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
f 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\(_{\text{reg}}\) cell-mediated immunosuppression by protecting T\(_{\text{reg}}\) 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
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 hepato-cellular 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...
| Tissues | Study scale | Country | Prognosis marker (experiment) | Results | Reference |
|---------|-------------|---------|-----------------------------|---------|-----------|
| Brain   | 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 |
|         | 95          | Japan   | NQO1 and GCLC expression    | Upregulation of NQO1 and GCLC correlates with short progression-free survival. | Kanamori et al., 2015 |
|         | 63          | Taiwan  | NRF2 (IHC)                  | Meningioma patients with high NRF2 expression tend to show short overall survival. | Tsai et al., 2016 |
| 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 |
|         | 235         | USA     | NRF2 (IHC)                  | High NRF2 expression is associated with short overall survival and relapse-free survival. | Solis et al., 2010 |
|         | 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 |
|         | 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 |
| 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 |
| Squamous cell carcinoma | 48 Japan | NRF2 mutation | Squamous cell carcinoma patients with NRF2 mutation have poor prognosis. | Shibata et al., 2008 |
|         | 94 USA      | KEAP1 (IHC) | Low or absent KEAP1 expression is associated with short overall survival and relapse-free survival. | Solis et al., 2010 |
| Esophageal squamous cell carcinoma | 82 Japan | NRF2 mutation | Cancer patients with NRF2 mutation show short overall survival. | Shibata et al., 2011 |
|         | 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 |
| 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 |
|         | 60 Japan    | NRF2 transcriptional profile (microarray) | NRF2-activating transcriptional profiles are associated with poor prognosis. | Shibata et al., 2010 |
| 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   | Phosphorylated-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.
| 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 growth | 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<sup>LSL-G12D</sup> mouse + Adeno-Cre | Mating with Nrf2 knockout mice | NRF2 deficiency inhibits Kras<sup>G12D</sup>-mediated lung carcinogenesis. | DeNicola et al., 2011 | 57           |
|        | Dish culture | 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<sup>LSL-G12D</sup>/.CCSP-Cre mouse | NRF2 Inhibitor (brusatol) | NRF2 inhibition by brusatol sensitizes Kras<sup>G12D</sup>-induced lung cancer to cisplatin treatment. | Tao et al., 2014 | 62           |
|        | Dish culture | A549 NSCLC cell line | NRF2 knockdown by siRNA | NRF2 knockdown inhibits cell growth and suppresses tumorigenesis. | Mitsuishi et al., 2012 | 63           |
|        | GEMM       | Kras<sup>LSL-G12D</sup>/.Tp53<sup>fl/fl</sup> 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 | 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<sup>fl/fl</sup>.Keap1<sup>1/1</sup> mouse + Adeno-Cre/Krt5<sup>CreERT2</sup> | 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<sup>G12V</sup> mouse | Nrf2/Keap1 knockdown by shRNA | NRF2 activation protects cancer stem cells from cisplatin treatment by glutathione production. | Oshimori et al., 2015 | 66           |
| 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 | 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 | NFR2 knockdown decreases proteasome subunit gene expression, impairing misfolded protein degradation, and suppresses anchorage-independent growth. | Chen et al., 2017 | 68 |
|        | Xenograft | | | | | |
| 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 | MIA PaCa2, Capan 2 (pancreatic cancer cell lines) | NRF2 disruption by CRISPR-CAS9 system Mating with Nfr2 knockout mice | NRF2 knockdown decreases sphere formation and tumorigenic activity. p62 accumulation extends survival period of KrasLSLG12D+/+;IKKaΔpan;Pdx1-Cre mice through NRF2 activation. | Todoric et al., 2017 | 69 |
|        | Xenograft | GEMM | | | | |
|        | Organoid culture | Human primary and metastatic tumor cells Suit2 PDA cell line | NRF2 knockdown by shRNA Mating with Nfr2 knockout mice | NRF2 deletion reduces tumor size and proliferation of organoids by protecting translation factors from oxidative stress. | Chio et al., 2016 | 70 |
|        | Xenograft | GEMM | | | | |
|        | GEMM | KrasLSLG12D+/−;Ptf1a-Cre mouse | Mating with Nfr2 knockout mice | NRF2 deletion reduces numbers of total PanN1-1a cells and their Ki67-positive ratios. | DeNicola et al., 2011 | 57 |
| Melanocyte | Dish culture | B16 melanoma cells | BSO treatment | Glutathione synthesis promoted by NRF2 confers temozolomide resistance. | Rocha et al., 2016 | 71 |
| Prostate | Dish culture | 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 |
|         | Xenograft | | | | | (Continues) |
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. 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. 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. 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, whereas these triple mutations in the pancreas do not cause cancers but result in fibrosis instead. 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). 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 active.
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. NRF2 also promotes serine synthesis from glucose by indirectly inducing genes in the serine synthesis pathway, namely PHGDH, PSAT1 and SHMT4, through ATF4 activation.

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. 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.

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 anticaner 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.

### Future Perspectives of Diagnostic and Therapeutic Strategies for NRF2-Addicted Cancers

Several NRF2 inhibitors have been reported for the treatment of NRF2-addicted cancers. 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. Another NRF2 inhibitor, halofuginone, was found to exert a chemosensitizing effect on NRF2-addicted cancer cells. 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...
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\(_{\text{reg}}\) 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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