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REVIEW ARTICLE

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NRF2 addiction in cancer cells

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The Kelch-like ECH-associated protein 1/nuclear factor erythroid-derived 2-like 2 (KEAP1-NRF2) system is a pivotal defense mechanism against oxidative and electrophilic stress. Although transient NRF2 activation in response to stress is beneficial for health, persistent NRF2 activation in cancer cells has deleterious effects on cancer-bearing hosts by conferring therapeutic resistance and aggressive tumorigenic activity on cancer cells. Because NRF2 increases the antioxidant and detoxification capability of cancer cells, persistently high levels of NRF2 activity enhance therapeutic resistance of cancer cells. NRF2 also drives metabolic reprogramming to establish cellular metabolic processes that are advantageous for cell proliferation in cooperation with other oncogenic pathways. As a result of these advantages, cancer cells with persistent activation of NRF2 often develop "NRF2 addiction" and show malignant phenotypes leading to poor prognoses in cancer patients. Inhibition of NRF2 is a promising therapeutic approach for NRF2-addicted cancers and NRF2 inhibitors are being actively developed. However, giving systemic NRF2 inhibitors might have undesirable effects on cancer-bearing hosts, considering the central roles of NRF2 in cytoprotection. To avoid these side-effects, new therapeutic targets besides NRF2 for NRF2-addicted cancers have been actively explored. This review introduces recent studies describing the development and characterization of NRF2-addicted cancers, as well as their potential therapeutic targets. Expected advances in diagnostic and therapeutic interventions for NRF2-addicted cancers are also discussed.

KEYWORDS
KEAP1, metabolic reprogramming, NRF2, therapeutic resistance, tumor microenvironment

1 | PHYSIOLOGICAL ROLES OF THE KEAP1-NRF2 SYSTEM

Living organisms are constantly interacting with their surrounding environment. Appropriate environmental responses are relevant for the maintenance of homeostasis and optimal health at cellular as

Abbreviations: FH, fumarate hydratase; GEMM, genetically engineered mouse model; IHC, immunohistochemistry; KEAP1, Kelch-like ECH-associated protein 1; MEF, mouse embryonic fibroblast; MDSC, myeloid-derived suppressor cell; NRF2, nuclear factor erythroid-derived 2-like 2; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; T_{reg}, regulatory T cell.

well as organismal levels. Many environmental stimuli disturb redox homeostasis and result in biomolecules undergoing chemical changes such as protein carbonylation, lipid peroxidation and nucleic acid oxidation, leading to functional alteration or impairment of biomolecules. The KEAP1-NRF2 system is an important defense mechanism against redox disturbances.[1] NRF2 is a potent transcription activator belonging to the Cap'n'Collar (CNC) transcription factor family, which is characterized by a unique CNC motif followed by a well-conserved basic region-leucine zipper (bZip) structure. Under normal conditions, NRF2 is constantly poly-ubiquitinated by the CUL3-KEAP1 E3 ubiquitin ligase complex and subjected to degradation by proteasomes. When cells are exposed to oxidative and/or electrophilic stress, highly reactive thiols in KEAP1 are directly modified, resulting in inactivation of the CUL3-KEAP1 complex and stabilization of NRF2. NRF2 then translocates to the nucleus and induces a battery of cytoprotective genes by binding to the antioxidant response element (ARE) by heterodimerization with small MAF proteins (Figure 1, "Transient NRF2 activation").[1]

Biochemical, biophysical and structural analyses showed that KEAP1 forms a cherry bob-like homodimer and interacts with a single NRF2 molecule at two binding sites, namely a DLG motif and an ETGE motif in the N-terminal region of NRF2 (Figure 1, "Transient NRF2 activation").[2-4] Appropriate interaction between KEAP1 and NRF2 is considered critical for efficient ubiquitination of NRF2, and modification of KEAP1 thiols by electrophiles is likely to induce conformational alterations in the overall structure of the CUL3-KEAP1-NRF2 complex and to suppress the ubiquitination of NRF2.

The physiological relevance of NRF2 has been shown by numerous studies using *Nrf2*-deficient mice and human cohort studies of SNP in the promoter region of the *NRF2* gene. *Nrf2*-deficient mice are generally susceptible to redox disturbances and easily develop drug toxicity.[5,6] Oxidative tissue damage after ischemia and reperfusion, including that resulting in noise-induced hearing loss, is effectively suppressed by NRF2 activation through its antioxidant function.[7] Further, NRF2 possesses potent anti-inflammatory activity and alleviates a variety of inflammatory conditions, including autoimmune diseases.[8,9]

In line with these protective roles of the KEAP1-NRF2 system, NRF2 activation effectively prevents chemical carcinogenesis by increasing antioxidant and detoxification capabilities.[10-13] NRF2 activation in cancer-bearing hosts is also beneficial as a result of the fact that it potentiates anticancer immunity. NRF2 effectively inhibits the activity of MDSC and prevents apoptotic T_{reg} cell-mediated immunosuppression by protecting T_{reg} cells from apoptosis.[14-16] Thus, NRF2 activation in the host is beneficial as a result of its suppressive effect on cancer initiation and its anticancer-cell activity.

2 | ABERRANT ACTIVATION OF NRF2 AS A STRONG PROGNOSTIC FACTOR IN CANCER PATIENTS

In contrast to the protective roles described above, persistent activation of NRF2 at high levels in normal cells has deleterious effects. *Keap1*-deficient mice die after weaning as a result of obstructive lesions in the upper digestive tract caused by epithelial hyperkeratosis.[17] *Keap1* deficiency in the developing kidney causes polyuria with low osmolality and bilateral hydronephrosis.[18] *Keap1* deficiency in bone marrow results in the exhaustion of hematopoietic stem cells.[19] These deleterious effects are all canceled by *Nrf2* disruption, indicating that excessive activation of NRF2 in normal cells is toxic and

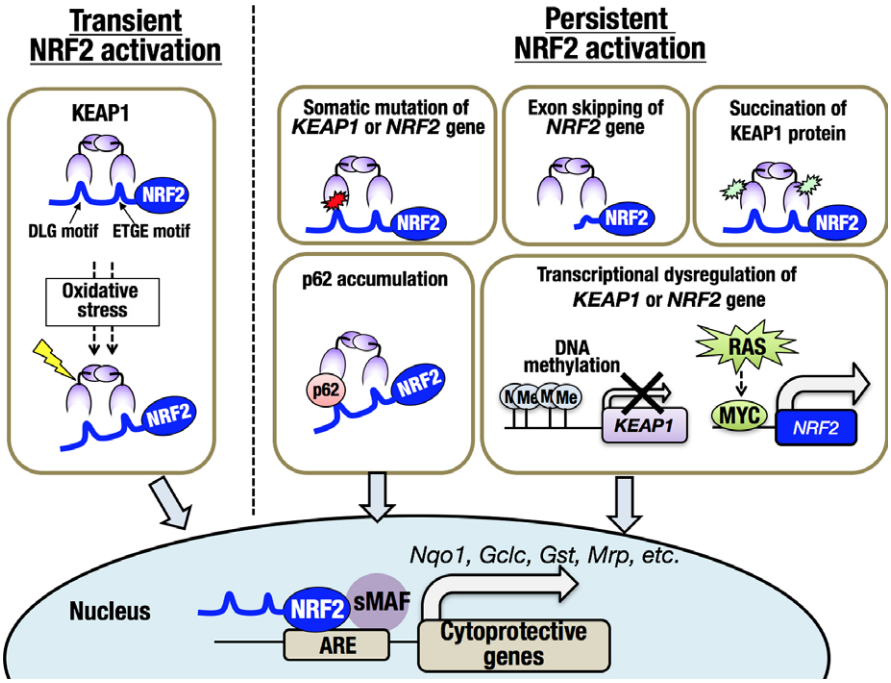

FIGURE 1 The KEAP1-NRF2 system in physiological and pathological conditions in cancer cells. NRF2 activation is triggered by exposure to oxidative or electrophilic stress, resulting in upregulation of cytoprotective genes (left; transient NRF2 activation). In cancer cells, the KEAP1-NRF2 regulatory system is frequently disrupted, resulting in the persistent activation of NRF2 (right; persistent NRF2 activation). ARE, antioxidant response element

suggesting that appropriate regulation of NRF2 by KEAP1 is required for organismal health.

However, this scenario is not applicable to cancer cells. NRF2 activation in cancer cells confers therapeutic resistance and aggressive tumorigenic ability on cancer cells, driving their malignant progression. Many clinical studies have indeed shown strong correlations between NRF2 activation in tumor tissues and poor clinical outcomes of patients (Table 1). In many studies, NRF2 accumulation was examined using immunohistochemistry, and high levels of NRF2 accumulation were found to be commonly associated with poor prognosis in various cancer types. Somatic mutations of *NRF2*, *KEAP1* and *CUL3* are also prognostic markers of non-small cell lung cancers, esophageal cancers and head and neck cancers.[27-29,31] Cancer tissue expression levels of NRF2 target genes, such as *NQO1* and *GCLC*, and those of downstream effectors of NRF2, such as *PHGDH*, *PSAT1* and *SHMT2*, are also well associated with clinical outcomes of cancer patients.[21,23,27,32,41] Thus, NRF2 and its downstream effectors are important prognostic factors in a wide range of cancers.

3 | VARIOUS CAUSES OF ABERRANT ACTIVATION OF NRF2

Multiple mechanisms that cause aberrant persistent activation of NRF2 have been reported, including genetic changes, epigenetic effects and altered protein-protein interactions (Figure 1, "Persistent NRF2 activation").

3.1 | Somatic mutations of *KEAP1* and *NRF2*

Somatic mutations of *KEAP1* and *NRF2* genes are one of the main causes of constitutive NRF2 activation. Mutations in *KEAP1*, which are generally mutually exclusive with those in *NRF2*, are frequently found in solid tumors, especially in the head and neck, lung and bladder.[46] Although *KEAP1* mutations are found in various positions in the coding region, most *NRF2* mutations are located in the DLG and ETGE motifs, which are critical for binding with KEAP1. The functional impacts of these mutations have been analyzed by co-crystallization of KEAP1 and the DLG/ETGE motifs of NRF2.[47,48]

3.2 | Exon skipping in *NRF2*

Aberrant *NRF2* transcripts with recurrent loss of exon 2 have been found in lung, head and neck squamous cell carcinoma and hepatocellular carcinoma.[49] NRF2 mutants that are translated from mRNA lacking exon 2 do not interact with KEAP1, resulting in persistent localization in the nucleus.

3.3 | *KEAP1* promoter methylation

Epigenetic alteration has been suggested as another cause of dysregulation of the KEAP1-NRF2 system. Inverse correlation between DNA methylation levels and *KEAP1* expression levels was reported in renal cell carcinoma.[50]

3.4 | p62 (SQSTM1) accumulation

p62 is one of the adaptor proteins that recognizes ubiquitinated substrate proteins for selective autophagy. Phosphorylated p62 has a higher affinity for KEAP1 than the non-phosphorylated form of p62, and competes with NRF2 for KEAP1 binding.[51] Aberrant accumulation of p62 is frequently observed in hepatocellular carcinoma, and causes persistent activation of NRF2.[52,53]

3.5 | Fumarate hydratase mutation

Fumarate hydratase is a Krebs cycle enzyme that catalyzes the conversion from fumarate to malate. Fumarate, which accumulates in *FH* deficiency, modifies KEAP1 thiols as a result of its electrophilic property and stabilizes NRF2. Type II papillary renal cell carcinoma, which is accompanied by *FH* mutations, shows elevated expression of NRF2 target genes and highly malignant phenotypes.[54,55]

3.6 | Transcriptional activation of the *NRF2* gene

Transcription levels of the *NRF2* gene influence protein levels of NRF2 in basal and induced conditions.[56] RAS signal activation induces the recruitment of MYC to the *NRF2* promoter and upregulates NRF2 transcription, which is suggested to enhance the tumorigenesis induced by the oncogenic KRAS mutant.[57]

4 | ESTABLISHMENT OF NRF2-ADDICTED CANCER CELLS

Because NRF2 confers great advantages on cancer cells, including therapeutic resistance, increased antioxidant capacity and aggressive tumorigenic ability, cancer cells with NRF2 activation often develop "NRF2 addiction", which is one of the forms of non-oncogene addiction. This state has been shown in human cancer cell lines and mouse cancer models with abundant accumulation of NRF2 (Table 2). Although persistent activation of NRF2 confers growth and survival advantages on cancer cells, leading to NRF2 addiction, excessive activation of NRF2 in normal cells is rather toxic, as described in Section 2. These results imply that certain prerequisites, which are not fully understood, enable the establishment of NRF2-addicted cancers.

An important observation for understanding the dominant role of NRF2 in driving aggressive cell proliferation is that nuclear accumulation of NRF2 is greatly enhanced in the presence of proliferative signals.[63,75,76] Whereas NRF2 is trapped by the CUL3-KEAP1 complex in the cytoplasm and ubiquitinated for degradation, NRF2 is also ubiquitinated by the CUL1-βTrCP complex after being phosphorylated by GSK3.[77,78] As GSK3 is phosphorylated by AKT and inactivated, CUL1-βTrCP complex-mediated degradation of NRF2 is inhibited in proliferating cells in which the PI3K-AKT pathway is

TABLE 1 Clinical studies analyzing the association between KEAP1-NRF2 status and cancer patient prognosis

Tissues		Study scale	Country	Prognosis marker (experiment)	Results	Reference	Reference no.
Brain	Glioma	75	China	NRF2 (IHC)	High NRF2 expression is correlated with age, tumor grade and onset time. It is also correlated with short disease-free survival and overall survival.	Zhao et al., 2015	20
	Anaplastic glioma	95	Japan	*NQO1* and *GCLC* expression	Upregulation of *NQO1* and *GCLC* correlates with short progression-free survival.	Kanamori et al., 2015	21
	Meningioma	63	Taiwan	NRF2 (IHC)	Meningioma patients with high NRF2 expression tend to show short overall survival.	Tsai et al., 2016	22
Lung	Non-small cell lung cancer	443	USA	Serine biosynthesis enzyme (*PHGDH, PSAT1 and SHMT2*)	Patients with tumors expressing high levels of serine biosynthesis enzymes, which are induced downstream of NRF2, show poor prognosis.	DeNicola et al., 2015	23
		235	USA	NRF2 (IHC)	High NRF2 expression is associated with short overall survival and relapse-free survival.	Solis et al., 2010	24
		330	USA	Somatic mutation of *KEAP1* or *NRF2*	Among patients with advanced *KRAS* mutant lung cancer, patients with co-mutations in *KEAP1*/NRF2 have shorter survival, shorter duration of initial chemotherapy and shorter overall survival from initiation of immune therapy than those with single *KRAS* mutation.	Arbour et al., 2018	25
		109	Japan	NRF2 (IHC)	Among patients not receiving irradiation or chemotherapy, high NRF2 expression is associated with short overall survival and relapse-free survival.	Inoue et al., 2012	26
	Adenocarcinoma	458	USA	NRF2 target gene signature*KEAP1* mutation	NRF2 target gene signature and *KEAP1* mutation are correlated with short survival.	Romero et al., 2017	27
	Squamous cell carcinoma	48	Japan	NRF2 mutation	Squamous cell carcinoma patients with NRF2 mutation have poor prognosis.	Shibata et al., 2008	28
		94	USA	KEAP1 (IHC)	Low or absent KEAP1 expression is associated with short overall survival and relapse-free survival.	Solis et al., 2010	24
Esophageal squamous cell carcinoma		82	Japan	NRF2 mutation	Cancer patients with *NRF2* mutation show short overall survival.	Shibata et al., 2011	29
		46	Japan	NRF2 (IHC)	Among patients who have undergone chemotherapy and curative surgery, high expression of NRF2 is correlated with lymph node metastasis and poor postoperative outcome.	Kawasaki et al., 2014	30
Head and neck squamous cell carcinoma		302	Canada	Somatic mutation of *KEAP1, CUL3* and *RBX1*	Mutations of *KEAP1, CUL3* or *RBX1* are associated with short median survival.	Martinez et al., 2015	31
		60	Japan	NRF2 transcriptional profile (microarray)	NRF2-activating transcriptional profiles are associated with poor prognosis.	Shibata et al., 2010	32

(Continues)

TABLE 1 (Continued)

Tissues	Study scale	Country	Prognosis marker (experiment)	Results	Reference	Reference no.
Breast cancer	106	Japan	NRF2 (IHC)	Patients with NRF2 accumulation show short disease-free survival and breast cancer-specific survival.	Onodera et al., 2014	33
Hepatocellular carcinoma	65	China	NRF2 (IHC)	Among patients who have undergone surgical resection without any neoadjuvant or adjuvant chemotherapy, high expression of NRF2 is correlated with short overall survival and disease-free survival.	Zhang et al., 2015	34
	107	China	Pphosphorylated-NRF2 (IHC)	Increased expression of phosphorylated NRF2 is associated with short overall survival and disease-free survival.	Chen et al., 2016	35
Bladder cancer	44	UK	NRF2 (IHC)	Among patients treated with radical cystectomy and chemotherapy, positive NRF2 staining is associated with short overall survival, bladder cancer-specific survival and recurrence-free survival.	Hayden et al., 2014	36
Pancreatic adenocarcinoma	69	Finland	KEAP1 (IHC)	Decreased KEAP1 expression is associated with short relapse-free survival and pancreatic cancer-specific survival.	Isohookana et al., 2015	37
	103	Finland	NRF2 (IHC)	Nuclear staining of NRF2 is associated with poor prognosis.	Soini et al., 2014	38
Cervical cancer	89	China	NRF2/KEAP1 (IHC)	Positive NRF2 staining and negative KEAP1 staining are both associated with poorly differentiated histology, lymph node metastasis and advanced FIGO stage.	Ma et al., 2015	39
Melanoma	121	Finland	NRF2 (IHC)	Nuclear NRF2 expression correlates with greater Breslow's depth, invasive phenotype, nodular growth and short melanoma-specific survival.	Hintsala et al., 2016	40
Ovarian cancer	64	USA	NRF2 target gene expression (microarray)	Patients with NRF2 pathway activation have high resistance to platinum-based therapy and have short overall survival.	Konstantinopoulos et al., 2011	41
	108	Taiwan	NRF2 (IHC)	High NRF2 expression is associated with short disease-free survival and overall survival.	Liew et al., 2015	42
Gastric cancer	175	Japan	NRF2 (IHC)	Positive NRF2 staining is associated with clinicopathological factors, including tumor size, tumor depth, lymph node metastases, lymphovascular invasion, undifferentiated histology, advanced stage, and chemoresistance.Positive NRF2 staining is associated with poor overall postoperative survival.	Kawasaki et al., 2015	43
	186	China	NRF2 (IHC)	NRF2 accumulation correlates with short overall survival and disease-free survival.	Hu et al., 2013	44
Colorectal cancer	76	China	NRF2/NQO1 (IHC)	High NRF2 or NQO1 expression correlates with Duke's stage and poor prognosis.	Ji et al., 2014	45

FIGO, The International Federation of Gynecology and Obstetrics; GEMM, genetically engineered mouse model; IHC, immunohistochemistry; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid-derived 2-like 2.

TABLE 2 Aberrant activation of NRF2 in cancer cells and their NRF2 dependency

Tissue	Experiment	Cancer cell line/mouse cancer model with NRF2 activation	Method of modulating the KEAP1-NRF2 pathway	Observations (with suggested mechanisms)	Reference	Reference no.
Brain	Xenograft	U251MG glioblastoma cell line	*NRF2* knockdown by shRNA	*NRF2* knockdown suppresses tumorigenic activity of U251MG cells.	Ji et al., 2013	58
	Dish culture Soft agar growth	F98/U87 glioblastoma cell line	NRF2 overexpression *KEAP1* knockdown by shRNA	NRF2 activation promotes cell proliferation, anchorage-independent growth and inhibits ferroptosis.	Fan et al., 2017	59
	Xenograft	Human primary glioblastoma	*NRF2* knockdown by shRNA	*NRF2* knockdown suppresses tumorigenic activity of glioma stem cells.	Zhu et al., 2013	60
Lung	Soft agar growthXenograft	NSCLC cell line	*PHGDH* knockdown by shRNA	Inhibition of PHGDH that is induced by NRF2 by ATF4 activation suppresses soft agar growth and tumorigenic activity of NSCLC cells.	DeNicola et al., 2015	23
	GEMM	$Kras^{LSL\text{-}G12D/+}$ mouse + Adeno-Cre	Mating with *Nrf2* knockout mice	NRF2 deficiency inhibits $Kras^{G12D}$-mediated lung carcinogenesis.	DeNicola et al., 2011	57
	Dish culture Xenograft	A549, ABC1, COR-L105 (NSCLC cell lines) KYSE70 (esophageal squamous cell carcinoma cell line)	NRF2 inhibitor (halofuginone)	NRF2 inhibition by halofuginone alleviates resistance to chemotherapy.	Tsuchida et al., 2017	61
	GEMM	$Kras^{LSL\text{-}G12D/+}$:CCSP-Cre mouse	NRF2 inhibitor (brusatol)	NRF2 inhibition by brusatol sensitizes $Kras^{G12D}$-induced lung cancer to cisplatin treatment.	Tao et al., 2014	62
	Dish culture Xenograft	A549 NSCLC cell line	NRF2 knockdown by siRNA NRF2 knockdown by direct injection of siRNA into tumors	*NRF2* knockdown inhibits cell growth and suppresses tumorigenesis.	Mitsuishi et al., 2012	63
	GEMM	$Kras^{LSL\text{-}G12D/+}$:$Tp53^{f/f}$ mouse + Adeno-Cre	*Keap1* disruption by CRISPR-CAS9 system	*Keap1* deletion accelerates lung tumorigenesis, and depends on glutaminolysis.	Romero et al., 2017	27
	Dish culture Xenograft	CALU1, HCC364, HCC827, MGH-065 (NSCLC cell line)	*Keap1* disruption by CRISPR-CAS9 system	*Keap1* deletion promotes cell survival in the presence of multiple inhibitors targeting the RT/Ras/MAPK pathway.	Krall et al., 2017	64
	GEMM	$Tp53^{f/f}$:$Keap1^{f/f}$ mouse + Adeno-Cre/$Krt5^{CreERT2}$	*Nrf2* knockdown by shRNA	*Keap1* deletion enhances therapeutic resistance and promotes aggressive proliferation and metastasis, which are canceled by NRF2 knockdown.	Jeong et al., 2017	65
Head and Neck	GEMM	TetO-$Hras^{G12V}$ mouse	*Nrf2*/*Keap1* knockdown by shRNA	NRF2 activation protects cancer stem cells from cisplatin treatment by glutathione production.	Oshimori et al., 2015	66

(Continues)

TABLE 2 (Continued)

Tissue	Experiment	Cancer cell line/mouse cancer model with NRF2 activation	Method of modulating the KEAP1-NRF2 pathway	Observations (with suggested mechanisms)	Reference	Reference no.
Breast	3D culture Soft agar growth Xenograft	MCF10A expressing active mutant of AKT2 MDA-MB-231, T47D, ZE-75-1 (breast cancer cell lines)	Inhibition of glutathione synthesis by BSO treatment	Glutathione synthesis stimulated by NRF2 activation contributes to spheroid formation, anchorage-independent growth and tumorigenesis and confers chemoresistance on cancer cells.	Lien et al., 2016	67
	Soft agar growth	Transformed human mammary epithelial cells	*NRF2* knockdown by shRNA	*NRF2* knockdown decreases proteasome subunit gene expression, impairing misfolded protein degradation, and suppresses anchorage-independent growth.	Chen et al., 2017	68
Liver	Xenograft	Huh1 HCC cell line	*p62* disruption and re-introduction of wild-type and serine mutant of p62	Phosphorylated p62 stabilizes NRF2 and promotes tumorigenesis.	Ichimura et al., 2013	51
Pancreas	Sphere formation Xenograft GEMM	MIA PaCa2, Capan 2 (pancreatic cancer cell lines) *Kras*$^{LSLG12D/+}$:*IKKa*$^{\Delta pan}$:*Pdx1*-Cre mouse	*NRF2* disruption by CRISPR-CAS9 system Mating with *Nrf2* knockout mice	*NRF2* knockdown decreases sphere formation and tumorigenic activity. p62 accumulation extends survival period of *Kras*$^{LSLG12D/+}$:*IKKa*$^{\Delta pan}$:*Pdx1*-Cre mice through NRF2 activation.	Todoric et al., 2017	69
	Organoid culture Xenograft GEMM	Human primary and metastatic tumor cells Suit2 PDA cell line *Kras*$^{LSLG12D/+}$:*Tp53*$^{R172H/+}$:*Pdx1*-Cre mouse	NRF2 knockdown by shRNA Mating with *Nrf2* knockout mice	*NRF2* deletion reduces tumor size and proliferation of organoids by protecting translation factors from oxidative stress.	Chio et al., 2016	70
	GEMM	*Kras*$^{LSLG12D/+}$:*Ptf1a*-Cre mouse	Mating with *Nrf2* knockout mice	*NRF2* deletion reduces numbers of total PanIN-1a cells and their Ki67-positive ratios.	DeNicola et al., 2011	57
Melanocyte	Dish culture Allograft	B16 melanoma cells	BSO treatment	Glutathione synthesis promoted by NRF2 confers temozolomide resistance.	Rocha et al., 2016	71
Prostate	Dish culture Xenograft	DU-145 prostate cancer cell line	*NRF2* knockdown by shRNA	Attenuation of NRF2 expression enhances the efficacy of chemotherapeutic drugs and ionizing radiation and reduces tumor volume.	Zhang et al., 2010	72

(Continues)

TABLE 2 (Continued)

Tissue	Experiment	Cancer cell line/mouse cancer model with NRF2 activation	Method of modulating the KEAP1-NRF2 pathway	Observations (with suggested mechanisms)	Reference	Reference no.
Kidney	GEMM	FH-deficient mouse	FH re-expression	FH deficiency induces NRF2 activation by KEAP1 inactivation.	Adam et al., 2011	54
	Dish culture	FH-deficient UOK262 cells derived from hereditary leiomyomatosis and renal cell carcinoma (HLRCC) patients			Ooi et al., 2011	55
	Xenograft	CCF-RC1 renal cell carcinoma cell line	*iASPP* knockdown by siRNA	NRF2 activation by iASPP accumulation confers 5-FU resistance and promotes proliferation and tumorigenesis.	Ge et al., 2017	73
MEF	Allograft	NRF2-addicted cancer model cell (MEF with SV40 and $HRAS^{G12V}$)	*Nrf2* knockdown by shRNA	Constitutive NRF2 activation as a result of Keap1 deletion enhances tumorigenesis by IL11 upregulation under the influence of the tumor microenvironment.	Kitamura et al., 2017	74

5-FU, fluorouracil; FH, fumarate hydratase; GEMM, genetically engineered mouse model; IHC, immunohistochemistry; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid-derived 2-like 2; NSCLC, non-small cell lung cancer.

active. For instance, PI3K-AKT activation caused by *Pten* deficiency in combination with *Keap1* deficiency in the mouse liver results in massive accumulation of NRF2 and NRF2-dependent proliferation of hepatocytes and cholangiocytes.[63,75,76] Thus, quantitative increases of the NRF2 protein under proliferative signals substantiates the dominant role played by NRF2 in leading cancer cells to NRF2 addiction.

Consistent with this observation, oncogenic mutations inducing proliferative signals are likely to convert the role of NRF2 from cellular guardian to cancer driver. Experimental results using genetically engineered mouse models have shown that simple stabilization and accumulation of NRF2 are not sufficient for making NRF2 a cancer driver.[18,19,79] Because *Keap1*-deficient mice are resistant to carcinogenesis, establishment of NRF2-addicted cancer models by *Keap1* mutation requires combining *Keap1* mutation with additional oncogenic mutations, such as activating mutations of *KRAS/HRAS* and loss-of-function of *TP53*.[27,65,74] These results suggest that NRF2 is a facultative cancer driver, able to confer malignant phenotypes on cancer cells only in the presence of active oncogenic signaling.

Intriguingly, the frequency of NRF2-addicted cancers possessing somatic mutations of *KEAP1* or *NRF2* is likely to vary from tissue to tissue. In The Cancer Genome Atlas (TCGA) database, *KEAP1* and *NRF2* genes are mutated in approximately 10%-30% of lung cancers, in combination with oncogenic mutations such as *KRAS* and *TP53*, whereas no mutations have been found in *KEAP1* or *NRF2* genes in the case of pancreatic cancers. In good agreement with these clinical observations, *Kras*:*Tp53*:*Keap1* triple mutations in the lung cause cancers showing aggressive proliferation,[27] whereas these triple mutations in the pancreas do not cause cancers but result in fibrosis instead.[80] These observations suggest that tissue-specific factors are likely to determine the prerequisites for NRF2-addicted cancer development.

5 | CHARACTERISTICS OF NRF2-ADDICTED CANCER CELLS

Although NRF2 inhibitors are expected to be promising therapeutic drugs for NRF2-addicted malignant cancers, giving systemic NRF2 inhibitors might cause undesirable effects as a result of the impaired protective functions of NRF2. Detailed characterization of NRF2-addicted cancers has been conducted to identify effective therapeutic targets besides NRF2 for NRF2-addicted cancers.

Several metabolic features of NRF2-addicted cancers have been described (Figure 2, left side). In proliferating cancer cells, NRF2 stabilization is enhanced and its transcriptional activation ability is augmented, resulting in the transcriptional activation of a wider range of NRF2 target genes (i.e., metabolic genes in addition to cytoprotective genes).[63] NRF2 activates genes encoding enzymes for NADPH production and the pentose phosphate pathway, and subsequently facilitates the metabolic flux of glucose into purine nucleotide synthesis and that of glutamine into glutaminolysis and glutathione synthesis. An NRF2-addicted lung cancer model generated by triple

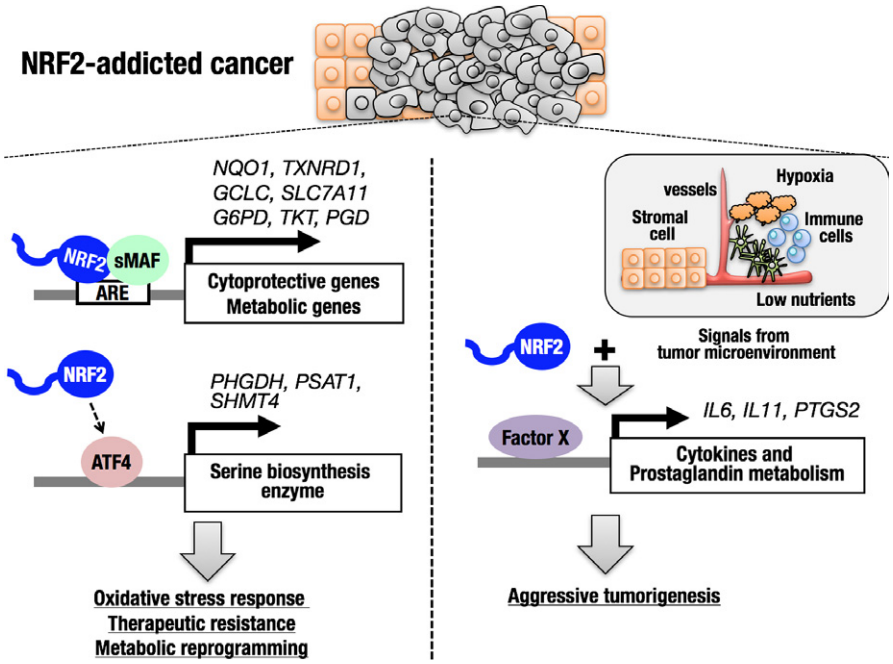

FIGURE 2 Transcriptional regulation by nuclear factor erythroid-derived 2-like 2 (NRF2) and its impacts on NRF2-addicted cancer cells. Canonical (left) and non-canonical (right) downstream genes of NRF2 in NRF2-addicted cancer cells. Direct target genes of NRF2 are upregulated by NRF2 irrespective of signals from the microenvironment (left). Non-canonical downstream genes are upregulated downstream of NRF2 only in the presence of signals from the microenvironment (right). ARE, antioxidant response element; IL, interleukin

mutations of the *Kras*, *Tp53* and *Keap1* genes in mice consistently showed a heavy dependence on glutaminolysis, showing a robust sensitivity to inhibition of SLC1A5, a glutamine transporter.[27] NRF2 also promotes serine synthesis from glucose by indirectly inducing genes in the serine synthesis pathway, namely *PHDGH*, *PSAT1* and *SHMT4*, through ATF4 activation.[23]

Novel downstream effectors of NRF2 in NRF2-addicted cancer models have been identified through the comparison of gene expression profiles in ordinary dish culture conditions and allograft tumor-forming conditions.[74] MEF obtained from wild-type and *Keap1*-null embryos were transformed by SV40 T antigen and oncogenic HRAS to establish WT-TR MEF and *Keap1*$^{-/-}$-TR MEF, respectively. Although cell growth in the culture-dish condition was comparable between WT- and *Keap1*$^{-/-}$-TR MEF, tumorigenic activity of *Keap1*$^{-/-}$-TR MEF was dramatically enhanced compared with WT-TR MEF, and the increased tumorigenic activity of *Keap1*$^{-/-}$-TR MEF was verified as NRF2 dependent. When gene expression profiles were compared between WT-TR MEF and *Keap1*$^{-/-}$-TR MEF in the culture-dish and tumor-forming conditions, canonical NRF2 target genes were all upregulated in *Keap1*$^{-/-}$-TR MEF in both conditions, whereas non-canonical genes encoding cytokines and prostaglandin-metabolizing enzymes were highly upregulated in *Keap1*$^{-/-}$-TR MEF in the tumor-forming condition only (Figure 2, right side). Among the non-canonical genes, *Il11* was found to be critical for the aggressive tumorigenic activity of *Keap1*$^{-/-}$-TR MEF, which is consistent with a clinical observation that expression levels of NRF2 and IL-11 are significantly correlated in breast cancer cases.[74]

Another intriguing difference in the gene expression profiles of WT-TR MEF and *Keap1*$^{-/-}$-TR MEF, unique to the tumor-forming condition, was the significant downregulation of genes encoding MHC class I and antigen-presentation factors in the tumors generated from *Keap1*$^{-/-}$-TR MEF. This result suggests that *Keap1*$^{-/-}$-TR MEF are likely to evade anticancer immunity, which might be an alternative advantage supporting the aggressive tumorigenesis of *Keap1*$^{-/-}$-TR MEF. Thus, the tumor microenvironment has a substantial impact on the expression levels of downstream effectors of NRF2 in NRF2-addicted cancer cells. Detailed mechanisms of the NRF2 contribution to tumorigenesis under various tumor microenvironments need to be clarified in future studies.

6 | FUTURE PERSPECTIVES OF DIAGNOSTIC AND THERAPEUTIC STRATEGIES FOR NRF2-ADDICTED CANCERS

Several NRF2 inhibitors have been reported for the treatment of NRF2-addicted cancers.[61,62] For example, brusatol, which is a plant-derived natural quassinoid, promotes poly-ubiquitination of NRF2, which reduces the NRF2 protein level without changing the transcription level of the *NRF2* gene.[62] Another NRF2 inhibitor, halofuginone, was found to exert a chemosensitizing effect on NRF2-addicted cancer cells.[61] Halofuginone represses prolyl-tRNA synthetase activity leading to translational inhibition. NRF2 protein level is effectively reduced by halofuginone, which is consistent with a short half-life of the NRF2 protein.

New potential therapeutic targets of NRF2-addicted cancers are being identified in addition to NRF2 inhibitors (Table 2). Some of them, such as glutathione synthesis, serine synthesis, the pentose phosphate pathway, and IL-11, are direct or indirect downstream effectors of NRF2 for mediating malignant phenotypes. In contrast

to these downstream effectors of NRF2, upstream regulators causing NRF2 activation, including p62 accumulation, FH mutation, and iASPP, are also possible therapeutic targets.

Recently, enhancement of anticancer immunity in cancer-bearing hosts has been shown to be very effective for eradicating cancers. Because NRF2 activation inhibits immunosuppressive events directed by MDSC and apoptotic T_{reg} cells,[14-16] giving NRF2 inducers to cancer-bearing hosts is expected to be an immunostimulatory therapy against cancer cells. A concern in treating cancer patients with NRF2 inducers is possible malignant progression as a result of NRF2 activation in cancer cells. However, the effects of NRF2 inducers on NRF2-addicted cancer cells are expected to be minimal, as NRF2 is already maximally activated in NRF2-addicted cancer cells, although intratumor heterogeneity must be carefully considered. Appropriate animal models need to be developed to evaluate the indication for NRF2 inducers for NRF2-addicted cancers.

Compared to active exploratory and mechanistic research on therapeutic targets, diagnostic biomarkers and surrogate markers have yet to be developed for NRF2-addicted cancers. Based on the unique metabolic activities of NRF2-addicted cancers, detailed metabolite analysis might lead to the identification of useful diagnostic markers. Diagnostic and therapeutic advances await further studies and technological improvements.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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