1*) and favoring a tumor-proliferative phenotype.²⁵³

Nrf2 and drug/radiotherapy resistance of cancer

A marked property of tumor cells exhibiting elevated Nrf2 activity is a profound protection from anticancer drugs or radiotherapy-induced cell death. Therefore, Nrf2 is a potent determinant of chemo- and/or radioresistance of cancer. This includes resistance of NSCLC, cervical cancer, endometrial serous carcinoma, or cholangiocarcinoma cells to treatment with cisplatin,^{177,254,255} pancreatic cancer cells to treatment with gemcitabine,²⁵⁶ colon and gastric cancer cells to treatment with 5-fluorouracil (5-FU),^{257,258} hepatocellular carcinoma cells to treatment with doxorubicin,²⁵⁹ or prostate cancer cells to treatment with mitoxantrone or topotecan.²⁶⁰

Additionally, anticancer drugs themselves can elicit Nrf2-dependent cytoprotection. For example, oxaliplatin²⁶¹ and PIs like bortezomib (BTZ) are strong inducers of Nrf2, and thereby can lead to chemoresistance. BTZ has been shown to induce drug resistance in neuroblastoma cells through Nrf2-dependent HO1 expression. Likewise, proteasome inhibition results in upregulation of proteasomal gene expression due to increased Nrf2 activation, and obviously also Nrf1 activation, which confers not only resistance to PIs but also further growth advantages to cancer cells.

A detailed secretome analysis identified a cluster of drug-metabolizing and prosurvival Nrf2 target genes in cancer stem cells, underscoring the pivotal role of Nrf2 in intrinsic chemoresistance.²⁶² For example, ABC transporters like MRP3, MRP4, or MRP5 are upregulated by Nrf2, facilitating the efflux of anticancer drugs like cisplatin, 5-FU, or gemcitabine out of cancer cells.^{256,263} In addition, Nrf2-inducible metabolizing enzymes, such as AKR1B10,²⁶⁴ affecting drugs

like daunorubicin or idarubicin,²⁵⁹ NQO1, or HO1 affecting drugs like gemcitabine, cisplatin, doxorubicin, or daunorubicin contribute to the detoxification of anticancer drugs and thereby to chemoresistance (collectively discussed by Na and Surh²⁶⁵). Furthermore, Nrf2-induced expression of survival genes like *BCL2* contributes to reduced apoptotic responses to anticancer drugs, and the Nrf2-dependent expression of proteasomal genes promotes growth and survival-related pathways depending on the ubiquitin/proteasome pathway.

Likewise, the sensitivity of cells to irradiation-induced cell damage and thereby cell death is under the control of Nrf2. A number of Nrf2 target genes, such as *HO1* and *PRX1*, have been shown to directly suppress cytotoxic effects by irradiation,²⁶⁵ which manifest through ROS formation and ROS-dependent DNA damage. Accordingly, radioresistance due to Nrf2 amplification²⁶⁶ has been reported for, eg, NSCLC, head and neck, pancreatic or prostate cancer, glioma, and esophageal squamous carcinoma.

Nrf2 inhibition in cancer therapy

As outlined earlier and demonstrated by numerous experimental and preclinical studies within the last decade, Nrf2 inhibitors represent rational tools to prevent malignant progression in cancer, whereas activators of Nrf2 are obviously more suited for cancer prevention. As long as the action of Nrf2 fits into still-homeostatic control of cellular and tissue integrity, its pharmacological activation by bioactive compounds seems to be a reasonable measure for prevention of diseases, like cancer. However, upon exposure to persistent oxidative stress (eg, during chronic inflammation) and/or when adapting a premalignant phenotype, cells may take advantage of Nrf2 activation to undergo malignant transformation. Therefore, the molecular mechanisms defining these two conditions need to be unraveled in order to assess the use of Nrf2 either as an activation target in prevention strategies or as an inhibition target in anticancer therapy. As outlined earlier, genetic and epigenetic alterations or long-lasting and/or insufficiently compensated environmental stress (toward ROS and xenobiotics) are conditions making Nrf2 protumorigenic. This property of Nrf2 then manifests even more when facing additional oncogenic pathways (Figure 3).

Regardless of the still-limited knowledge of the exact modalities by which Nrf2 switches from being anti- to protumorigenic, numerous experimental attempts have been made to block tumor growth and progression or to improve anticancer therapy through inhibiting Nrf2. There have been many Nrf2 short hairpin RNA (shRNA)- or small interfering

RNA-based studies^{197,226,240,254,255,267} in cell-culture settings or using mouse models that demonstrated sensitization of tumor cells to apoptosis, suppression of tumor-cell proliferation or lower invasiveness, and reduced tumor vascularization. Furthermore, reexpression of miR-507, -634, -450a, and -129-5p has been shown to suppress esophageal squamous cell carcinoma through dampening Nrf2 signaling.¹⁵⁹

However, while all these studies indicated substantial benefits from genetic silencing of Nrf2 to reduce proliferation, viability, and chemo/radioresistance of cancer cells, its immediate application is expected to be limited by insufficient delivery of such nucleic acids as miRNAs or shRNAs into cells. In this respect, small molecules are preferable for clinical applications. More emphasis has now been given to Nrf2 inhibition by using pharmacological compounds affecting Nrf2 activation.

Strategies for pharmacological Nrf2 inhibition

A number of studies have already reported Nrf2-inhibitory effects of established compounds, but validation of these effects has either proven unsuccessful or a transfer into clinical application that way has seemed unlikely. For example, all-*trans*-retinoic acid and other RAR α ligands are able to inhibit Nrf2,⁹² but the significance of the negative interference between RAR α and Nrf2 is not clear, because a positive effect of retinoic acids on Nrf2 has been reported as well.²⁶⁸ Nevertheless, it would be interesting to see whether inhibiting Nrf2 in retinoid-based chemotherapy might act synergistically with other anticancer drugs. Tarumoto et al reported that ascorbic acid, a reducing reagent, can overcome resistance to the anticancer drug imatinib by inhibiting Nrf2 activity,²⁶⁹ but this effect was indirect. Kweon et al showed that epigallocatechin 3-gallate can inhibit Nrf2 activity and thereby HO1 expression in A549 NSCLC cells, but only at rather high concentrations,²⁷⁰ limiting its safe use in the clinic.

When considering novel, naturally derived compounds for inhibiting Nrf2, one should keep in mind that these may have considerable toxicities. As an example, the mycotoxin ochratoxin A is an efficient inhibitor of Nrf2, but exerts nephrotoxicity and kidney carcinogenic effects.²⁷¹ Therefore, depending on the cellular context, biological drugs may be inappropriate or at least strongly limited in their use in clinical settings. Therefore, the number of reports on more or less specific Nrf2 inhibitors that may be biologically safe in such attempts is still quite few.

One of the first reports on drug-mediated Nrf2 inhibition described a chemosensitizing effect of brusatol, a quassinoid

compound from an evergreen shrub from Southeast Asia, on A549 NSCLC cells.²⁷² Brusatol potently and reversibly inhibited Nrf2 through an increasing effect on Keap1-mediated polyubiquitination of Nrf2, thereby promoting Nrf2 degradation and suppressing Nrf2 activity. In that way, brusatol decreased Nrf2 target gene expression and increased anticancer drug-induced but not basal apoptosis. Moreover, brusatol sensitized A549 tumors for chemotherapy with cisplatin in a murine xenograft model in an Nrf2-dependent fashion, without obvious toxicity. Interestingly, even in the presence of certain mutant-Keap1 variants, brusatol exerted Nrf2 inhibitory effects, thus having the potential to compensate for an impaired Keap1-inhibitory function. However, previous studies have shown that brusatol can activate NF- κ B.²⁷³ Thereby, on the one hand, brusatol may lead to increased growth and survival of tumor cells through its inducing effect on the NF- κ B pathway. On the other hand, through the reported negative interference of NF- κ B with Nrf2 activation,^{189,190} brusatol may inhibit Nrf2 via NF- κ B. Therefore, brusatol can exert differential effects on two crucial stress- and tumor-related pathways depending on its dose and presumably the tumor type as well. Another property of brusatol – the rapid reversibility of Nrf2 inhibition – may further limit its efficacy in cancer therapy. This would imply the need for an extended administration of the drug to efficiently target the amplified Nrf2. In addition, a compensatory increase of Nrf2 activity could occur as a rebound effect from brusatol treatment.

Similarly in A459 cells, the vegetable-derived flavonoid luteolin has been shown to suppress Nrf2 activation independently of Keap1 through an enhanced turnover of Nrf2 mRNA. Along with decreasing Nrf2 activity, luteolin augmented the responsiveness of A459 and also MCF7 breast cancer or Caco2 colon cancer cells to anticancer drug (oxaliplatin, bleomycin, doxorubicin)-induced apoptosis, and thereby acted as a potent chemosensitizer. However, this Nrf2-inhibitory effect was limited to a particular dose of luteolin, and may considerably depend on the cellular context, because this compound has been shown to efficiently induce Nrf2 activation in several other tumor cell lines, thereby acting cytoprotectively.^{274,275} Moreover, protection from azoxymethane/dextran sulfate sodium-induced colorectal cancer in mice by luteolin has been ascribed to its Nrf2-inducing properties.²⁷⁶ Therefore, the use of luteolin as an Nrf2 inhibitor in chemosensitization may be hampered by its dual effects on Nrf2.

The synthetic compound IM3829 (cyclohexyl-ethoxy-aniline) was demonstrated to enhance the responsiveness

of NSCLC cells to radiotherapy-induced apoptosis through inhibiting Nrf2 and expression of its target genes. As observed with luteolin, IM3829 affects the stability of Nrf2 mRNA independently of Keap1. In a mouse tumor xenograft model, IM8329 potently enhanced the efficacy of radiotherapy.²⁷⁷ Further studies are needed to demonstrate the safety of this compound for its use as radiosensitizer.

The coffee-derived alkaloid trigonelline has been previously shown to exert a profound inhibitory effect on Nrf2.²⁷⁸ A recent study further reported inhibition of Nrf2 activation in several pancreatic cancer cell lines by trigonelline.¹³⁸ It was shown that trigonelline is capable of preventing the nuclear accumulation of Nrf2, an effect insensitive to treatment with the nuclear export inhibitor leptomycin B. Thereby, trigonelline potently inhibited the expression of Nrf2 target genes, including those of proteasomal subunit proteins. This was accompanied by a decrease of proteasome activity, making pancreatic cancer cells more sensitive to anticancer drugs as well as to death ligands, eg, TRAIL. In a mouse xenograft tumor model, trigonelline treatment also exerted a profound Nrf2 inhibitory and a marked chemosensitizing effect without any measurable toxicity. A major advantage of using this substance in ongoing studies is given by the fact that trigonelline has been already tested in human clinical trials and is well tolerated.^{279,280}

Another study reported suppression of Nrf2 in pancreatic cancer cells by the PI3K-p110a inhibitor PIK-75.²⁵⁶ By reducing Nrf2 protein levels, PIK-75 decreased the expression of Nrf2 target genes, including *MRP5*, the expression of which is induced by anticancer drugs like gemcitabine. PIK-75 treatment augmented the apoptosis of pancreatic cancer cells and potentiated gemcitabine toxicity through blocking Nrf2-dependent *MRP5* expression. Accordingly, a marked reduction of *in vivo* tumor growth in a mouse xenograft model was observed after PIK-75 treatment, and a synergistic effect of PIK-75 was observed when given in combination with gemcitabine.²⁵⁶ PIK-75 is thus a promising drug, as it has a great impact on tumor-cell viability.

The same holds true for the natural flavonoids chrysin and apigenin, which have been described as effective adjuvant sensitizers to reduce anticancer-drug resistance of HCC cells by downregulating Nrf2 activation.^{171,259} Through affecting the PI3K/Akt- and Erk-dependent activation of Nrf2, these compounds have been shown to increase the responsiveness to anticancer drugs. This effect could be attributed to decreased expression of Nrf2 targets like HO-1, *MRP5*, and *AKR1B10*. Another natural flavonoid, wogonin, has been shown to decrease Nrf2 protein levels and thereby

expression of HO-1 and NQO1 in MCF7 breast cancer cells, accompanied by sensitization to doxorubicin.²⁸¹ This action of wogonin is obviously also dependent on suppression of the PI3K/Akt/Nrf2 pathway. However, it still needs to be validated whether these PI3K/Akt interfering compounds are safe in clinical settings.

Another approach investigated the effect of treatment with 2-deoxy-D-glucose (2-DG) and 6-aminonicotinamide (6-AN) on the radiosensitivity of head and neck squamous carcinoma cells. It was shown that 2-DG/6-AN treatment reduced Nrf2 activity through upregulated Keap1 expression, thereby decreasing Nrf2-dependent resistance to radiotherapy.²⁸²

Most if not all of these strategies described so far deal with substances that affect other pathways as well. The use of them may be thus restricted to tumor cells exhibiting Nrf2 amplification, whereas in other conditions in chemo/radioresistant cancer cells, eg, amplified NF- κ B, these drugs should rather be excluded from cancer therapy. More data are thus needed to assess under what conditions these compounds will have beneficial effects, eg, in chemosensitization, and when they will not. The ongoing search for other drugs – in particular, those having greater selectivity for inhibiting Nrf2 – is certainly an important task. However, it is quite obvious that plant-derived substances are very often limited in their specificity for a selected target. Owing to the broad target reactivity, these substances have been evolutionarily conserved to provide greatest protection of the host from environmental threats. Synthetic derivatives with greater target selectivity are therefore needed to get more reliable Nrf2 inhibitors.

Conclusion and outlook

Regardless of the dual role of Nrf2 in oncogenesis, deregulated Nrf2 activity in tumors essentially contributes to the manifestation of cancer hallmarks and associates with poor prognosis and therapy resistance. This undeniably underscores the dark side of Nrf2. From experimental data using mouse models, it has become clear that Nrf2 affects tumorigenesis in a time- and context-dependent fashion. However, it could also be learned that a generalized prediction of the ultimate outcome of tumor development with respect to the Nrf2 status is rather difficult. This is exemplified by two studies on kRas-driven cancer in mice. On the one hand, it was reported that Nrf2 prevents the initiation of chemically induced lung cancer, but favors its malignant progression later on depending on mutated kRas.¹⁶³ On the other hand, it was shown that Nrf2 exerts its protumorigenic effects at

an earlier stage of pancreatic carcinogenesis initiated by mutated kRas.¹³ Given the advantages transformed cells can gain from Nrf2 activation, greater survival and growth-favoring metabolic effects may pave the way quite early for local tumor development. Later on, the multiple effects of Nrf2 on tumor vascularization, invasiveness, and cancer stem cell self-renewal may contribute to cancer progression and metastasis formation as well. It is therefore evident that the decision to make use of Nrf2 as an activation or inhibition target for a given therapy requires an accurate estimation of its different roles during each step of tumorigenesis, which can vary from tumor to tumor.

Certainly, the use of Nrf2 as an inducible target in cancer-prevention strategies is still an attractive option when considering, eg, acute/temporary exposure to environmental hazards like aflatoxin, benzo(a)pyrene, or diesel exhaust. However, long-term application seems not to be suitable as long as it is not fully known how Nrf2 is kept under homeostatic control and when/how this control gets lost. Nrf2 can be regarded instead as a useful marker for the assessment of particular cancer phenotypes, such as chemo- and radioresistance or autophagy deficiency. Under these conditions, Nrf2 gain-of-function mutations or amplified Nrf2-inducing signaling pathways directly relate to the malignant phenotype, whereas Keap1 mutations may have additional effects. Therefore, predicting the clinical outcome of alterations in the Keap1/Nrf2 pathway may be sometimes difficult, and from the plethora of data it is also clear that cross talk with other tumor-related pathways has a pivotal role in shaping the effects of Nrf2 on the cellular phenotype. This includes effects on proliferation and senescence, which are particularly relevant in oncogenic kRas background on the one hand and p53 status on the other hand. While mutated kRas drives proliferative pathways, accompanied by ROS formation giving rise to additional growth advantages through the induction of Nrf2-dependent cytoprotection, certain p53 mutants could still negatively interfere with Nrf2, but others might not. Likewise, NF- κ B, albeit having the potential of directly or indirectly inducing Nrf2, can competitively suppress Nrf2 too. Therefore, during inflammation, Nrf2 can add its cytoprotective actions to the prosurvival effect of NF- κ B, but Nrf2 and NF- κ B can also negatively interfere with each other. As another example, nuclear hormone receptors, such as RARs, ER α , or the ARs exert complex interactions with the Keap1/Nrf2 pathway, and thereby define the role of Nrf2 to act as either a tumor suppressor or tumor promoter.

In view of the complexity of the cross talk between Nrf2 and its numerous signaling-network partners, it is clear that

we still know too little about the direction in which Nrf2 may act during tumorigenesis to judge its general use as a therapeutic inhibition target. Nevertheless, its temporary use as a sensitizer in chemo/radiotherapy will be certainly advantageous if really specific and safe-in-use Nrf2 inhibitors are established. As already outlined, most existing Nrf2-inhibiting compounds do not fit these criteria so far. On the one hand, these compounds can have severe cytotoxicities when affecting vital tissue-protection mechanisms, eg, in the kidney. On the other hand, drugs affecting upstream pathways like the PI3K/Akt pathway can exert antitumor effects independently of Nrf2. In addition, possible limitations of manipulating the Keap1/Nrf2 pathway may relate to the numerous actions of Keap1 independently of controlling Nrf2. Therefore, Keap1 influences NF- κ B activation through interfering with I κ B kinase phosphorylation, PPAR γ , and several other pathways. Therefore, much effort is still needed in the search for novel compounds and engineered small molecules that target Nrf2 more specifically.

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Disclosure

The authors report no conflicts of interest in this work.

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