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Importance of the Keap1-Nrf2 pathway in NSCLC: Is it a possible biomarker? (Review)

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Abstract. Worldwide, lung cancer remains the most common cause of cancer-related mortality, with non-small cell lung cancer (NSCLC) accounting for 85% of all diagnosed lung cancer cases. Chemotherapy is considered the standard of care for patients with advanced NSCLC; however, the tumors can develop mechanisms that inactivate these drugs. Comparative genomic analyses have revealed that disruptions in the kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid-2-related factor-2 (Nrf2) pathway are frequent in NSCLC, although Nrf2 mutations occur less frequently than Keap1 mutations. As the Keap1-Nrf2 pathway appears to be a primary regulator of key cellular processes that aid to resist the action of chemotherapy drugs, the clinical implementation of Nrf2 inhibitors in patients with advanced NSCLC may be a useful therapeutic approach for patients harboring KEAP1-NRF2 mutations. The aim of the present review was to highlight findings of how constitutive Nrf2 activation may be a specific biomarker for predicting patients most likely to benefit from classical chemotherapy drugs, overall improving patient survival rate.

Contents

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1. Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, with a 5-year overall survival rate of <15% (1). Non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer, representing ~85% of cases, and its incidence has risen steadily over the past few decades, particularly in women (2). Although target-directed therapy has been demonstrated to improve chemotherapy response among NSCLC patients (3), for the numerous NSCLC patients with an unknown mutation status or with no apparent gene mutations, platinum-based regimens are the standard of care recommended by the European Society for Medical Oncology Clinical Practise Guidelines (4); however, chemotherapy for advanced inoperable NSCLC is generally palliative. The major factor contributing to failure of chemotherapy in the treatment of lung cancer is development of drug resistance (5).

The lung is continuously exposed to chemicals and carcinogens that cause oxidative stress to lipids, proteins and DNA. In response, lung cells must activate cytoprotective mechanisms to defend and protect themselves against these insults (6). Lung cancer tumors seem to inherit this ability, which affords them protection against insults that generate oxidative stress or against the toxicity of xenobiotics produced in the microenvironmental conditions of tumour growth (7). The kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid-2-related factor-2 (Nrf2) pathway is a key determinant for cells in coping with oxidative stress (8). In response to environmental or endogenous insults, the Keap1-Nrf2 pathway increases the expression of a series of cytoprotective/defensive proteins that protect cells against oxidative stress and promote cell survival (9).

Previous data indicate that abnormal states of the Keap1-Nrf2 pathway exist in lung cancer (10-12). Elevated Nrf2 levels and Keap1 dysfunction have been frequently identified in lung cancer, and it is possible that they are associated with tumour progression, cytoprotection, resistance to chemotherapeutic drugs and poor prognosis (13).

The current review summarizes recent advancements in our understanding of alterations in the Keap1-Nrf2 pathway in NSCLC, its role in drug resistance, and discusses its usefulness as a potential biomarker. An improved understanding of the role served by the Keap1-Nrf2 pathway in the regulation of

cytoprotective mechanisms may aid the search for novel anticancer targets that afford decreased tumour defense systems and increased sensitivity to treatments.

2. Keap1-Nrf2 pathway

The Keap1-Nrf2 pathway serves a central role in protecting cells from oxidative and/or electrophilic stress. Nrf2, also known as NFE2L2, belonging to the cap'n'collar subfamily of the basic leucine zipper transcription factors, is the primary regulator of the inducible cell defense system, which mediates the expression of >200 oxidative stress-related genes (14), including those transcribing antioxidant proteins, proteasome subunits, chaperones, growth factors and their receptors, certain transcription factors, phase I and II detoxification enzymes, and drug efflux pumps that can accelerate the metabolic inactivation of antitumor agents and decrease intracellular drug concentrations (8,15,16).

Nrf2 activity is tightly regulated by Keap1, which is a cytoplasmic adaptor protein of the Cullin3 (Cul3)-based E3-ligase (17). Under normal physiological conditions, Keap1 constitutively targets Nrf2 for polyubiquitination and degradation by the 26S proteasome (18). However, upon oxidative stress, Keap1 is inactivated and the ubiquitination of Nrf2 halted, which leads to the accumulation of Nrf2 in the cytoplasm. Consequently, Nrf2 is translocated into the nucleus via the importin-α5/importin-β1 import pathway, where it induces the transcription of a series of antioxidant responsive element (ARE)-responsive genes and ultimately leads to the activation of the defensive system as well as mechanisms of chemoradiation resistance (19) (Fig. 1).

Several KEAP1 and NRF2 gene mutations which disrupt the Keap1-Nrf2 interaction and result in Nrf2 overexpression have been identified in several cancer tissues, including lung, breast, bladder, ovarian and liver, and numerous types of cancer cell line (10,20-23).

3. Mechanisms of Keap1-Nrf2 pathway deregulation

Distinct mechanisms have been described for the activation of Nrf2 in NSCLC, which include:

i) Somatic mutations: Gain-of-function mutations in NRF2 and loss-of-function mutations in KEAP1 (8). Initially, KEAP1 gene mutations (G430C and G364C) were identified in a human lung adenocarcinoma cell line (NCI-H1648), each involving a glycine to cysteine substitution in the Kelch-repeat domain of Keap1 (20). These adenocarcinoma cells exhibited reduced affinity of Keap1 to Nrf2 and in consequence a constitutive activation of Nrf2 was observed (18). Subsequent to this, other somatic mutations have also been identified in the Kelch or intervening linker (IVR) domains of the Keap1 protein in NSCLC cell lines (NL20, A549, H460, H1435, H23, H358, H1993, H1395, H838, H1299 and H292) and in tissues from NSCLC patients (10,11).

ii) KEAP1 hypermethylation: DNA methylation by DNA methyltransferases in the promoter region of KEAP1 may affect its expression and hinder its ability to bind to Nrf2, resulting in Nrf2 activation (12,24). Conversely, CpG methylation of the NRF2 promoter appears to downregulate NRF2 expression indirectly (25,26).

iii) Accumulation of $p21^{Cip1/WAF1}$ and $p62^{lck}$ may disrupt the Keap1-Nrf2 complex (27): In response to injuries producing reactive oxygen species (ROS), cells generate an Nrf2-dependent anti-oxidant response in an attempt to repair the ROS- and/or electrophile-induced damages. However, if the ROS provoke DNA damage, the transcription factor p53 may be activated, which induces cell cycle arrest to allow time for the DNA repair (28). It is this point where the cyclin-dependent kinase inhibitor $p21^{Cip1/WAF1}$ (a direct downstream target of p53) may associate with the DLG motif of Nrf2, inhibiting Keap1-dependent Nrf2 ubiquitination, resulting in stabilization of the Nrf2 protein and, ultimately, leading to the enhanced expression of oxidative stress-related genes (29). However, if DNA is unrepaired, a second response of the cell may occur and p53-induced apoptosis is promoted. Under this condition, p53 appears to suppress the transcription of target genes of Nrf2 (30). However, impairment of autophagy in cancer cells is usually accompanied by accumulation of p62/sequestosome 1 protein. This protein, targeted directly onto the Kelch-repeat domain of Keap1 via its STGE motif, may thereby disrupt the Keap1-Nrf2 complex (31). Again, this interaction may cause a decrease in the ubiquitination of Nrf2 and elicit as a result an increase in Nrf2 stability (29,32).

iv) Transcriptional upregulation of NRF2 by oncogenes: Oncogenes including $C\text{-}MYC^{ERT12}$, $K\text{-}RAS^{G12D}$ and $BRAF^{V619E}$ may increase the transcriptional level of NRF2 and NRF2-regulated genes, resulting in a marked increase of cytoprotection of tumour cells (33).

v) Metabolic activation of Nrf2 by Kreb's cycle intermediates: In the Kreb's cycle, fumarate modifies cysteine residues within Keap1, which disrupts the ability to ubiquitinate Nrf2 and in consequence prolongs activation of Nrf2 (34). In addition, defects in fumarate hydratase may stimulate an importin-mediated nuclear transport of Nrf2 and the transcription of antioxidant enzymes through the succination of Keap1 (35). Furthermore, it has been documented that metabolic reprogramming of both catabolic and anabolic pathways appear to be driven by Keap1-Nrf2 aberrations. For example, DeNicola *et al* (36) demonstrated that activation of Nrf2 regulated serine and glycine metabolism and was linked with clinical aggressiveness in NSCLC, and Mitsuishi *et al* (37) showed that Nrf2 redirected glucose and glutamine into anabolic pathways, particularly under the sustained activation of phosphatidylinositide 3-kinase (PI3K)-protein kinase B (PKB)/Akt signaling, which was advantageous for proliferation and survival in A549 cells.

vi) Loss of exon 2 in the NRF2 gene: Aberrant NRF2 transcripts may result from exon 2 skipping, which translates an Nrf2 protein isoform missing either the DLG or ETGE motifs of the regulatory Nrf2-ECH homology (Neh)2 domain, resulting in persistent Nrf2 localization in the nucleus (38,39);

vii) In recent years, there has been recognition that the repression of Nrf2 by Cul1-β-transducin repeat-containing protein (β-TrCP) E3 ubiquitin ligase is augmented by glycogen synthase kinase-3β (GSK-3β). Thus, Nrf2 may be regulated by GSK-3β through the creation of a DSGIS motif-containing phosphodegron present in the Neh6 domain of Nrf2 that is recognized by Cul13-β-TrCP (40). Conversely, the phosphorylation of Nrf2 by GSK-3β may be inhibited by growth factor signaling through the PI3K-Akt pathway (41).

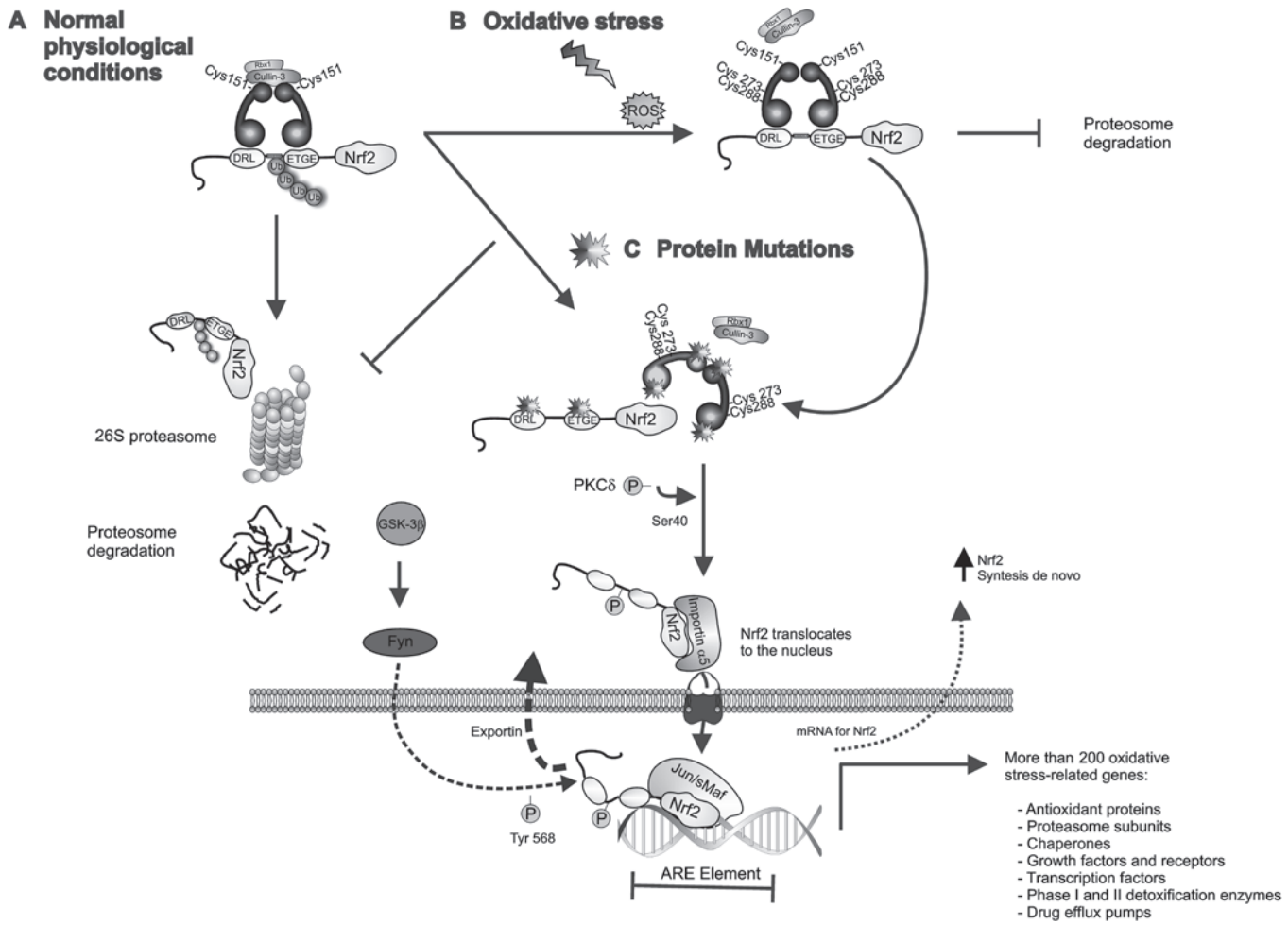

Figure 1. Keap1-Nrf2 pathway. (A) Under physiological/basal conditions, the Keap1-Cullin3 complex promotes ubiquitin E3-ligase activity and proteasomal degradation of Nrf2. (B) Upon exposure to chemicals or ROS, reactive cysteine residues of Keap1 are modified leading to a disruption of the Keap1-Nrf2 complex and the release of Nrf2, which also allows *de novo* synthesized Nrf2 to accumulate in the cytoplasm. The phosphorylation of Ser-40 in the Neh2 domain of Nrf2 by PKCδ results in Nrf2 activation and translocation to the nucleus, where Nrf2-mediated transcriptional activity of ARE-responsive genes is initiated. GSK-3β phosphorylates the tyrosine kinase Fyn and induces its nuclear accumulation. Fyn phosphorylates Nrf2 at tyrosine-568, facilitating its nuclear export and degradation. (C) Loss-of-function mutations in Keap1 or Nrf2 lead to constitutive activation of Nrf2 by disrupting the Keap1-Nrf2 interaction. In the nucleus, Nrf2 heterodimerizes with sMaf proteins and regulates the expression of >200 oxidative stress-related genes. ARE, antioxidant response element; Fyn, proto-oncogene Src-family tyrosine kinase; GSK-3β, glycogen synthase kinase 3; Keap1, kelch-like ECH-associated protein 1; sMaf, musculoaponeurotic fibrosarcoma oncogene; Nrf2, nuclear factor erythroid-2-related factor-2; PKCδ, protein kinase C-δ; ROS, reactive oxygen species.

4. Keap1-Nrf2 pathway disruption and prognosis

There is increasing data to suggest that KEAP1-NRF2 mutational status is associated with poor prognosis and chemotherapeutic resistance in NSCLC (10). For example, Inoue *et al* (42) examined the expression of Nrf2 by immunohistochemical analyses, in clinical tissue samples from 109 NSCLC cases, and observed that higher nuclear accumulation of Nrf2 correlated with worse lung cancer-specific survival. By immunohistochemistry, Yang *et al* (43) analyzed the Nrf2 expression status of 60 patients with stage IIIB or IV NSCLC and compared the response to platinum-based treatments in both groups. Although positive staining for Nrf2 was found in nearly all cases to varying degrees, patients with stage IV disease exhibited higher Nrf2 expression than patients with stage IIIB disease (P=0.017). Interestingly, patients with <75% positive staining achieved a higher response rate than those with 75-100% positive staining (P=0.003; r=0.447), suggesting that Nrf2 expression may be a useful index to predict the efficacy of platinum-based treatments (43).

Interestingly, the results of several studies of NSCLC tumour samples have also indicated that the occurrence of NRF2 or KEAP1 mutation is mutually exclusive and associated with different histologies. Solis *et al* (44) studied 304 tumour tissue samples [190 adenocarcinomas (ADCs) and 114 squamous cell carcinomas (SqCCs)] following adjuvant treatment. They detected that nuclear Nrf2 expression in 26% of the NSCLC samples was significantly more common in SqCC (38%) than in ADC (18%; P<0.0001) and established an association of a nuclear Nrf2 abundance with worse progression-free survival. Li *et al* (45) reported that the frequency of Keap1 alterations was significantly higher in papillary ADC tumors (60%) than that reported previously for NSCLC (3-19%) (46). In a recent study, Frank *et al* (47) analyzed the tumour tissues of 1,391 patients with NSCLC and identified that the frequency for Nrf2 mutations was 3.5% (n=49), while for Keap1 mutations was 11.3% (n=157). Nrf2 mutations were

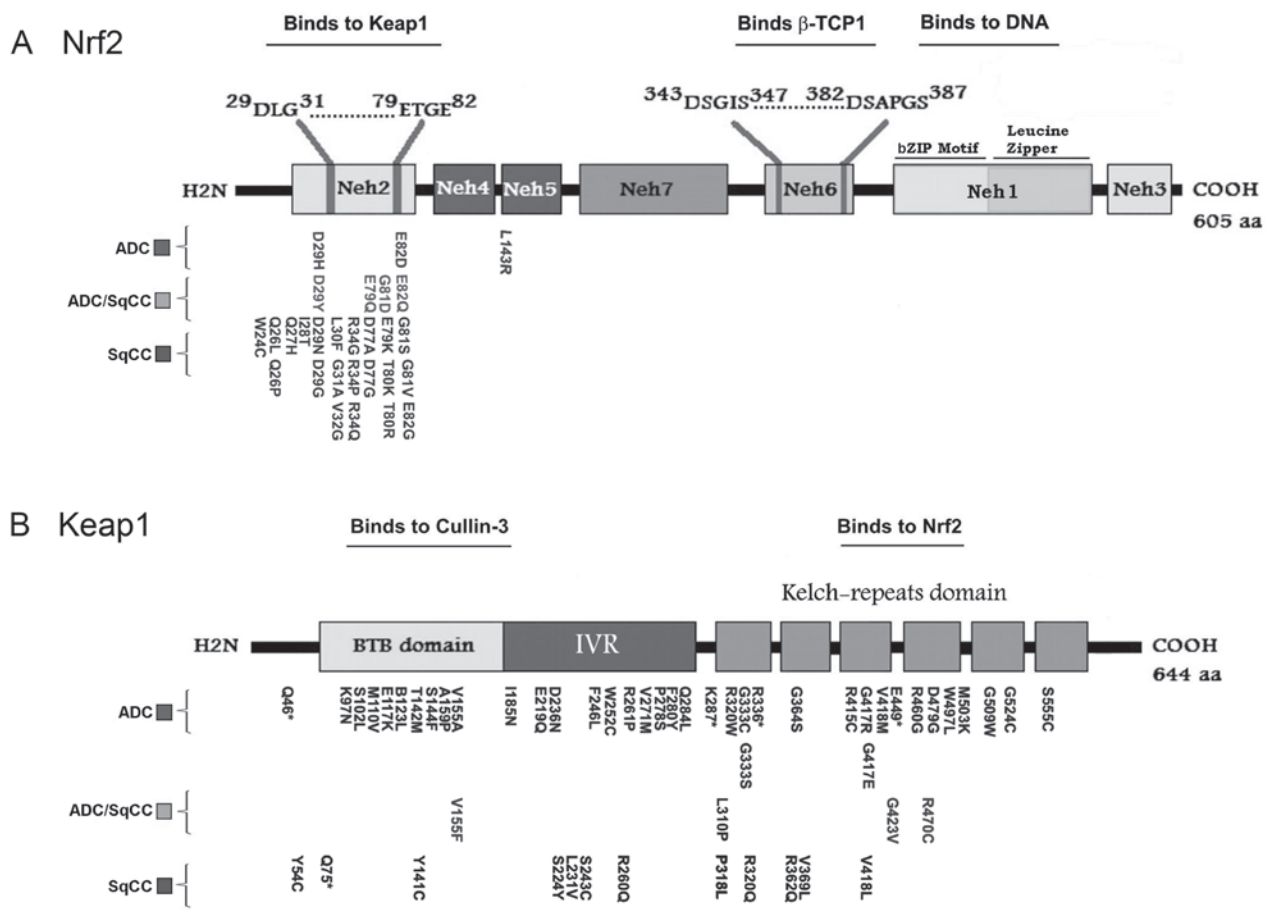

Figure 2. Schematic representation of domain architecture of the Keap1 and Nrf2 proteins and mutations found in NSCLC. (A) Human NRF2 is a polypeptide of 605 amino acids and contains 7 Neh domains. Neh1 contains the signature CNC motif, which is a highly conserved bZIP domain for DNA binding. The Neh1 CNC-bZIP domain is responsible for dimerization with sMaf protein and is required for binding to ARE sequences in DNA; the Neh2 domain controls the interaction with Keap1 through the DLG and ETGE motifs; the Neh6 domain contains two binding sites (DSGIS and DSAPGS motifs), and the phosphorylation of the DSGIS motif by GSK-3β increases binding ability to the β-TrCP1 adaptor protein. (B) Human Keap1 is a polypeptide of 644 amino acids. The BTB domain is required for the formation of Keap1 homodimers as well as the recruitment of Cul3-based E3-ligase. The Kelch-repeat domain controls Nrf2 interaction. The region between the BTB and Kelch repeat domains constitutes the IVR. Amino acid positions of the identified mutations of Nrf2 and Keap1 are shown for ADC, ADC/SqCC and SqCC. All the indicated mutations are listed in the Catalogue of Somatic Mutations in Cancer database (http://cancer.sanger.ac.uk/cosmic). ADC, adenocarcinoma; ADC/SqCC, adenocarcinoma/squamous cell carcinoma; ARE, antioxidant response element; bZIP, basic leucine zipper; BTB, broad complex, tram-track and bric-a-brac; CNC, cap'n'collar; Cul3, Cullin3; GSK-3β, glycogen synthase kinase 3; IVR, intervening linker; KEAP1, kelch-like ECH-associated protein 1; sMaf, musculoaponeurotic fibrosarcoma oncogene; Neh, Nrf2-ECH homologous structure; NRF2, nuclear factor erythroid-2-related factor-2; SqCC, squamous cell carcinoma; β-TrCP1, β-T-complex protein 1.

most frequent in SqCC (59.2%) whereas Keap1 mutations were predominantly detected in ADC (72.2%). Furthermore, patients with tumors containing KEAP1 mutation presented a worse Eastern Cooperative Oncology Group performance status and the response on application to different chemotherapy regimens was notably poor (47). A large-scale genomic study involving The Cancer Genome Atlas Research Network examined the exome sequences and copy number profiles of 660 ADC and 484 SqCC tumour/normal tissue pairs (48). This report estimated a KEAP1-NRF2 mutation frequency of 34% in 178 tumors from affected individuals and revealed that the KEAP1 gene was significantly mutated exclusively in ADC, whereas NRF2 was significantly mutated in SqCC. CUL3, the protein product of which is a known interaction partner of Keap1, also reached statistical significance as a mutated gene in the lung SqCC cohort (48).

Studies have also investigated whether the frequency of KEAP1-NRF2 mutations differs by race and ethnicity. Ohta *et al* (11) reported loss of heterozygosity (LOH) in KEAP1 in 5 of 65 (8%) Japanese patients with lung cancer, and Singh *et al* (10) performed a systematic analysis of the KEAP1 genomic locus in a Caucasian population of 54 cases of NSCLC samples, and in 12 lung cancer cell lines, in which deletions, insertions, missense mutations of KEAP1 and LOH at 19p13.2 were commonly found. The KEAP1 somatic mutations were detected in 19% (10 of 54) of all lung cancer cases and in 26% (9 of 35) of ADC cases (10).

Genomic profiling analysis has suggested the prevalence of NRF2 exon deletions to be 1-2% in a panel of 113 NSCLC cell lines (38), and the existence of two NRF2 mutation 'hot-spots' in ~10% of patients with SqCC, which may enable the transcription factor to evade Keap1-mediated repression (38,49). Besides, the mutation burden in Keap1 is spread across the protein domains, while those in Nrf2 only occur in codons for amino acids around the DLG and ETGE motifs (the domains of Nrf2 that interact with Keap1) (10,11,39,50) (Fig. 2).

Despite these data, the frequency of KEAP1 or NRF2 mutation in NSCLC remains uncertain, which thus warrants further

studies to provide discriminating differences between the NSCLC subtypes as well as a comparison between racial/ethnic groups.

5. Co-occurring aberrations

Comparative genomic analyses have determined that co-occurring genomic aberrations are present in 83.7% of patients with tumors containing a mutation in Nrf2 and 87.3% of those with a mutation in Keap1 (47). Mutations in the gene for p53 (TP53) are the most common concurrent aberration, with a frequency of 40.8% in tumors harboring mutations in Nrf2 and 44.9% in tumors with mutations in Keap1. Similar distribution patterns have been observed for epidermal growth factor receptor (EGFR) mutations, with a frequency of 6.1% reported in tumors with mutation in Nrf2 and 6.3% in those with mutation in Keap1. For c-MET amplification, a mutation frequency of 26.3% has been observed in tumors with Nrf2 mutation, while for Keap1 mutation the frequency was 18.3% (47). NSCLC with Keap1 mutation which also harbors an activating KRAS mutation is associated with worse prognosis compared with KRAS-mutated patients without Keap1 mutation (51). Interestingly, patients with ADC that have high mutational load and which could benefit from anti-programmed cell death-1 treatment have shown a high prevalence of Keap1 mutations (52). By contrast, SqCC and mutations in Nrf2 have been associated with high programmed death-ligand 1 expression (53).

Altogether these studies further suggest that in NSCLC, the mutations in KEAP1 or NRF2 may each give rise to a different subset of cancer, opening the opportunity to select a novel series of druggable molecules distinct between the two main lung cancer histological types, for overall improved treatment. Therefore, it appears necessary to implement next-generation sequencing (NGS)-based multiplex diagnostics for cancer, which is capable of capturing and amplifying ~10,000 human exons in a single multiplex reaction. Through use of such novel technology, an improved understanding may be gained of the molecular interactions of mutations in KEAP1 and NRF2 with co-occurring, targetable genetic aberrations.

6. Crosstalk between the Keap1-Nrf2 and EGFR pathways

In lung ADC the tyrosine kinase activity of EGFR is frequently overexpressed or highly activated and has been associated with growth, survival and therapeutic resistance (54). EGFR aberrations can overactivate downstream pro-oncogenic signaling pathways, including the PI3K/Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways, leading to cell growth and proliferation (55). As a result, EGFR has become viewed as an important specific molecule for targeted therapy with specific tyrosine kinase inhibitors (TKIs) (56). In cancer cells, EGFR-mediated stimulation of the PI3K/Akt pathway may inhibit the constitutive activity of GSK-3β, decreasing the GSK-3β-dependent degradation of Nrf2, enabling it to translocate to the nucleus, where Nrf2-mediated transcriptional activity of cytoprotective genes is initiated (57). Conversely, the activation of GSK-3β by inhibition of PI3K/Akt was reported to decrease Nrf2 protein levels in human A549 lung cells that lack functional Keap1 (48). Activation of the PI3/Akt pathway provides a rationalized explanation of how ARE-driven genes may be induced by aberrant activity of growth factor receptors, and of why this pathway may be implicated as an important mechanism in resistance to EGFR inhibitors (58).

Furthermore, EGFR itself may directly regulate Nrf2 activity in cancer cells. Huo *et al* (59) showed that nuclear EGFR induced phosphorylation and ubiquitination of Keap1, leading to stabilization of Nrf2 and stimulation of transcriptional activity, which contributed to cancer cell resistance to chemotherapy. Thus, the function of the EGFR-PI3K-AKT pathway in regulating the Keap1-Nrf2 axis may have an important role not only in cancer cell growth but also in the expression of genes that confer drug resistance in human cancers (60).

Notably, in lung tumors, mutations of EGFR or overactivity of Nrf2 appear to be mutually exclusive (61); it has been observed that the frequency of nuclear Nrf2 expression is significantly higher in EGFR wild-type ADC (21%) than in tumors with EGFR mutation (0%) (44,62). In this regard, it has been speculated that the lack of nuclear Nrf2 expression in ADC containing EGFR mutations may be an important factor that contributes to the chemosensitivity observed with platinum-based chemotherapy (44). This crosstalk between the EGFR-PI3K-AKT and Keap1-Nrf2 pathways may explain why for certain cases of NSCLC, mortality rate remains among the highest of all cancers, despite the availability of improved therapeutics including EGFR-TKIs (56).

The interaction of mutations in KEAP1 and NRF2 with co-occurring targetable genetic aberrations requires an improved understanding, and highlights the need for NGS-based molecular cancer diagnostics to cover co-occurring mutations at least in the setting of clinical research.

7. Drug resistance and clinical implications

There is data to suggest that the activation of the Keap1-Nrf2 pathway is associated with the emergence of resistance to chemotherapy drugs via transcriptional activation of genes conferring resistance to such drugs, including: Multidrug resistance-associated protein 1 [also known as ATP binding cassette (ABC) subfamily C member 1] and γ-glutamylcysteine synthetase genes, which may be involved in resistance against cisplatin and alkylating agents (63,64); breast cancer resistance protein (also known as ABC subfamily G member 2), which is considered to mediate the efflux of gefitinib (an EGFR inhibitor), conferring resistance to TKI regardless of EGFR mutation (65-67); and several other cytoprotective genes (heme oxygenase 1, NAD(P)H quinone dehydrogenase 1 and glutathione S-transferases) (68).

In support of these observations, it has been reported that the inhibition of Nrf2 in lung cancer cells resulted in enhanced intracellular accumulation of carboplatin and etoposide, and consequently in enhanced chemotherapy-induced cell death (69,70). Furthermore, Tian *et al* (71) reported that small interfering RNA knockdown of Nrf2 significantly disrupted Nrf2 signaling *in vitro* and led to sensitization of the H292 cell line to platinum-based drugs, and Singh *et al* (70) demonstrated that xenografts derived from Nrf2-silenced lung cancer cells had an enhanced response to carboplatin *in vivo* compared with control knockdown cells.

In clinical practice, the repercussion of deregulation in the Keap1-Nrf2 pathway is a negative effect for patients with NSCLC, generally manifesting as reduction in overall survival and poorer prognosis. Several reports support this; for example, Solis *et al* (44) reported that Nrf2 overexpression [P=0.0139; hazard ratio (HR), 1.75] or low or absent Keap1 expression (P=0.0181; HR, 2.09) was associated with worse overall survival in SqCC. Takahashi *et al* (62) identified that KEAP1 gene mutations were likely associated with a worse prognosis and lower postoperative disease-free survival rate in pathological stage I-II NSCLC. These observations were also confirmed in other reports, where the nuclear expression of Nrf2 was indicated to serve a role in resistance to platinum-based treatment in SqCC (61,72,73). A previous meta-analysis of microarray data on the expression signatures of 240 NRF2-mediated genes identified a group of 50 genes that predicted a worse clinical outcome in 60% of NSCLC cohorts analyzed (72). In a recent study of patients with NSCLC, the median overall survival of patients with Keap1 mutation was 19.1 months (95% CI, 1.8-36.3 months), and 14.0 months (95% CI, 5.6-22.3 months) for those patients with Nrf2 mutation (47).

Overall, the available data indicates that Keap1-Nrf2 pathway activation serves an important role in the acquisition of resistance to chemotherapy in NSCLC, and provides an explanation for the poor outcomes observed clinically. It has therefore been suggested that the dysregulation of Keap1-Nrf2 pathway may be a clinically useful biomarker of prognosis in lung cancer patients (74,75).

8. Future directions and conclusions

In recent years, pharmaceutical companies have focused on Keap1-Nrf2 pathway targets in order to identify novel and effective molecules capable of inhibiting the Keap1-Nrf2 interaction (76). An obvious advantage of targeting Keap1 or Nrf2 molecules would be the expected increase in effectiveness of standard antitumor chemotherapies; targeting of the detoxification pathways involved in detoxification of various chemotherapy drugs would also be required to reduce the possibility of alternative or redundant detoxification pathway activation. Two reports in the last 5 years have provided a comprehensive summary of current literature relevant to small-molecule modulators of the Keap1-Nrf2 pathway and their usefulness as potential preventive and therapeutic agents (77,78).

Recently, the inhibition of NRF2 has become a promising therapeutic approach for cancers displaying Nrf2 overactivation, and consequently, the clinical implementation of Nrf2 inhibitors in NSCLC patients with Keap1-Nrf2 pathway deregulation may be a useful therapeutic strategy (76). There have been few Nrf2 inhibitors identified to date. Brusatol, a quassinoid isolated from the *Brucea javanica* shrub, has been identified as a unique inhibitor of the Nrf2 through enhancing ubiquitination and degradation of Nrf2 (79). Notably, treatment with brusatol sensitized a broad spectrum of cancer cells to antitumor drugs, reduced tumour burden and improved survival in murine A549 xenograft models (80,81). Nevertheless, while the use of targeted inhibitors may be a novel therapeutic strategy to treat several types of cancer, it is noteworthy that administration of systemic NRF2 inhibitors may have undesirable effects on cancer patients, considering the central roles of NRF2 in cytoprotection (82).

Novel potential therapeutic targets in cancers exhibiting Keap1-Nrf2 pathway deregulation are being identified in addition to Nrf2 inhibitors. Some of these targets, including the glutathione synthesis, serine synthesis and pentose phosphate pathways, and IL-11, are direct or indirect downstream effectors of Nrf2 in mediating malignant phenotypes. However, efforts are still required to search for novel compounds and engineered small molecules that target Nrf2 more specifically, and to establish Nrf2 inhibitors suitable for safe clinical use and temporary use as a sensitizer in chemo- and radiotherapy regimens.

To date, considerable progress has been made to understand the mechanisms involved in the fine regulation of the Keap1-Nrf2 pathway and its downstream genes. However, due to the complexity of the cross-talk between Nrf2 and its numerous signaling-network partners, further studies are still required to determine the optimum markers for predicting the clinical outcome of patients with NSCLC. An improved understanding of the molecular mechanisms activating the Keap1-Nrf2 pathway may provide a basis for improvement in the selection of patients with poor prognosis, to be treated only with supportive care and thus avoiding unnecessary adverse effects and complications of systemic chemotherapy.

In conclusion, the findings reviewed herein suggest that evaluation of Keap1-Nrf2 pathway dysregulation in patients with NSCLC may provide predictive biomarkers of chemotherapy resistance and improve the accuracy of diagnosis.

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Authors' contributions

RBR was responsible for all aspects involved in the design, performance and writing of the literature review.

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Competing interests

The author declares that they have no competing interests.

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