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Development of targeted therapy of NRF2^{high} esophageal squamous cell carcinoma

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ABSTRACT

Esophageal squamous cell carcinoma (ESCC) is a deadly disease and one of the most aggressive cancers of the gastrointestinal tract. As a master transcription factor regulating the stress response, NRF2 is often mutated and becomes hyperactive, and thus causes chemo-radioresistance and poor survival in human ESCC. There is a great need to develop NRF2 inhibitors for targeted therapy of NRF2^{high} ESCC. In this review, we mainly focus on three aspects, NRF2 inhibitors and their mechanisms of action, screening novel drug targets, and evaluation of NRF2 activity in the esophagus. A research strategy has been proposed to develop NRF2 inhibitors using human ESCC cells and mouse models.

1. Introduction

Esophageal cancer is expected to affect 19,260 adults and cause 15,530 deaths in the US in 2021 [1]. In the world, it is the seventh most prevalent cancer and the sixth leading cause of cancer-related death, with more than 604,100 new cases and 544,076 deaths in 2020 [2]. Two main histological types of esophageal cancer exist, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma. Human ESCC develops from precancerous lesions, and its histopathology follows a step-wise pattern of hyperplasia, dysplasia, and squamous cell carcinoma (SCC). The 5-year survival rate for ESCC is ~18%, a number that reflects late diagnosis, the aggressiveness of the disease, and a lack of effective treatment strategies [3,4]. Esophageal adenocarcinoma has become the predominant type of esophageal cancer in Western countries, and gastroesophageal reflux disease and obesity are the main risk factors. Barrett's esophagus is a precancerous lesion that may further develop into dysplasia and adenocarcinoma [5]. Similar to ESCC, adenocarcinoma has a very poor prognosis. Thus, there is a great need to further elucidate the molecular mechanisms and develop more effective treatment strategies for esophageal cancer.

Nuclear factor (erythroid-derived 2)-like 2 (*NRF2* or *NFE2L2*) mutations are commonly seen in ESCC, with frequencies between 5% and 30% [6]. As a major cellular defense mechanism, the NRF2 signaling pathway regulates the expression of enzymes involved in detoxification and anti-oxidative stress response. NRF2 forms heterodimers with small MAF proteins and binds to the antioxidant response elements (ARE) of target genes when cells are exposed to oxidative stress or xenobiotics. Kelch-like ECH-associated protein 1 (KEAP1) inhibits the function of NRF2 by retaining NRF2 in the cytoplasm and facilitating its ubiquitination-dependent degradation under normal physiological conditions. Oxidative stress and electrophilic modification of KEAP1 results in NRF2 nuclear translocation and ARE-dependent gene transcription (Fig. 1) [7]. In addition to the KEAP1-CUL3 E3 ubiquitin ligase complex, NRF2 can also be ubiquitinylated by β-TrCP-CUL1 or HRD1 for

Abbreviations: ARE, antioxidant response element; ESCC, esophageal squamous cell carcinoma; KEAP1, Kelch-like ECH-associated protein 1; NRF2/NFE2L2, nuclear factor erythroid 2-related factor 2; SCC, squamous cell carcinoma.

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proteasomal degradation [8,9].

The NRF2 signaling pathway is a double-edged sword in the context of carcinogenesis. Chemical or genetic activation of NRF2 induces cytoprotective enzymes conferring protection against chemical carcinogenesis in multiple models. *Nrf2*$^{-/-}$ mice are more susceptible to chemical carcinogenesis than wild-type counterparts [10,11]. On the other hand, cancer cells can hijack the NRF2 signaling pathway for their survival through mechanisms that lead to constitutive activation of NRF2 signalings, such as somatic mutations of *KEAP1/NRF2/CUL3*, accumulation of disruptor proteins, skipping of *NRF2* exon 2, KEAP1 succinylation, *KEAP1* hypermethylation, increased NRF2 expression, and electrophilic attack of KEAP1 by oncometabolites. NRF2 hyperactivation promotes cell proliferation and metabolic reprogramming, accelerates distant metastases, and confers chemo- and radio-resistance [12,13]. *NRF2* and *KEAP1* are classified as high-confidence cancer driver genes [14]. Although not yet realized, the NRF2 signaling pathway is regarded as a tractable molecular target for cancer therapy [15]. We have previously reviewed the functional role of NRF2 in ESCC [16].

Using gene microarray data of human ESCC (GEO23400; $n = 53$) [17], we performed clustering analysis with a list of esophageal NRF2 target genes [18] and identified two subtypes, NRF2^{high} cases ($n = 17$) and NRF2^{low} cases ($n = 36$) [19]. Similarly, human ESCC can be clustered into NRF2^{Mut} and NRF2^{WT} cases according to DNA mutations. NRF2^{Mut} ESCC was associated with a significantly worse prognosis than NRF2^{WT} ESCC [20]. These data suggest subtyping human ESCC according to the NRF2 status is essential for targeted therapy, and potent NRF2 inhibitors are highly desirable.

The esophagus is a unique organ site for studies on NRF2 hyperactivation in cancer. Genetic activation of NRF2 in *Keap1*$^{-/-}$ mice results in robust phenotypes in the esophagus, esophageal hyperplasia and hyperkeratosis [21]. To date, all transcriptionally impacted genes downstream of *Keap1* knockout are NRF2 responsive, although it remains possible that other KEAP1 substrates could alter transcription by an NRF2-independent mechanism. Overexpression of KEAP1 substrates (e.g., WTX, PALB2, SQSTM1, DPP3, CDK20) containing competitive binding motifs (e.g., ETGE, DLG) results in NRF2 displacement and subsequent NRF2 activation [22–29]. However, in the esophagus, NRF2 is the most important KEAP1 substrate as the esophageal phenotype of *Keap1*$^{-/-}$ mice can be completely rescued in *Nrf2*$^{-/-}$;*Keap1*$^{-/-}$ and *K5Cre;Nrf2*$^{fl/fl}$;*Keap1*$^{-/-}$ mice [21,30].

It should be noted that in addition to ESCC, genetic mutations and copy-number alterations of the NRF2 signaling pathway are commonly seen in human cancers, most notably non-small cell lung cancer, uterine carcinoma, head and neck cancer, and bladder carcinoma [31]. Thus, mechanistic studies on and therapeutic development for NRF2^{high} ESCC will also likely benefit patients of these cancers.

2. NRF2 inhibitors and their mechanisms of action

At least 5 strategies have been proposed to target the NRF2 signaling pathway for cancer therapy: (1) transcriptional downregulation of *NRF2*; (2) increased degradation of *NRF2* mRNA or decreased translation; (3) enhancement of NRF2 degradation through up-regulation/activation of E3 ubiquitin ligase complexes for NRF2; (4) blocking the dimerization of NRF2 with small MAF proteins; and (5) blocking the NRF2-sMAF DNA-binding domain [7]. Many small-molecule compounds with NRF2-inhibitory activities have been reported in the literature (Table 1). However, some NRF2-inhibitory compounds, i.e., 4-methoxychalcone, apigenin, ascorbic acid, BET bromodomain inhibitor JQ1, chrysin, cryptotanshinone, epigallocatechin 3-gallate, luteolin, metformin, N-*N*-dimethylformamide, trichostatin A, triptolide, valproic acid, and wogonin, in fact, activate NRF2 expression and/or activity in some other experimental settings. These compounds have been well-reviewed in the literature and may have limited potential for further drug development [15,32,33]. For example, apigenin significantly sensitized doxorubicin-resistant BEL-7402 cells to doxorubicin by dramatically reducing NRF2 expression at both the mRNA and protein levels through downregulation of PI3K/Akt pathway [34]. On the contrary, apigenin activated NRF2 nuclear translocation, nuclear NRF2-ARE binding activity, ARE-dependent luciferase activity, and expression NRF2 target genes in rat primary hepatocytes and human hepatoma HepG2 cells [35,36].

High-throughput screening of chemical compound libraries containing natural products, synthetic compounds, and FDA-approved drugs is a popular approach to identify candidate compounds or compound classes with NRF2-inhibitory activities [37–41]. Apart from small molecule inhibitors, siRNA is also promising for targeting NRF2 [42,43]. However, siRNA formulation and delivery into esophageal epithelial cells in vivo are major hurdles in drug development. Following the screen, compounds are subject to validation of their NRF2-inhibitory activities in cultured cells and animal models. However, in vitro screen is subject to limitations. Variations in culture conditions (e.g., seeding density, cell cycle, growth status, medium, confluence, cell line divergence from the original sources), affect drug response in the cells [44].

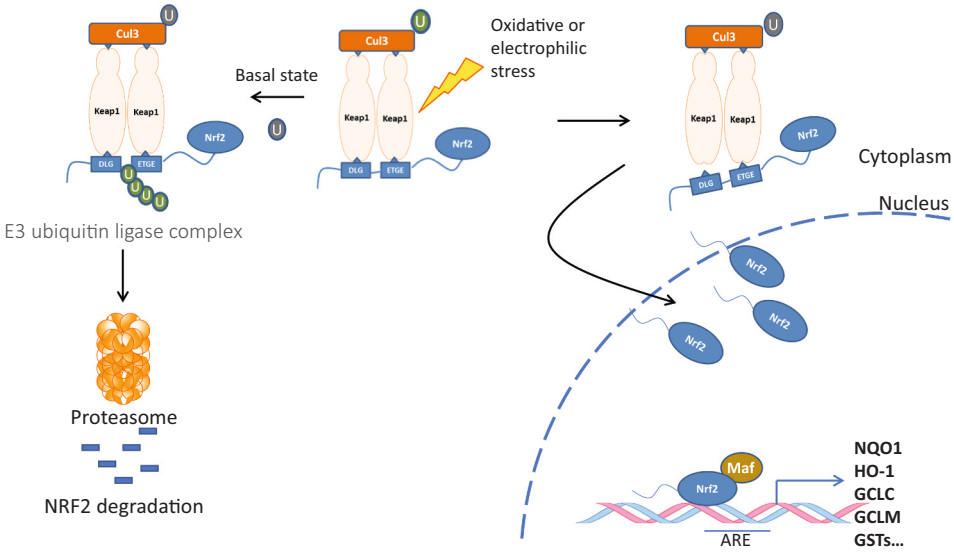

Fig. 1. NRF2 signaling pathway. Under basal conditions, NRF2 is bound to KEAP1, which is a CUL3-based E3 ubiquitin ligase adapter that regulates NRF2 ubiquitination and proteasomal degradation. When cells are exposed to oxidative or electrophilic stress, KEAP1 changes its conformation and releases NRF2. Then NRF2 translocates into the nucleus, forms a heterodimer with its obligatory partner (small MAF proteins), binds to the ARE, and activates the transcription of downstream genes, such as antioxidative genes and phase II detoxification enzymes.

Table 1
NRF2 inhibitors.

Compound	Mechanisms of Action	Note
(E)-3-(3,5-dimethoxyphenyl)-1-(2-methoxyphenyl)prop-2-en-1-one [90]	Unknown	
1-(4-(tert-Butyl)benzyl)-3-(4-chlorophenyl)-N-hydroxy-1H pyrazole-5-carboxamide [91]	Unknown	
3′,4′,5′,5,7-pentamethoxy flavone [92]	Upregulates KEAP1 expression and inhibits ERK	
Ailanthone [93,94]	Post-translational mechanisms, i.e., increase of KEAP1 and decrease of UCHL1 deubiquitinase	
All-trans-retinoic acid [95–97]	Inhibits NRF2-ARE binding through a direct interaction between NRF2 and RARα	
AEM1 [37]	Unknown	High-throughput screen
Bexarotene [96,98]	RXRα activation which interacts with the Neh7 domain of NRF2 and antagonizes ARE-dependent mRNA expression	RXRα-specific ligand
Brusatol [99–101]	Global translation inhibitor	
Camptothecin [102]	May inhibit NRF2 transcription, translation and/or promoting mRNA degradation	DNA topoisomerase I inhibitor
Clobetasol propionate [38] [103]	Binds to glucocorticoid receptor and recruits a corepressor to suppress ARE-dependent transcription; Prevents NRF2 nuclear translocation; Promotes NRF2 degradation by promoting β-TrCP-dependent degradation in a glucocorticoid receptor and GSK3-dependent manner	High-throughput screen
Corvallatoxin (digoxigenin, cymarin) [104]	Activates GSK3β and thus promotes NRF2 degradation	Na^+/K^+-ATPase inhibitor
Costunolide [105]	Inhibits telomerase	
Halofuginone [106]	Inhibits prolyl-tRNA synthetase and thus global translation	
HER2 antibodies (Trastuzumab, Pertuzumab) [107]	*NRF2* promoter hypermethylation	
Homoharringtomine [108]	Stabilizes GC-rich sequence in 5′-UTR of *NRF2* DNA	
HSP90 inhibitors (17-AAG, 17-DMAG, IPI-504) [80]	Metabolism by NRF2 target genes (NQO1 and TXNRD1) into more potent HSP90 inhibitors	
IM3829 (4-(2-Cyclohexylethoxy)aniline) [109]	Inhibits NRF2 nuclear translocation	
Isoniazid [110,111], Ethionamide [112]	Activates SIRT1; Inhibits NRF2 nuclear translocation by inhibiting ERK1 phosphorylation	Anti-tubercular drug
K67 and its derivatives [113,114]	Inhibits KEAP1-phospho-p62 protein-protein interaction and thus promotes NRF2 degradation	
Malabaricone-A [115]	Unknown	
Mitomycin C [81]	Bioactivation by NRF2 target genes (cytochrome P450 reductase, NQO1 and PPP enzymes) causes DNA damage	
ML385 [39]	Binds to the Neh1 domain of NRF2 and thus interferes with the DNA binding activity of NRF2-MAFG complex	High-throughput screen
Ochratoxin A [116–118]	Inhibits NRF2 nuclear translocation, NRF2-ARE binding, and histone acetylation, and increases miR-132	
Sorafenib [119]	Unknown	Inhibitor of multiple kinases (VEGFR, PDGFR and RAF)
Tetrahydrocarbazoles [120]	May stabilize KEAP1-NRF2 interaction	
Trigonelline [121,122]	Inhibits NRF2 nuclear translocation	
Vorinostat [123]	Downregulates c-Myc, increases KEAP1 expression, and inhibits NRF2 nuclear translocation	
• PHA-767491 • AZ-628 • SL-327 [41]	Inhibits NRF2 nuclear translocation	• Cdc7/CDK9 inhibitor • RAF inhibitor • MEK inhibitor
• Stattic • Grassypeptolide A • Cardiac glycosides (lantoside C, strophanthidin, peruvoside, proscillaridin, ouabain) • Emetin and anisomycin • Actin-disrupting agents (lyngbyabellin A, dolastatin 12) [38,40]	• STAT3 inhibitor • May inhibit DPP8 and DPP4 and thus promote NRF2 degradation • May inhibit Na^+,K^+-ATPase pump and thus decrease KEAP1 phosphorylation [124], or through HIF pathway • Protein synthesis inhibitor • Possibly interferes actin polymerization and NRF2 nuclear translocation	High-throughput screen
• Antimetabolites (e.g., methotrexate) [38]		High-throughput screen

While the NRF2-inhibitory activity of many compounds can be validated using ESCC cell lines in vitro, they have rarely been validated in proper animal models. Organ-specific animal models are needed to determine whether these compounds are effective at nontoxic doses to suppress ESCC. In order to develop NRF2 inhibitors for $NRF2^{high}$ ESCC, CRISPR-Cas9 was used to create a novel mouse carrying the $Nrf2^{E79Q}$ mutation within the endogenous *Nfe2l2* locus, which is the most commonly observed activating mutation in human cancer and is known to activate NRF2 [45]. This mouse line allows conditional esophagus-specific activation of NRF2 when crossed with *K14Cre* line or *Sox2CreER* line [46]. Four weeks after tamoxifen exposure, $Sox2CreER;LSL\text{-}Nrf2^{E79Q/+}$ mice developed strong $NRF2^{high}$-driven esophageal phenotype in the esophagus, similar to $Keap1^{-/-}$ mice (data not shown). Combination of *Nrf2* mutation and esophagus-specific carcinogen exposure (e.g., 4-nitroquinoline 1-oxide, *N*-nitrosomethylbenzylamine) is expected to generate $NRF2^{high}$ ESCC in mice for testing the cancer therapeutic efficacy of NRF2 inhibitors.

To our best knowledge, none of the NRF2 inhibitors have entered clinical trials for targeted therapy. It remains necessary to identify more novel NRF2 inhibitors and clarify their mechanisms of action. Such compounds must be potent in the target cells with acceptable toxicity profiles and act through proper mechanisms. Halofuginone and brusatol inhibit NRF2 through inhibition of global protein synthesis, thus decreasing enthusiasm for further clinical development.

3. Drug targets in NRF2^{high} ESCC

Historically transcription factors are viewed as "undruggable". This is mainly because of the challenges associated with targeting either the protein–DNA or protein–protein interactions that mediate their functions, as opposed to more tractable active sites of enzymes or receptors [47]. While multiple approaches have been developed to target transcription factors, some other genes may represent viable therapeutic targets if they prove functionally critical. For example, NR0B1 was identified as a selectively expressed protein in NRF2^{high} lung cancer and small molecules that disrupted NR0B1 protein complexes inhibited NRF2-dependent lung cancer growth [48]. To find additional drug targets for NRF2^{high} ESCC, we downloaded the publicly available omics data of human cancer cell lines among which 22 human ESCC cell lines were included (Table 2; Supplementary Material). Using the RNAseq data and a list of differentially expressed genes in NRF2^{high} ESCC [19], we performed clustering analysis and principal component analysis to subtype ESCC cell lines into two clusters, NRF2^{high} and NRF2^{low} (Fig. 2A, B). Among the NRF2^{high} ESCC cell lines, KYSE70 (*NFE2L2*^{W24C}), KYSE180 (*NFE2L2*^{D77V};*KEAP1*^{P278Q}), KYSE520 (*NFE2L2*^{T80I}), OE21 (*NFE2L2*$^{G81S/D318H}$), TE6 (*NFE2L2*$^{F71\_D77del}$), and TE11 (*NFE2L2*^{D29G}) are NRF2^{Mut} cells, and KYSE510, TE9, and TT are NRF2^{WT} cells. KYSE510 has an NRF2^{high} status probably due to *PIK3CA*^{E545K} mutation. TE9 has been reported to express a high level of NRF2 [49].

To validate the clustering analysis, we compared the metabolomics data of NRF2^{high} ESCC cells and NRF2^{low} ESCC cells. Several metabolites (e.g., glutathione oxidized, glutathione reduced, NADP) are significantly higher, and several others (e.g., 6-phosphogluconate, glycine, aspartate, glutamate) are significantly lower in NRF2^{high} ESCC cells than NRF2^{low} ESCC cells. These observations are in agreement with several studies on the functional role of hyperactive NRF2 in cellular metabolomics [19,49,50]. Using the combined RNAi screen data of 20 ESCC cell lines (9 NRF2^{high} and 11 NRF2^{low}), we also found that NRF2^{high} ESCC cells were more dependent on the *NFE2L2* gene than NRF2^{low} ESCC cells (Fig. 2C). These data support the clustering result of NRF2^{high} and NRF2^{low} ESCC cells.

We then compared gene dependency scores of NRF2^{high} ESCC cells with those of NRF2^{low} ESCC cells using data from two screening techniques, RNAi and CRISPR. It is generally believed that these two techniques are complementary especially when an improved bioinformatics tool is used to eliminate the off-target effect of RNAi [51]. The precision of the two libraries in detecting essential genes is similar and combining data from both screens improves performance, even though results from these two screens show little correlation, which can be partially explained by the identification of distinct essential biological processes with each technology [52,53]. In total, 9 genes (*ABL1, ALDH3A1, C1QTNF9B, CASC3, DDX4, EXOSC3, GPAA1, SNAP47, SYT2*) were found to have higher dependency (i.e., lower dependency scores) in NRF2^{high} ESCC cells by both screens. For example, ABL1, as a non-receptor tyrosine kinase, is known to be essential and actionable for lung cancer cell survival [54]. ABL1 promotes NRF2 nuclear localization in kidney cancer cells [55]. ALDH3A1 oxidizes various aldehydes to the corresponding acids. NRF2 is known to regulate ALDH3A1 expression in pancreatic cancer cells [56]. ALDH3A1 is strongly expressed in human ESCC tissue, but barely detectable in the non-malignant esophageal epithelium. Knockdown of *ALDH3A1* in ESCC cells suppresses cell viability and clonogenic capacity as well as tumor growth in vivo [57]. These data suggest that ABL1 and ALDH3A1 are potential drug targets for NRF2^{high} ESCC.

There is a strong rationale to target kinases in combination with NRF2 inhibition for NRF2^{high} ESCC. Multiple signaling pathways, for example, PI3K/AKT/mTOR can activate NRF2 [58]. AKT can also increase the stability of NRF2 by activating p21 which disrupts the NRF2-KEAP1 interaction, and by inhibiting GSK3β which leads to reduced NRF2 phosphorylation, prevents its nuclear translocation, and promotes its ubiquitination and degradation [23,59]. On the other hand, NRF2 overexpression is known to activate PI3K/AKT signaling in melanocytes and hepatocytes [60,61]. NRF2 directly regulates mTOR transcription when the PI3K pathway is intact, whereas this function is lost when PI3K is activated [62]. It should be noted that keratinocyte-specific deficiency of *Pten* resulted in AKT activation and esophageal hyperplasia and hyperkeratosis [63], similar to the NRF2^{high}-driven esophageal phenotype. Recently, the NRF2 and PI3K pathways have been shown to synergize in driving non-small-cell lung cancer which was associated with metabolic reprogramming and altered immune microenvironment [64]. These data suggest that the NRF2 and PI3K pathways play a synergistic role in promoting carcinogenesis through mutually reciprocal positive reinforcement. In fact, in a pan-cancer analysis of TCGA datasets, the strongest co-occurrence of affected pathways was between genomic alterations of the NRF2 and PI3K pathways. These co-occurring alterations appeared most frequently in lung cancer, ESCC, head and neck SCC, and uterine carcinoma [65].

Synthetic lethality is another potential approach to identify additional drug targets for NRF2^{high} ESCC [66,67]. Using isogenic ESCC cells with varying NRF2 status and high-throughput screening technologies, this approach may open up novel therapeutic opportunities.

Table 2
Multi-omics data of human ESCC cell lines.

Data	Spreadsheet in Supplementary Material	Weblink	Reference
RNAseq	CCLE_RNAseq-genes-rpkm_ESCC	https://depmap.org/portal/download/	[125]
Metabolomics	Metabolomics data	https://portals.broadinstitute.org/ccle/data	[126]
CRISPR gene dependency screen	Achilles CRISPR Dependency	https://depmap.org/portal/download/	[127]
RNAi gene dependency screen	Combined RNAi Dependency	https://depmap.org/portal/download/	[51]
PRISM drug sensitivity screen	PRISM Drug Sensitivity	https://depmap.org/portal/download/	[71]
CTRP V2 drug sensitivity screen	CTRP V2	https://depmap.org/portal/download/	[69]
GDSC1 drug sensitivity screen	GDSC1	https://depmap.org/portal/download/	[68,70]
GDSC2 drug sensitivity screen	GDSC2	https://depmap.org/portal/download/	[68,70]
Protein-Protein Interaction and drug sensitivity	Original data not available	https://mutanome.lerner.ccf.org	[79]

4. Drug candidates for NRF2^{high} ESCC

In recent years, several large-scale drug sensitivity databases have become publically available. These databases provide a huge information base for further data mining that may lead to discovery of new drug candidates or drug repurposing opportunites [68–71]. Using drug sensitivity data from three databases (PRISM, CTRP V2, and GDSC), we compared drug sensitivity scores of NRF2^{high} ESCC cells in comparison to NRF2^{low} ESCC cells (Supplementary Material). From the PRISM database, we found NRF2^{high} ESCC cells are significantly more sensitive to 62 drugs than NRF2^{low} ESCC cells. These 62 drugs mainly fall into two classes, microtubule inhibitors and kinase inhibitors (especially Aurora A kinase inhibitors). For example, alisertib (an Aurora A kinase inhibitor) is known to induce oxidative stress and inhibit the expression of NRF2 in osteosarcoma cell lines [72]. Crizotinib (an inhibitor of ALK and c-Met) induces hepatotoxicity to reduce cancer cell viability, by

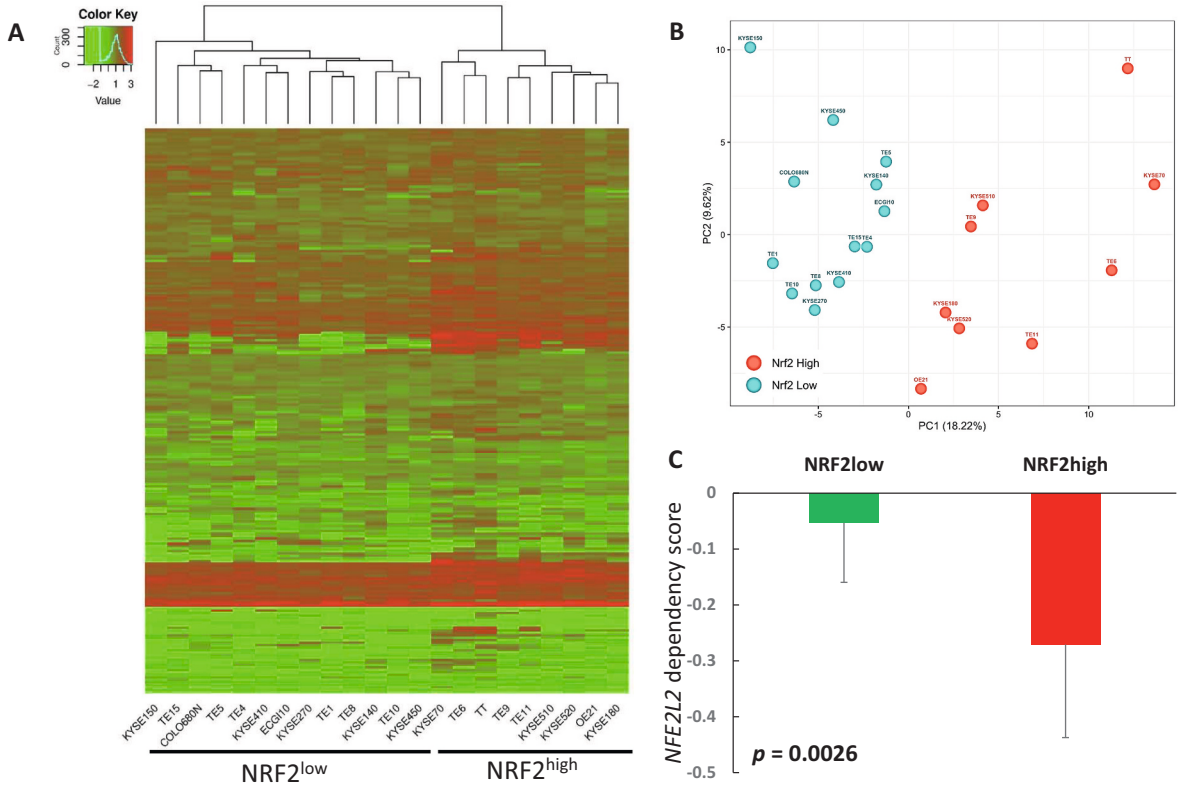

Fig. 2. Subtyping 22 ESCC cells into two subtypes, NRF2^{high} and NRF2^{low}, using RNAseq data. (A) Clustering analysis; (B) Principal component analysis; (C) Significant dependency on the *NFE2L2* gene by NRF2^{high} ESCC cells as compared to NRF2^{low} ESCC cells. Student's *t*-test is used for statistical evaluation of *NFE2L2* gene dependency score using the original data from the combined RNAi screen.

activating oxidative stress responses, stimulating mitochondrial apoptosis and necrosis, accumulating reactive oxygen species, and inhibiting NRF2 signaling [73]. Apatinib (a VEGFR2 tyrosine kinase inhibitor) promotes oxidative stress-dependent apoptosis, by suppressing glutathione and NRF2 signaling in ovarian cancer cells [74].

From the CTRP V2 database, we found 4 drugs are more sensitive for NRF2^{high} ESCC cells than NRF2^{low} ESCC cells: fluorouracil, leptomycin B, BRD-K71781559, tanespimycin. From GDSC1 and GDSC2 databases, we found motesanib and navitoclax, respectively. Motesanib (sorafenib) is a multi-kinase inhibitor that selectively inhibits EGFR1, VEGFR2, and VEGFR3, and inhibits tumor growth. A study of sorafenib in the treatment of advanced gastric and gastroesophageal cancers showed that when combined with docetaxel and cisplatin, the regimen was effective in reducing tumor size and increase progression-free survival and overall survival [75]. Lung cancer cells that were sensitive to trametinib plus navitoclax (a BCL-xL/BCL-2 inhibitor) expressed higher levels of NRF2 than did those that were resistant [76]. Navitoclax also induces apoptosis and synergizes with chemotherapy by targeting stemness pathways in esophageal cancer [77,78].

Furthermore, in a recent study on the pharmacogenomics landscape of protein-protein interaction (PPI)-perturbing mutations, NRF2-KEAP1 PPI was identified as one of the top PPIs among 470 putative PPIs in a pan-cancer analysis of 33 cancer types. Sensitivity to 8 drugs was found higher in cancer cells with mutant NRF2-KEAP1 PPI as compared with those with wild-type NRF2-KEAP1 PPI: 17-AAG, docetaxel, temsirolimus, JNJ-2684165, midostaurin, mitomycin C, ZG-10, and embelin [79]. Interestingly, both 17-AAG and mitomycin C have been reported in the literature to be more potent for NRF2^{high} cancer cells than NRF2^{low} cancer cells acting through synthetic lethality [80,81].

5. Monitoring NRF2 activity in ESCC

When NRF2 inhibitors are used for clinical trials on NRF2^{high} ESCC, there will be a need to assess the NRF2 activity in the cancer tissue to serve two clinical needs: (1) Diagnosis of NRF2^{high} ESCC: We currently depend on exome sequencing to detect gene mutations, RNAseq to elucidate mRNA expression profiles, or quantitative multiplex immunohistochemistry to evaluate protein expression, for selecting cancer patients for targeted therapy. Although this tissue-based invasive approach has been widely used and is reasonably successful in clinical practice, they all require invasive biopsy. In addition, more or less they downplay tumor heterogeneity by using one piece of tumor or biopsy tissue to represent the whole tumor. (2) Evaluation of the efficacy of NRF2 inhibitors: The efficacy of cancer-targeted therapy is assessed in the same way as traditional cancer therapy, and clinicians depend on symptoms, biomarkers, and radiology to guide treatment planning. More efficient and non-invasive tools that can reveal molecular changes as well as functional abnormalities are highly needed (Table 3).

We have generated an NRF2^{high} mRNA signature that is responsive to NRF2 inhibition and an NRF2^{high} protein signature. Algorithms will be generated for the assessment of NRF2 activity in ESCC tissue samples. In addition, hyperactive NRF2 is known to cause metabolic reprogramming in the esophagus through transcriptional regulation of metabolic genes, for example, glucose transporter/enzymes (GLUT1, HK1, HK2) and acetate enzyme (ACSS2) [19,82]. NRF2 also positively regulates the expression of a monocarboxylate transporter (MCT1) in mouse skeletal muscle, colonic epithelial cells, and mouse liver [83,84]. It has become feasible to use PET/CT imaging tools with radionuclides to evaluate whether an individual's cancer is NRF2^{high} before treatment and whether an NRF2 inhibitor successfully hits NRF2 after treatment. For example, ^{18}F-FDG, is widely used for clinical staging and follow-up of human cancer including ESCC. A multicenter prospective trial showed

Table 3
Approaches for the evaluation of NRF2 activity in the esophagus.

Sample	Assay	Measurement	Pros	Cons
Surgical/biopsy specimens	Whole exome sequencing	*NRF2, KEAP1, CUL3* mutations	Accurately reflect the NRF2 status in cancer tissues	Invasive procedure to harvest tissue samples; Potential variations due to tumor heterogeneity; High quality of tissue samples;
	RNAseq or qPCR	$NRF2^{high}$ signature		
	Quantitative multiplex IHC	Nuclear localization of NRF2 and overexpression of NRF2 and its target genes		
	NanoString	A combination of DNA, mRNA, proteins		
Live animal or human patients	PET/CT	Radionuclide avidity	Reflect the functional status of NRF2 in the whole cancer tissues; Non-invasive and convenient for clinical follow-up	Possible lack of specificity for NRF2 status

that ^{18}F-FDG PET/CT had a sensitivity of 79% and a specificity of 95% for stage IV human ESCC, while early-stage T1 and T2 tumors tended to have minimal or no FDG uptake [85]. ^{18}F-FDG is transported into the cells via glucose transporters on the membrane (e.g., GLUT1) and metabolized in the cytosol into ^{18}F-FDG-6P by hexokinases (e.g., HK1, HK2), which are overexpressed in NRF2 hyperactive tissues [86]. In patients with lung SCC, the maximum standardized uptake value (SUVmax) was significantly higher in $NQO1^{high}$ tumors than $NQO1^{low}$ tumors (NQO1 is a bona fide NRF2 target) [87]. GLUT1 expression is significantly correlated with SUVmax of ^{18}F-FDG in human ESCC tissues [88]. ^{11}C-acetate is transported into the cells via monocarboxylate transporters on the membrane (e.g., MCT1), and converted into ^{11}C-acetyl-CoA by acyl-CoA synthetase short-chain family members (e.g., ACSS2). ^{11}C-acetyl-CoA will then be used for de novo lipogenesis, histone acetylation, and the tricarboxylic acid cycle. ^{11}C-acetate PET/CT is primarily used for detecting prostate cancer due to the poor uptake of ^{18}F-FDG and the proximity of the prostate to the bladder [89].

We have conducted a preliminary imaging study on wild-type and *Sox2CreER;LSL-Nrf2*^{E79Q} mice using ^{18}F-FDG/^{11}C-acetate PET imaging in combination with contrast-enhanced CT. Both ^{18}F-FDG uptake and ^{11}C-acetate uptake were significantly elevated in the $NRF2^{high}$ esophagus as compared to the control. Ex vivo autoradiography of ^{11}C-acetate showed dramatic accumulation in the $NRF2^{high}$ esophagus (data not shown). Further research is ongoing to develop dual ^{18}FDG/^{11}C-acetate PET for assessment of NRF2 activity in the esophagus. Although the idea remains exploratory or speculative, metabolic imaging warrants further studies in consideration of the critical role of NRF2 in metabolism.

6. Conclusion

There is a pressing need for NRF2 inhibitors for targeted therapy of $NRF2^{high}$ ESCC and other $NRF2^{high}$ cancers. Promising inhibitors and novel drug targets have been identified and await further validation, in particular, in animal models. NRF2 activity assays with tissue-based methods and imaging tools are highly needed. Further research is expected to develop the inhibitors and elucidate their mechanisms of action for $NRF2^{high}$ ESCC.

Conflict of interest statement

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Comparison of $NRF2^{high}$ and $NRF2^{low}$ ESCC cells. This file contains the RNAseq data (22 cell lines), metabolomics data (22 cell lines), combined RNAi gene dependency screen data (cell viability as the readout, 20 cell lines), Achilles CRISPR gene dependency screen data (cell viability as the readout, 16 cell lines), PRISM drug sensitivity data (viability as the readout, 20 cell lines), CTRP V2 drug sensitivity data (cell viability as the readout, 20 cell lines), GDSC1 drug sensitivity data (cell viabilility IC_{50} and AUC as readouts, 22 cell lines), and GDSC2 drug sensitivity data (cell viabilility IC_{50} and AUC as readouts, 20 cell lines). Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellsig.2021.110105.

References

[1] R.L. Siegel, K.D. Miller, H.E. Fuchs, et al., Cancer statistics, 2021, CA Cancer J. Clin. 71 (2021) 7–33.
[2] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 71 (2021) 209–249.
[3] X. Kang, K. Chen, Y. Li, et al., Personalized targeted therapy for esophageal squamous cell carcinoma, World J. Gastroenterol. 21 (2015) 7648–7658.
[4] Y. Liu, Z. Xiong, A. Beasley, et al., Personalized and targeted therapy of esophageal squamous cell carcinoma: an update, Ann. N. Y. Acad. Sci. 1381 (2016) 66–73.
[5] A.K. Rustgi, H.B. El-Serag, Esophageal carcinoma, N. Engl. J. Med. 371 (2014) 2499–2509.
[6] Cancer Genome Atlas Research N, Analysis Working Group: Asan U, Agency BCC, Integrated genomic characterization of oesophageal carcinoma, Nature 541 (2017) 169–175.
[7] M.R. de la Vega, M. Dodson, E. Chapman, et al., NRF2-targeted therapeutics: new targets and modes of NRF2 regulation, Curr. Opin. Toxicol. 1 (2016) 62–70.
[8] L. Baird, M. Yamamoto, The molecular mechanisms regulating the KEAP1-NRF2 pathway, Mol. Cell. Biol. 40 (2020).
[9] B. Harder, T. Jiang, T. Wu, et al., Molecular mechanisms of Nrf2 regulation and how these influence chemical modulation for disease intervention, Biochem. Soc. Trans. 43 (2015) 680–686.
[10] A. Lan, W. Li, Y. Liu, et al., Chemoprevention of oxidative stress-associated oral carcinogenesis by sulforaphane depends on NRF2 and the isothiocyanate moiety, Oncotarget 7 (2016) 53502–53514.
[11] A. Ohkoshi, T. Suzuki, M. Ono, et al., Roles of Keap1-Nrf2 system in upper aerodigestive tract carcinogenesis, Cancer Prev. Res. (Phila.) 6 (2013) 149–159.
[12] M. Yamamoto, T.W. Kensler, H. Motohashi, The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis, Physiol. Rev. 98 (2018) 1169–1203.
[13] M. Rojo de la Vega, E. Chapman, D.D. Zhang, NRF2 and the hallmarks of cancer, Cancer Cell 34 (2018) 21–43.
[14] D. Tamborero, A. Gonzalez-Perez, C. Perez-Llamas, et al., Comprehensive identification of mutational cancer driver genes across 12 tumor types, Sci. Rep. 3 (2013) 2650.
[15] J. Zhu, H. Wang, F. Chen, et al., An overview of chemical inhibitors of the Nrf2-ARE signaling pathway and their potential applications in cancer therapy, Free Radic. Biol. Med. 99 (2016) 544–556.
[16] S. Ma, C. Paiboonrungruan, T. Yan, et al., Targeted therapy of esophageal squamous cell carcinoma: the NRF2 signaling pathway as target, Ann. N. Y. Acad. Sci. 1434 (1) (2018) 164–172.
[17] H. Su, N. Hu, H.H. Yang, et al., Global gene expression profiling and validation in esophageal squamous cell carcinoma and its association with clinical phenotypes, Clin. Cancer Res. 17 (2011) 2955–2966.
[18] H. Chen, J. Li, H. Li, et al., Transcript profiling identifies dynamic gene expression patterns and an important role for Nrf2/Keap1 pathway in the developing mouse esophagus, PLoS One 7 (2012), e36504.
[19] J. Fu, Z. Xiong, C. Huang, et al., Hyperactivity of the transcription factor NRF2 causes metabolic reprogramming in mouse esophagus, J. Biol. Chem. 294 (2019) 327–340.

[20] Y. Cui, H. Chen, R. Xi, et al., Whole-genome sequencing of 508 patients identifies key molecular features associated with poor prognosis in esophageal squamous cell carcinoma, Cell Res. 30 (2020) 902–913.
[21] N. Wakabayashi, K. Itoh, J. Wakabayashi, et al., Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation, Nat. Genet. 35 (2003) 238–245.
[22] N.D. Camp, R.G. James, D.W. Dawson, et al., Wilms tumor gene on X chromosome (WTX) inhibits degradation of NRF2 protein through competitive binding to KEAP1 protein, J. Biol. Chem. 287 (2012) 6539–6550.
[23] W. Chen, Z. Sun, X.J. Wang, et al., Direct interaction between Nrf2 and p21(Cip1/WAF1) upregulates the Nrf2-mediated antioxidant response, Mol. Cell 34 (2009) 663–673.
[24] B.E. Hast, D. Goldfarb, K.M. Mulvaney, et al., Proteomic analysis of ubiquitin ligase KEAP1 reveals associated proteins that inhibit NRF2 ubiquitination, Cancer Res. 73 (2013) 2199–2210.
[25] M. Komatsu, H. Kurokawa, S. Waguri, et al., The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1, Nat. Cell Biol. 12 (2010) 213–223.
[26] S.C. Lo, M. Hannink, PGAM5, a Bcl-XL-interacting protein, is a novel substrate for the redox-regulated Keap1-dependent ubiquitin ligase complex, J. Biol. Chem. 281 (2006) 37893–37903.
[27] J. Ma, H. Cai, T. Wu, et al., PALB2 interacts with KEAP1 to promote NRF2 nuclear accumulation and function, Mol. Cell. Biol. 32 (2012) 1506–1517.
[28] K.M. Mulvaney, J.P. Matson, P.F. Siesser, et al., Identification and characterization of MCM3 as a kelch-like ECH-associated protein 1 (KEAP1) substrate, J. Biol. Chem. 291 (2016) 23719–23733.
[29] Q. Wang, J. Ma, Y. Lu, et al., CDK20 interacts with KEAP1 to activate NRF2 and promotes radiochemoresistance in lung cancer cells, Oncogene 36 (2017) 5321–5330.
[30] T. Suzuki, S. Seki, K. Hiramoto, et al., Hyperactivation of Nrf2 in early tubular development induces nephrogenic diabetes insipidus, Nat. Commun. 8 (2017) 14577.
[31] E.W. Cloer, D. Goldfarb, T.P. Schrank, et al., NRF2 activation in cancer: from DNA to protein, Cancer Res. 79 (2019) 889–898.
[32] D. Lastra, R. Fernandez-Gines, G. Manda, et al., Perspectives on the clinical development of NRF2-targeting drugs, Handb. Exp. Pharmacol. 264 (2021) 93–141.
[33] E. Panieri, L. Saso, Potential applications of NRF2 inhibitors in cancer therapy, Oxidative Med. Cell. Longev. 2019 (2019) 8592348.
[34] A.M. Gao, Z.P. Ke, J.N. Wang, et al., Apigenin sensitizes doxorubicin-resistant hepatocellular carcinoma BEL-7402/ADM cells to doxorubicin via inhibiting PI3K/Akt/Nrf2 pathway, Carcinogenesis 34 (2013) 1806–1814.
[35] C.S. Huang, C.K. Lii, A.H. Lin, et al., Protection by chrysin, apigenin, and luteolin against oxidative stress is mediated by the Nrf2-dependent up-regulation of heme oxygenase 1 and glutamate cysteine ligase in rat primary hepatocytes, Arch. Toxicol. 87 (2013) 167–178.
[36] X. Paredes-Gonzalez, F. Fuentes, S. Jeffery, et al., Induction of NRF2-mediated gene expression by dietary phytochemical flavones apigenin and luteolin, Biopharm. Drug Dispos. 36 (2015) 440–451.
[37] M.J. Bollong, H. Yun, L. Sherwood, et al., A small molecule inhibits deregulated NRF2 transcriptional activity in cancer, ACS Chem. Biol. 10 (2015) 2193–2198.
[38] E.J. Choi, B.J. Jung, S.H. Lee, et al., A clinical drug library screen identifies clobetasol propionate as an NRF2 inhibitor with potential therapeutic efficacy in KEAP1 mutant lung cancer, Oncogene 36 (2017) 5285–5295.
[39] A. Singh, S. Venkannagari, K.H. Oh, et al., Small molecule inhibitor of NRF2 selectively intervenes therapeutic resistance in KEAP1-deficient NSCLC tumors, ACS Chem. Biol. 11 (2016) 3214–3225.
[40] J.H. Matthews, X. Liang, V.J. Paul, et al., A complementary chemical and genomic screening approach for druggable targets in the Nrf2 pathway and small molecule inhibitors to overcome cancer cell drug resistance, ACS Chem. Biol. 13 (2018) 1189–1199.
[41] H.Y. Liu, A.Z. Tuckett, M. Fennell, et al., Repurposing of the CDK inhibitor PHA-767491 as a NRF2 inhibitor drug candidate for cancer therapy via redox modulation, Investig. New Drugs 36 (2018) 590–600.
[42] L. Ambrosio, M. Argenziano, M.A. Cucci, Carbosilane dendrimers loaded with siRNA targeting Nrf2 as a tool to overcome cisplatin chemoresistance in bladder cancer cells, Antioxidants (Basel) (2020) 9.
[43] P.C. Li, M.J. Tu, P.Y. Ho, et al., Bioengineered NRF2-siRNA is effective to interfere with NRF2 pathways and improve chemosensitivity of human cancer cells, Drug Metab. Dispos. 46 (2018) 2–10.
[44] C. Hatzis, P.L. Bedard, N.J. Birkbak, et al., Enhancing reproducibility in cancer drug screening: how do we move forward? Cancer Res. 74 (2014) 4016–4023.
[45] T. Shibata, T. Ohta, K.I. Tong, et al., Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 13568–13573.
[46] B.M. Bowman, S.A. Montgomery, T.P. Schrank, et al., A conditional mouse expressing an activating mutation in NRF2 displays hyperplasia of the upper gastrointestinal tract and decreased white adipose tissue, J. Pathol. 252 (2020) 125–137.
[47] J.H. Bushweller, Targeting transcription factors in cancer - from undruggable to reality, Nat. Rev. Cancer 19 (2019) 611–624.
[48] L. Bar-Peled, E.K. Kemper, R.M. Suciu, et al., Chemical proteomics identifies druggable vulnerabilities in a genetically defined cancer, Cell 171 (2017).
[49] Y. Kitano, Y. Baba, S. Nakagawa, et al., Nrf2 promotes oesophageal cancer cell proliferation via metabolic reprogramming and detoxification of reactive oxygen species, J. Pathol. 244 (2018) 346–357.
[50] D. Saigusa, I.N. Motoike, S. Saito, et al., Impacts of NRF2 activation in non-small-cell lung cancer cell lines on extracellular metabolites, Cancer Sci. 111 (2020) 667–678.
[51] J.M. McFarland, Z.V. Ho, G. Kugener, et al., Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration, Nat. Commun. 9 (2018) 4610.
[52] A. Schuster, H. Erasimus, S. Fritah, et al., RNAi/CRISPR screens: from a pool to a valid hit, Trends Biotechnol. 37 (2019) 38–55.
[53] D.W. Morgens, R.M. Deans, A. Li, et al., Systematic comparison of CRISPR/Cas9 and RNAi screens for essential genes, Nat. Biotechnol. 34 (2016) 634–636.
[54] E. Testoni, N.L. Stephenson, P. Torres-Ayuso, et al., Somatically mutated ABL1 is an actionable and essential NSCLC survival gene, EMBO Mol. Med. 8 (2016) 105–116.
[55] C. Sourbier, C.J. Ricketts, S. Matsumoto, et al., Targeting ABL1-mediated oxidative stress adaptation in fumarate hydratase-deficient cancer, Cancer Cell 26 (2014) 840–850.
[56] H.Q. Duong, K.S. You, S. Oh, Silencing of NRF2 reduces the expression of ALDH1A1 and ALDH3A1 and sensitizes to 5-FU in pancreatic cancer cells, Antioxidants (Basel) (2017) 6.
[57] Y.Y. Jiang, Y. Jiang, C.Q. Li, et al., TP63, SOX2, and KLF5 establish a core regulatory circuitry that controls epigenetic and transcription patterns in esophageal squamous cell carcinoma cell lines, Gastroenterology 159 (2020).
[58] M. Dodson, M.R. de la Vega, A.B. Cholanians, et al., Modulating NRF2 in disease: timing is everything, Annu. Rev. Pharmacol. Toxicol. 59 (2019 Jan 6) 555–575.
[59] E.C. Lien, C.A. Lyssiotis, A. Juvekar, et al., Glutathione biosynthesis is a metabolic vulnerability in PI(3)K/Akt-driven breast cancer, Nat. Cell Biol. 18 (2016) 572–578.
[60] J.M. Shin, M.Y. Kim, K.C. Sohn, et al., Nrf2 negatively regulates melanogenesis by modulating PI3K/Akt signaling, PLoS One 9 (2014), e96035.
[61] D. Liu, Y. Zhang, Y. Wei, et al., Activation of AKT pathway by Nrf2/PDGFA feedback loop contributes to HCC progression, Oncotarget 7 (2016) 65389–65402.
[62] G. Bendavit, T. Aboulkassim, K. Hilmi, et al., Nrf2 transcription factor can directly regulate mTOR: linking cytoprotective gene expression to a major metabolic regulator that generates redox activity, J. Biol. Chem. 291 (2016) 25476–25488.
[63] A. Suzuki, S. Itami, M. Ohishi, et al., Keratinocyte-specific Pten deficiency results in epidermal hyperplasia, accelerated hair follicle morphogenesis and tumor formation, Cancer Res. 63 (2003) 674–681.
[64] S.A. Best, D.P. De Souza, A. Kersbergen, et al., Synergy between the KEAP1/NRF2 and PI3K pathways drives non-small-cell lung cancer with an altered immune microenvironment, Cell Metab. 27 (2018).
[65] F. Sanchez-Vega, M. Mina, J. Armenia, et al., Oncogenic signaling pathways in the cancer genome atlas, Cell 173 (2018).
[66] Y. Akimov, T. Aittokallio, Re-defining synthetic lethality by phenotypic profiling for precision oncology, Cell. Chem. Biol. 28 (2021) 246–256.
[67] J. Setton, M. Zinda, N. Riaz, Synthetic lethality in cancer therapeutics: the next generation, Cancer Discov. 11 (7) (2021) 1626–1635.
[68] F. Iorio, T.A. Knijnenburg, D.J. Vis, et al., A landscape of pharmacogenomic interactions in cancer, Cell 166 (2016) 740–754.
[69] B. Seashore-Ludlow, M.G. Rees, J.H. Cheah, et al., Harnessing connectivity in a large-scale small-molecule sensitivity dataset, Cancer Discov. 5 (2015) 1210–1223.
[70] W. Yang, J. Soares, P. Greninger, et al., Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells, Nucleic Acids Res. 41 (2013) D955–D961.
[71] C. Yu, A.M. Mannan, G.M. Yvone, et al., High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines, Nat. Biotechnol. 34 (2016) 419–423.
[72] N.K. Niu, Z.L. Wang, S.T. Pan, et al., Pro-apoptotic and pro-autophagic effects of the Aurora kinase a inhibitor alisertib (MLN8237) on human osteosarcoma U-2 OS and MG-63 cells through the activation of mitochondria-mediated pathway and inhibition of p38 MAPK/PI3K/Akt/mTOR signaling pathway, Drug Des. Devel. Ther. 9 (2015) 1555–1584.
[73] L. Guo, H. Gong, T.L. Tang, et al., Crizotinib and sunitinib induce hepatotoxicity and mitochondrial apoptosis in L02 cells via ROS and Nrf2 signaling pathway, Front. Pharmacol. 12 (2021), 620934.
[74] X. Sun, J. Li, Y. Li, et al., Apatinib, a novel tyrosine kinase inhibitor, promotes ROS-dependent apoptosis and autophagy via the Nrf2/HO-1 pathway in ovarian cancer cells, Oxidative Med. Cell. Longev. 2020 (2020) 3145182.
[75] W. Sun, M. Powell, P.J. O'Dwyer, et al., Phase II study of sorafenib in combination with docetaxel and cisplatin in the treatment of metastatic or advanced gastric and gastroesophageal junction adenocarcinoma: ECOG 5203, J. Clin. Oncol. 28 (2010) 2947–2951.
[76] X. Zhang, R. Zhang, H. Chen, et al., KRT-232 and navitoclax enhance trametinib's anti-cancer activity in non-small cell lung cancer patient-derived xenografts with KRAS mutations, Am. J. Cancer Res. 10 (2020) 4464–4475.
[77] Q. Chen, S. Song, S. Wei, et al., ABT-263 induces apoptosis and synergizes with chemotherapy by targeting stemness pathways in esophageal cancer, Oncotarget 6 (2015) 25883–25896.
[78] Q.H. Lin, F.C. Que, C.P. Gu, et al., ABT-263 induces G1/G0-phase arrest, apoptosis and autophagy in human esophageal cancer cells in vitro, Acta Pharmacol. Sin. 38 (2017) 1632–1641.
[79] F. Cheng, J. Zhao, Y. Wang, et al., Comprehensive characterization of protein-protein interactions perturbed by disease mutations, Nat. Genet. 53 (2021) 342–353.

[80] L. Baird, T. Suzuki, Y. Takahashi, et al., Geldanamycin-derived HSP90 inhibitors are synthetic lethal with NRF2, Mol. Cell. Biol. 40 (2020).
[81] L. Baird, M. Yamamoto, NRF2-dependent bioactivation of mitomycin C as a novel strategy to target KEAP1-NRF2 pathway activation in human cancer, Mol. Cell. Biol. 41 (2021).
[82] J.O. Odera, Z. Xiong, C. Huang, et al., NRF2/ACSS2 axis mediates the metabolic effect of alcohol drinking on esophageal squamous cell carcinoma, Biochem. J. 477 (2020) 3075–3089.
[83] K. Diehl, L.A. Dinges, O. Helm, et al., Nuclear factor E2-related factor-2 has a differential impact on MCT1 and MCT4 lactate carrier expression in colonic epithelial cells: a condition favoring metabolic symbiosis between colorectal cancer and stromal cells, Oncogene 37 (2018) 39–51.
[84] G. Shen, C. Xu, R. Hu, et al., Modulation of nuclear factor E2-related factor 2-mediated gene expression in mice liver and small intestine by cancer chemopreventive agent curcumin, Mol. Cancer Ther. 5 (2006) 39–51.
[85] R. Goel, R.M. Subramaniam, J.W. Wachsmann, PET/Computed tomography scanning and precision medicine: esophageal cancer, PET Clin. 12 (2017) 373–391.
[86] M.S. Yates, Q.T. Tran, P.M. Dolan, et al., Genetic versus chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression profiles between Keap1 knockout and triterpenoid-treated mice, Carcinogenesis 30 (2009) 1024–1031.
[87] S.Y. Park, S.J. Lee, J.H. Han, et al., Association between 18F-FDG uptake in PET/CT, Nrf2, and NQO1 expression and their prognostic significance in non-small cell lung cancer, Neoplasma 66 (2019) 619–626.
[88] Y. Hirose, H. Kaida, A. Kawahara, et al., Molecular biological correlation of fluorine-18 fluorodeoxyglucose uptake in esophageal squamous cell carcinoma, Nucl. Med. Commun. 37 (2016) 1053–1061.
[89] L.M. Deford-Watts, A. Mintz, S.J. Kridel, The potential of (1)(1)C-acetate PET for monitoring the fatty acid synthesis pathway in tumors, Curr. Pharm. Biotechnol. 14 (2013) 300–312.
[90] E.H. Kim, H. Jang, J.L. Roh, A novel polyphenol conjugate sensitizes cisplatin-resistant head and neck cancer cells to cisplatin via Nrf2 inhibition, Mol. Cancer Ther. 15 (2016) 2620–2629.
[91] J. Zhang, L. Su, Q. Ye, et al., Discovery of a novel Nrf2 inhibitor that induces apoptosis of human acute myeloid leukemia cells, Oncotarget 8 (2017) 7625–7636.
[92] X. Hou, X. Bai, X. Gou, et al., 3',4',5',5,7-pentamethoxyflavone sensitizes Cisplatin-resistant A549 cells to cisplatin by inhibition of Nrf2 pathway, Mol. Cells 38 (2015) 396–401.
[93] M.A. Cucci, M. Grattarola, C. Dianzani, et al., Ailanthone increases oxidative stress in CDDP-resistant ovarian and bladder cancer cells by inhibiting of Nrf2 and YAP expression through a post-translational mechanism, Free Radic. Biol. Med. 150 (2020) 125–135.
[94] M. Daga, S. Pizzimenti, C. Dianzani, et al., Ailanthone inhibits cell growth and migration of cisplatin resistant bladder cancer cells through down-regulation of Nrf2, YAP, and c-Myc expression, Phytomedicine 56 (2019) 156–164.
[95] M. Valenzuela, C. Glorieux, J. Stockis, et al., Retinoic acid synergizes ATO-mediated cytotoxicity by precluding Nrf2 activity in AML cells, Br. J. Cancer 111 (2014) 874–882.
[96] H. Wang, K. Liu, M. Geng, et al., RXRalpha inhibits the NRF2-ARE signaling pathway through a direct interaction with the Neh7 domain of NRF2, Cancer Res. 73 (2013) 3097–3108.
[97] X.J. Wang, J.D. Hayes, C.J. Henderson, et al., Identification of retinoic acid as an inhibitor of transcription factor Nrf2 through activation of retinoic acid receptor alpha, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19589–19594.
[98] J. Wu, H. Wang, X. Tang, Rexinoid inhibits Nrf2-mediated transcription through retinoid X receptor alpha, Biochem. Biophys. Res. Commun. 452 (2014) 554–559.
[99] B. Harder, W. Tian, J.J. La Clair, et al., Brusatol overcomes chemoresistance through inhibition of protein translation, Mol. Carcinog. 56 (2017) 1493–1500.
[100] A. Olayanju, I.M. Copple, H.K. Bryan, et al., Brusatol provokes a rapid and transient inhibition of Nrf2 signaling and sensitizes mammalian cells to chemical toxicity-implications for therapeutic targeting of Nrf2, Free Radic. Biol. Med. 78 (2015) 202–212.
[101] D. Ren, N.F. Villeneuve, T. Jiang, et al., Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 1433–1438.
[102] F. Chen, H. Wang, J. Zhu, et al., Camptothecin suppresses NRF2-ARE activity and sensitises hepatocellular carcinoma cells to anticancer drugs, Br. J. Cancer 117 (2017) 1495–1506.
[103] D.V. Kratschmar, D. Calabrese, J. Walsh, et al., Suppression of the Nrf2-dependent antioxidant response by glucocorticoids and 11beta-HSD1-mediated glucocorticoid activation in hepatic cells, PLoS One 7 (2012), e36774.
[104] J. Lee, J.S. Kang, L.B. Nam, et al., Suppression of NRF2/ARE by convallatoxin sensitises A549 cells to 5-FU-mediated apoptosis, Free Radic. Res. 52 (2018) 1416–1423.
[105] F. Ahmad, D. Dixit, V. Sharma, et al., Nrf2-driven TERT regulates pentose phosphate pathway in glioblastoma, Cell Death Dis. 7 (2016), e2213.
[106] K. Tsuchida, T. Tsujita, M. Hayashi, et al., Halofuginone enhances the chemo-sensitivity of cancer cells by suppressing NRF2 accumulation, Free Radic. Biol. Med. 103 (2017) 236–247.
[107] H.S. Khalil, S.P. Langdon, A. Goltsov, et al., A novel mechanism of action of HER2 targeted immunotherapy is explained by inhibition of NRF2 function in ovarian cancer cells, Oncotarget 7 (2016) 75874–75901.
[108] J.S. Kang, J. Lee, L.B. Nam, et al., Homoharringtonine stabilizes secondary structure of guanine-rich sequence existing in the 5'-untranslated region of Nrf2, Bioorg. Med. Chem. Lett. 29 (2019) 2189–2196.
[109] S. Lee, M.J. Lim, M.H. Kim, et al., An effective strategy for increasing the radiosensitivity of human lung cancer cells by blocking Nrf2-dependent antioxidant responses, Free Radic. Biol. Med. 53 (2012) 807–816.
[110] Y. Chen, P. Xue, Y. Hou, et al., Isoniazid suppresses antioxidant response element activities and impairs adipogenesis in mouse and human preadipocytes, Toxicol. Appl. Pharmacol. 273 (2013) 435–441.
[111] A.K. Verma, A. Yadav, J. Dewangan, et al., Isoniazid prevents Nrf2 translocation by inhibiting ERK1 phosphorylation and induces oxidative stress and apoptosis, Redox Biol. 6 (2015) 80–92.
[112] H. Peng, H. Wang, P. Xue, et al., Suppression of NRF2-ARE activity sensitizes chemotherapeutic agent-induced cytotoxicity in human acute monocytic leukemia cells, Toxicol. Appl. Pharmacol. 292 (2016) 1–7.
[113] D. Yasuda, M. Nakajima, A. Yuasa, et al., Synthesis of Keap1-phosphorylated p62 and Keap1-Nrf2 protein-protein interaction inhibitors and their inhibitory activity, Bioorg. Med. Chem. Lett. 26 (2016) 5956–5959.
[114] D. Yasuda, T. Ohe, K. Takahashi, et al., Inhibitors of the protein-protein interaction between phosphorylated p62 and Keap1 attenuate chemoresistance in a human hepatocellular carcinoma cell line, Free Radic. Res. 54 (2020) 859–871.
[115] A. Manna, S. De Sarkar, S. De, et al., The variable chemotherapeutic response of Malabaricone-A in leukemic and solid tumor cell lines depends on the degree of redox imbalance, Phytomedicine 22 (2015) 713–723.
[116] C. Boesch-Saadatmandi, A.E. Wagner, A.C. Graeser, et al., Ochratoxin A impairs Nrf2-dependent gene expression in porcine kidney tubulus cells, J. Anim. Physiol. Anim. Nutr. (Berl.) 93 (2009) 547–554.
[117] C. Cavin, T. Delatour, M. Marin-Kuan, et al., Reduction in antioxidant defenses may contribute to ochratoxin A toxicity and carcinogenicity, Toxicol. Sci. 96 (2007) 30–39.
[118] A. Limonciel, P. Jennings, A review of the evidence that ochratoxin A is an Nrf2 inhibitor: implications for nephrotoxicity and renal carcinogenicity, Toxins (Basel) 6 (2014) 371–379.
[119] S. Zhou, W. Ye, X. Duan, et al., The noncytotoxic dose of sorafenib sensitizes Bel-7402/5-FU cells to 5-FU by down-regulating 5-FU-induced Nrf2 expression, Dig. Dis. Sci. 58 (2013) 1615–1626.
[120] P. Chikkegowda, B.C. Pookunoth, V.R. Bovilla, et al., Design, synthesis, characterization, and crystal structure studies of Nrf2 modulators for inhibiting cancer cell growth in vitro and in vivo, ACS Omega 6 (2021) 10054–10071.
[121] A. Arlt, S. Sebens, S. Krebs, et al., Inhibition of the Nrf2 transcription factor by the alkaloid trigonelline renders pancreatic cancer cells more susceptible to apoptosis through decreased proteasomal gene expression and proteasome activity, Oncogene 32 (2013) 4825–4835.
[122] U. Boettler, K. Sommerfeld, N. Volz, et al., Coffee constituents as modulators of Nrf2 nuclear translocation and ARE (EpRE)-dependent gene expression, J. Nutr. Biochem. 22 (2011) 426–440.
[123] A. Leone, M.S. Roca, C. Ciardiello, et al., Vorinostat synergizes with EGFR inhibitors in NSCLC cells by increasing ROS via up-regulation of the major mitochondrial porin VDAC1 and modulation of the c-Myc-NRF2-KEAP1 pathway, Free Radic. Biol. Med. 89 (2015) 287–299.
[124] L.B. Nam, Y.S. Keum, Regulation of NRF2 by Na( )/K( )-ATPase: implication of tyrosine phosphorylation of Src, Free Radic. Res. 54 (2020) 883–893.
[125] M. Ghandi, F.W. Huang, J. Jane-Valbuena, et al., Next-generation characterization of the cancer cell line encyclopedia, Nature 569 (2019) 503–508.
[126] H. Li, S. Ning, M. Ghandi, et al., The landscape of cancer cell line metabolism, Nat. Med. 25 (2019) 850–860.
[127] F.M. Behan, F. Iorio, G. Picco, et al., Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens, Nature 568 (2019) 511–516.