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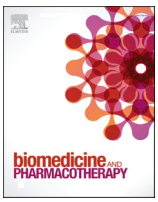

Nuclear factor kappa B expression in non-small cell lung cancer

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ABSTRACT

In this mini-review, we discuss the role of NF-κB, a proinflammatory transcription factor, in the expression of genes involved in inflammation, proliferation, and apoptosis pathways, and link it with prognosis of various human cancers, particularly non-small cell lung cancer (NSCLC). We and others have shown that NF-κB activity can be impacted by post-translational S-glutathionylation through reversible formation of a mixed disulfide bond between its cysteine residues and glutathione (GSH). Clinical data analysis showed that high expression of NF-κB correlated with shorter overall survival (OS) in NSCLC patients, suggesting a tumor promotion function for NF-κB. Moreover, NF-κB expression was associated with tumor stage, lymph node metastasis, and 5-year OS in these patients. NF-κB was over-expressed in the cytoplasm of tumor tissue compared to adjacent normal tissues. S-glutathionylation of NF-κB caused negative regulation by interfering with DNA binding activities of NF-κB subunits. In response to oxidants, S-glutathionylation of NF-κB also correlated with enhanced lung inflammation. Thus, S-glutathionylation is an important contributor to NF-κB regulation and clinical results highlight the importance of NF-κB in NSCLC, where NF-κB levels are associated with unfavorable prognosis.

1. Introduction

Non-small cell lung cancer (NSCLC) is a leading cause of cancer death in men and women worldwide, with a million deaths annually [1]. Even though surgery in conjunction with targeted and adjuvant therapies are useful treatments, currently the overall prognosis of the patients is still poor [2]. Therefore, promising prognostic biomarkers that contribute to the development of effective prevention and/or therapy in NSCLC could improve survival. The pro-inflammatory transcription factor, nuclear factor kappa B (NF-κB) contributes to malignancy through impacting cell senescence, apoptosis, metabolism, stress responses, and tumorigenesis [3–7]. In adenocarcinoma and squamous cell carcinomas of the lung, NF-κB activation induced by smoking regulates the expression of downstream pathways such as COX-2 [8], cyclin D1, matrix metalloproteinase-9 [9,10], thereby promoting cancer cell proliferation and survival [11]. Five members of the NF-κB family have been identified in mammals, forming various homo- or heterodimers subject to post-translational modifications, which are crucial for activation and translocation to the nucleus. S-glutathionylation is a reversible redox-sensitive post-translational modification that can regulate the function of NF-κB at multiple steps [12–16]. In the present review, we summarize recent studies on S-glutathionylation of NF-κB and evaluate the relationship of Nf-κB with clinical parameters of NSCLC, including patient prognosis.

2. Overview and components of NF-kB family

NF-kB was described in 1986 by Sen and Baltimore [17] as a nuclear transcription factor in B-cells that bound to the B-site of the immunoglobulin k enhancer. Over the years, the term NF-κB has been applied to different protein complexes made of homo- or heterodimers of five distinct proteins: p65 (RelA); p105/p50 (NF-kB1); p100/p52 (NF-kB2); c-Rel; and RelB [18]. In contrast to the other family members, NF-kB1 and NF-kB2 are synthesized as pro-forms (p105 and p100) and then proteolytically processed to p50 and p52. These proteins contain an N-terminal Rel homology domain (RHD), a highly conserved sequence of approximately 300 amino acids, which is responsible for DNA binding, dimerization, and nuclear localization. p65, c-Rel and RelB contain

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a C-terminal transcription activation domain (TAD), critical for the positive regulation of transcription function of downstream target genes. However, the carboxyl terminus of p50 and p52 do not contain TAD, and consequently, heterodimers need to be formed with proteins containing TAD to develop transcription factor functions. Instead, the *C*-terminal domains of p50 and p52 contain 6–7 ankyrin repeats responsible for sequestration in the cytosol, a characteristic particular to NF-kB inhibitory proteins (IkB) [19]. The most common form of cytoplasmic NF-kB appears to be the p50/p65/IkBα complex. NF-kB regulates the expression of some 500 genes that not only regulate immune and inflammatory responses but also play an important role in growth and apoptotic pathways of normal cells. Abnormalities, or disorders, of NF-kB are associated with several pathological conditions, including chronic inflammation, neurodegenerative diseases, atherosclerosis, immunodeficiency, and cancer. Therefore, transcriptional activities of NF-kB influence several human pathological conditions.

3. Signaling via the NF-kB system

The NF-kB complex is activated by phosphorylation, ubiquitination, and consecutive proteasomal degradation of the IkB component. Removal of IkB enables the p50/p65 heterodimer to translocate to the nucleus where transactivation is facilitated by specific DNA binding [20,21]. NF-kB is regulated by two pathways: the classical, NF-κB essential modulator (NEMO)-dependent and the non-classical, NEMO-independent (Fig. 1). Components of the classical pathway have been well described, while the non-classical pathway is less studied. It is subject to regulation by RelA/p50 or c-Rel/p50 and RelB/p52, respectively [22]. Cells exposed to, for example, bacteria, viruses, endotoxins, oxidative stress or radiation, are subject to activation of NF-kB with subsequent stimulation of canonical or non-canonical stress response pathways, impacting inflammatory responses [23].

3.1. Classical NF-κB signaling

Inflammatory stimuli such as TNF, IL-1, LPS [24] activate the NF-κB essential modulator (NEMO) and IKKα/β complexes, where IKKα/β phosphorylates NF-κB-bound IκBs, causing ubiquitination and proteasomal degradation of IκBs [25,26]. Free IκBs also undergo constitutive degradation via a ubiquitin-independent proteasomal degradation pathway. As IκBs are released from cytosolic NF-κB, free NF-κB is then available to translocate to the nucleus where it binds to κB sites on DNA and activates gene expression [27]. The IKKs consist of IKKα, IKKβ, and IKKγ. Two IKKγ molecules linked through disulfide bonds between Cys54 and Cys347 [28] form NEMO to which IKKα and IKKβ bind in the resting state [29,30]. IκBα, β, and ε are themselves NF-κB target genes, along with p100 that can form higher-molecular-weight complexes that inhibit NF-κB [31].

3.2. Non-classical NF-κB signaling

Developmental stimuli exemplified by members of the TNF-receptor superfamily lymphotoxin β (LTβ), CD40 ligand (CD40L), and B-cell activating factor (BAFF) [32] activate the complex of

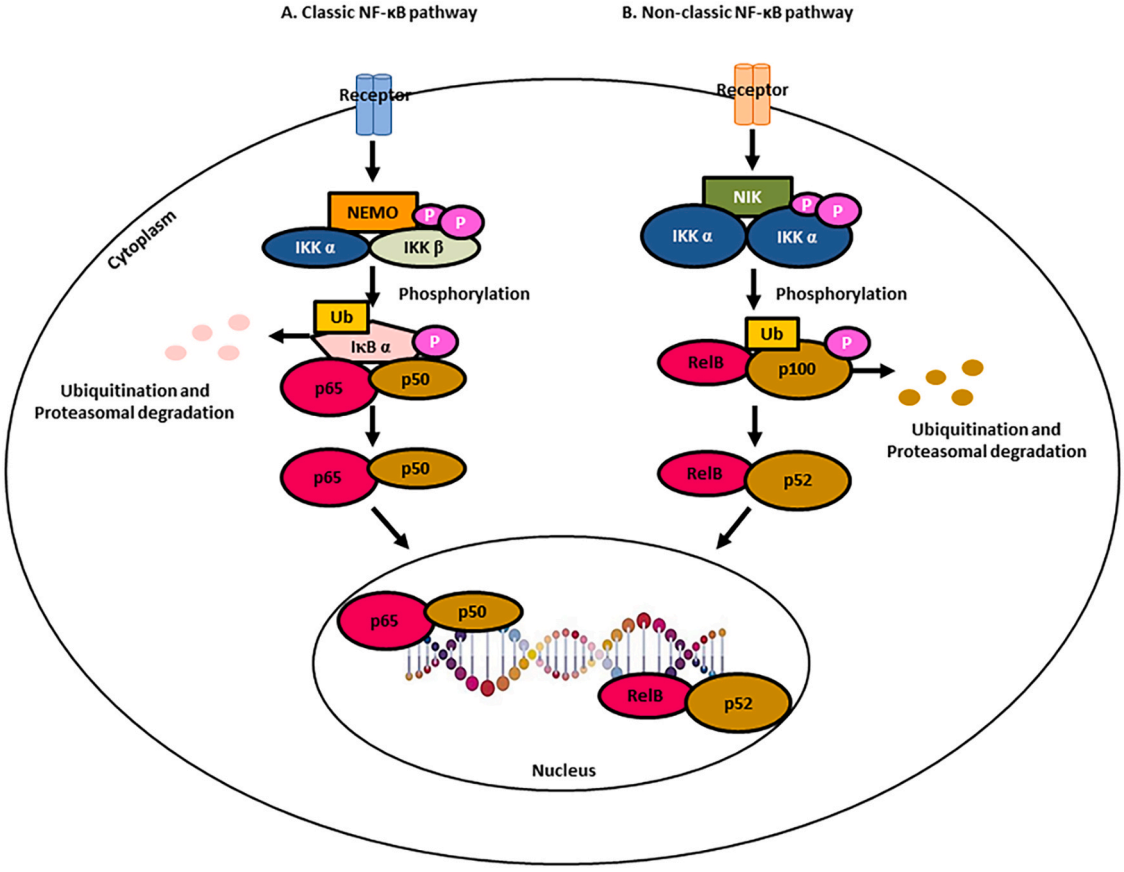

Fig. 1. NF-κB activation pathways. A. The classical NF-κB pathway. Activated complex NEMO and IKKα/β phosphorylates NFκB-bound IκBs, targeting them for ubiquitination and proteasomal degradation. Free IκBs also undergo constitutive degradation via a ubiquitin-independent proteasomal degradation pathway. As IκBs are degraded, free NFκB is then able to translocate to the nucleus where it binds to κB sites on DNA and activates gene expression. B. The non-classical NF-κB pathway. Activated NIK/IKKα complex phosphorylates p100. Upon phosphorylation, p100 is processed into p52 and is then available to bind RelB, creating a dimer that localizes to the nucleus and binds DNA to activate transcription. In addition, phosphorylation of p100 also results in its partial degradation releasing bound NFκB dimers for nuclear localization and gene activation.

NF-κB-inducing-kinase 1 (NIK1)/IKKα that phosphorylates p100, resulting in its ubiquitination and processing to p52 [11,33,34], able to bind RelB, creating a dimer that localizes to the nucleus and binds DNA to activate transcription [35,36]. Most p100 is found in a higher-molecular weight inhibitory complex (IκBδ). Active NIK1/IKKα complex also phosphorylates the p100 within IκBδ, resulting in its partial degradation and releasing bound NF-κB dimers for nuclear localization and gene activation [31].

Since NF-κB is a stress response factor, the stimulus and cell type involved determines whether downstream pathways lead to cell death or survival [37]. Activation of NF-κB has been found in a variety of human tumors, including lymphoma, colorectal cancer, esophageal cancer, head and neck cancer, breast cancer, hepatocellular carcinoma, and prostate cancer et al. [38–44]. During tumorigenesis, activation of NF-κB can be influenced by mutation [45] and NF-κB over-expression in certain solid tumors has been associated with poor prognosis [46]. NF-κB may promote development and progression of malignancy in several ways [47–52]: (i) anti-apoptosis: NF-κB can induce the expression of inhibitors of apoptosis (IAPs) and anti-apoptotic bcl-2 family members, up-regulate the expression of proteins related to the death receptor pathways and inhibit apoptosis. It can also inhibit p53-induced apoptosis by interacting with the tumor suppressor gene p53. (ii) promoting proliferation, invasion, and metastasis of tumor cells: NF-κB induces proliferation, invasion, and metastasis through modulation of cell cycle-related genes (cyclinsD1, D2 D3 E and c-myc), increasing expression of adhesion factors and invasion-related proteins, and angiogenesis factor. (iii) NF-κB up-regulation associated with tumor therapy resistance. (iv) influence the tumor microenvironment. By activating the expression of genes in tumor cells or cells in the tumor microenvironment, various cytokines were produced to involve in extracellular matrix dissolution, angiogenesis, bone tissue destruction, etc. (v) NF-κB is an important factor in inflammatory responses contributing to tumorigenesis. In contrast, NF-κB has also been reported to contribute to tumor suppression. Rocha and colleagues demonstrated that p53 regulated the activity of p52 subunit of NF-κB to inhibit the cyclin D1 promoter by switching p52-Bcl-3 activator complexes to p52-histone deacetylase (HDAC) repressor complexes [53]. In addition, they also showed that tumor suppressor alternative reading frame (ARF) bound to, and sequestered, the p53 inhibitor Mdm2 to activate p53 in response to oncogene expression. ARF can also regulate the p65 subunit of NF-κB to turn it into a p65-HDAC repressor, independent of p53 and Mdm2 [54]. These contrasting functions illustrate the importance of the cell or tissue type and whether there is underlying pathology.

4. Redox regulation/S-glutathionylation of NF-κB

Redox regulation through S-glutathionylation is involved in modulating the activity of NF-κB and its downstream signaling pathways. Under physiological conditions, intracellular redox homeostasis is generally maintained towards a reduced state with high glutathione (GSH)/glutathione disulfide (GSSG) ratios, acting as a major redox buffer in the cytosol [55–57]. Optimal GSH/GSSG ratios regulate cell survival and the pools of protein and non-protein thiols. S-glutathionylation is characterized by the reversible formation of a mixed disulfide bond between a protein cysteine residue and GSH, resulting in an increased molecular mass and enhanced negative charge; this reaction not only protects Cys from further oxidative damage but also controls protein tertiary structure [58–62]. In addition, reversible S-glutathionylation plays a key role in cell signaling and the crosstalk between proteins and other molecules, where the glutathionylation cycle can regulate signal transduction [60,63,64]. Protein S-glutathionylation takes place mainly in cytoplasm, endoplasmic reticulum, and mitochondria [65]. Within the cytoplasm, S-glutathionylation regulates a number of transcription factors in addition to NF-κB, such as erythroid 2-related factor 2 (Nrf-2) and activator protein-1 (AP-1) [66]. NF-κB, as a central regulator of immunity, is subject to multiple regulation by redox. S-glutathionylation negatively regulates the NF-κB pathway at multiple sites by introducing negative charges to DNA binding sites. Moreover, glutaredoxin (Grx) can activate NF-κB by catalyzing deglutathionylation and facilitating activation of survival genes [14,15] (Fig. 2). Pineda-Molina et al. found that S-glutathionylation of Cys62 of NF-κB p50 subunit prevents binding of the transcription factor to κB sites in the promoter regions of genes [14]. Changing the GSH/GSSG ratio from 100 to 0.1 caused inhibition of the DNA binding of p50WT subunit but had no effect on a DNA binding domain mutant (C62S). S-glutathionylation of inhibitory kappa kinase (IKK)-β inhibited phosphorylation of IκBα and further nuclear translocation of p65 (RelA). In alveolar macrophages, exposure to hydrogen peroxide caused S-glutathionylation of Cys179 of IKK-β causing IKKβ inactivation, subsequently preventing IκBα degradation and NF-κB translocation. Grx reversed this process and restored kinase activity [13]. Reynaert et al. precipitated S-glutathionylated proteins from either WT or Cys-179-mutant cell lysates and showed that H_2O_2 increased S-glutathionylation of WT IKK-β but not the Cys-179- mutant; in addition, they over-expressed cytosolic Grx1, resulting in a decrease in H_2O_2-induced S-glutathionylation of IKK-β. Knockdown of Grx1 sensitized cells to inhibition of IKK-β by H_2O_2, leading to repression of NF-κB transcriptional activity. Taken together, S-glutathionylation of Cys-179 of IKK-β by H_2O_2 is indeed responsible for inhibition of IKK-β activity and inhibitory effects of H_2O_2 can be circumvented by Grx1, allowing activation of IKK-β and NF-κB. NF-κB signaling is also a major survival pathway against hypoxia-induced apoptosis. In pancreatic cancer cells, hypoxia-induced the DNA binding of p65 subunit of NF-κB and NF-κB transactivation, which was blocked by the GSH precursor N-acetyl cysteine (NAC) [12]. In addition, dithiothreitol (DTT) increased p65 binding to DNA in hypoxic cells treated with NAC, but not with *N*-ethylmaleimide (NEM). Grx reversed the inhibition of p65 DNA binding in hypoxic cells treated with NAC, which confirmed that p65-SSG was formed under hypoxia and NAC treatment. Knock-down of Grx in cells also increased protein S-glutathionylation with hypoxia plus NAC and prevented NAC-induced NF-κB inactivation. S-glutathionylation of the p65 subunit of NF-κB, which is mainly responsible for transcriptional activation, has been shown to inhibit p65-NF-κB binding to DNA, resulting in decreased resistance to hypoxia in pancreatic cancer cells supplemented with GSH via NAC [15]. IκBα can be modified by the formation of a mixed disulfide at Cys189 both in vitro and in HeLa cells, reversible by dithiothreitol (DTT) and GSH. S-glutathionylation of Cys189 of IκBα inhibits phosphorylation by IKK and weakens ubiquitination, degradation, and subsequent activation of NF-κB, presumably through structural alterations [16].

There is evidence that members of the GST family, particularly GSTO1–1, contribute to redox regulation of NF-κB signaling. Through knockdown experiments and after LPS stimulation in macrophages, the absence of GSTO1–1 hindered nuclear translocation of NF-κB. This suggests that NF-κB may rely on GSTO1–1 for dissociation from IκBα, potentially through a process involving deglutathionylation [67]. In addition, endogenous GSTP1 can catalyze S-glutathionylation of IKKβ in response to LPS stimulation, causing inhibition of IKKβ kinase activity. This establishes the significance of IKKβ as a mediator of NF-κB signaling in relation to GSTP1 [68,69]. Praeruptorin B (Pra-B) has a significant impact on the S-glutathionylation of IKKβ induced by receptor activator of NF-κB ligand (RANKL), with the upregulation of GSTP1 playing a pivotal role in this process. This leads to the inhibition of p65 nuclear translocation. Furthermore, reversal of these effects upon siRNA-mediated knockdown of GSTP1 and inhibition of glutathione synthesis provides evidence that Pra-B enhances the S-glutathionylation of IKKβ through upregulation of GSTP1, ultimately resulting in the suppression of NF-κB nuclear translocation [70]. Collectively, these findings suggested that S-glutathionylation can control activation of NF-κB at multiple levels. Overall, emergent research on redox regulation in the NF-κB pathway highlights the importance of S-glutathionylation as a post-translational modification involved in modulating NF-κB

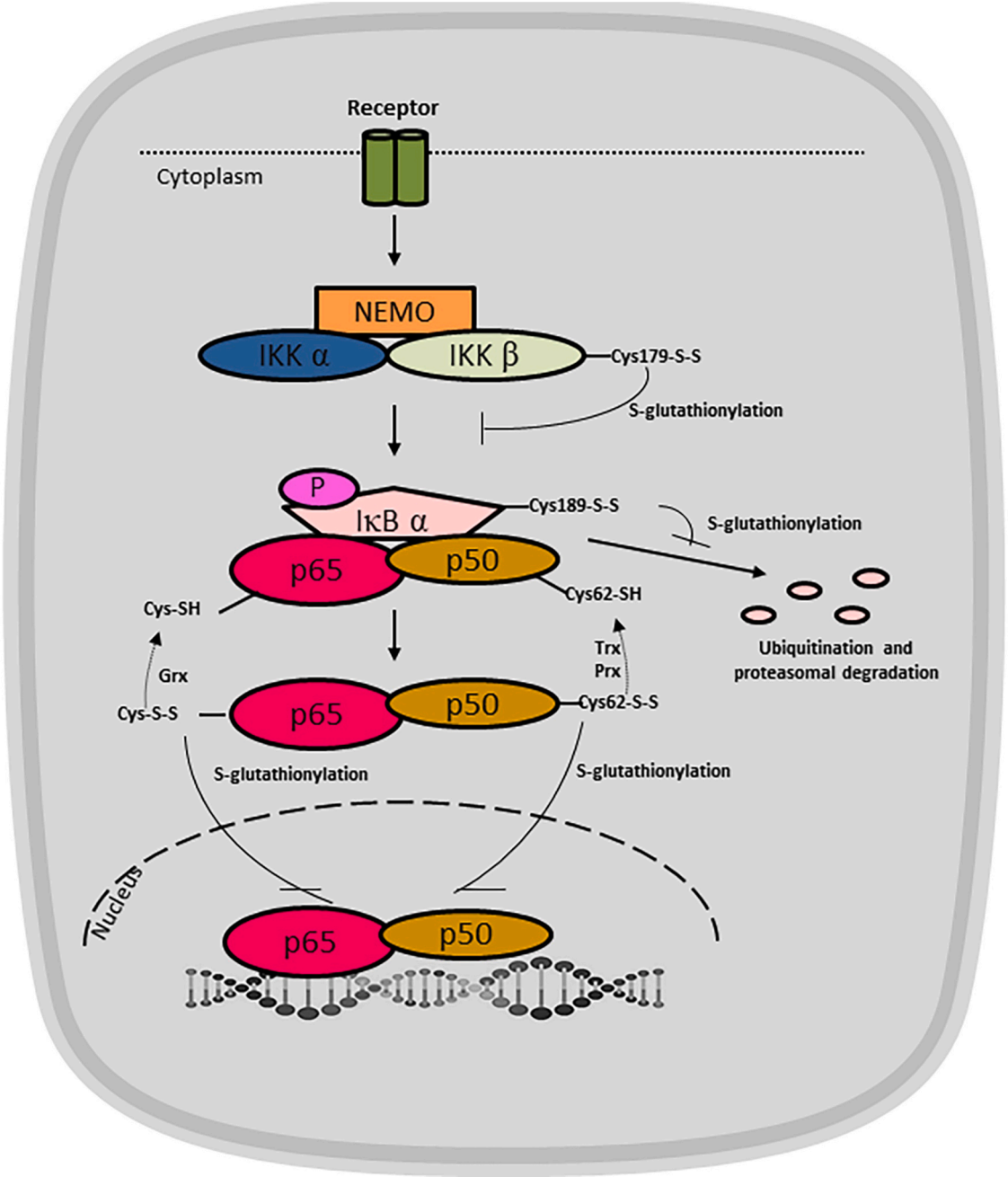

Fig. 2. S-glutathionylation of NF-κB. S-glutathionylation downregulates NF-κB pathway at four distinct levels: (1) S-glutathionylation of cysteine 179 of IKKβ, preventing its phosphorylation and activation. (2) S-glutathionylation of cysteine 189 of IκB, thus preventing its degradation and release of free NF-κB. (3) S-glutathionylation of cysteine 62 of the p50 subunit of NF-κB, thereby inhibiting its DNA binding, which can be restored by p50 reduction via antioxidant enzymes, such as thioredoxin (Trx) or peroxiredoxin (Prx). (4). S-glutathionylation of p65, preventing its DNA binding and favoring p65 nuclear export, which can be reversed by glutaredoxin (Grx).

activity and subsequent expression of downstream genes. Further details could elucidate how threshold mechanisms controlled by altered redox conditions impact NF-κB-mediated pathways.

5. NF-κB in non-small cell lung cancer (NSCLC)

Expression patterns of different NF-κB subunits in NSCLC tissues have been described [71,72]. Compared with normal lung, all NF-κB subunits in tumor tissue were more highly expressed. While RelB was most prevalent, RelA, p50, and p52 were present in normal bronchial and alveolar epithelial cells resected with tumor. All NF-κB subunits were also highly expressed in tissues adjacent to the tumor. In tumor tissues and tumor stroma, RelA, RelB, and p50 were more prevalent than p52, but there were no differences in expression of different NF-κB subunits in NSCLC tissues of different pathologies [73]. Proliferating cell nuclear antigen (PCNA) staining revealed that subunits of NF-κB were distributed differently in cells of tumor tissues and stroma. RelB was most highly expressed in the nucleus and cytoplasm, while other NF-κB subunits were only weakly expressed in the cytoplasm of tumors [73]. In addition, it was found that high expression areas of RelB, corresponded with low p50. In tumor stroma, RelA and p50 were most prevalent and located in the interstitial nucleus, while RelB and p52 were weakly expressed. Only p52 was consistently expressed in tumor tissues and tumor stroma [74]. While the nuclear expression of NF-κB p65 did not

exhibit a significant correlation with overall survival or disease-free survival in NSCLC patients, a pronounced elevation in activated nuclear NF-κB p65 expression was observed in lung cancers [75].

There is a growing body of preclinical data from in vivo models on the role of NF-κB in NSCLC. In a transgenic mouse model increased expression of a specific NF-κB inhibitor in airway epithelial cells showed that both lung inflammation and tumor formation were decreased [76]. An increased nuclear translocation of NF-κB subunit p65 occurred in both mice and human lung cancer cells with activated oncogenic K-ras^{G12D} and depletion of p53, also confirmed in vivo in tumors established with activated K-ras and loss of p53 [77]. Additionally, mice with knockout of the tumor suppressor gene *Gprc5a* (G-protein coupled receptor class C group 5 member A) elucidated how activation of NF-κB is linked with expression of various cytokines and chemokines [78]. Two independent mouse models of tobacco smoke exposure showed that tumor growth is enhanced in myeloid cells by activating IKKβ-dependent production of cytokines, such as IL-6 and TNF-α [79]. In an NF-κB inducible transgenic mouse model, inflammation, in addition to lung tumor formation were enhanced by regulatory T lymphocytes (Tregs) in airway epithelial cells [80]. Evidence derived from a Kras^{G12D}IKKβ$^{-/-}$ mouse model showed that depletion of IKKβ significantly attenuated tumor proliferation and prolonged survival [81]. Hopewell et al. elucidated that NF-κB plays a key role in mediating immune surveillance and promoting antitumor T cell responses in both murine and human lung cancer [82]. Downregulation of another subunit of NF-κB, IKKα, contributes to the promotion of pulmonary inflammation and development of lung squamous cell carcinomas (SCCs) [83]. By regulating NF-κB and STAT3 pathways to induce cell proliferation and tumorigenicity in vitro and in vivo, macrophages promote malignant transformation of human bronchial epithelial cells [84]. In addition, Theanine derivatives (TFC and TNC) acting through the NF-κB pathway can significantly inhibit the growth of invasive Lewis lung cancer (LLC) and A549 tumor cells [79], implying that activation of the NF-κB pathway promotes development of NSCLC. The combination of an NF-κB inhibitor and TNF-related apoptosis-inducing ligand (TRAIL) significantly promotes apoptosis of lung cancer cells resistant to TRAIL both in vitro and in vivo [77]. Additionally, the oncogene URGCP (up-regulator of cell proliferation) activates the expression of MMP-9 by activating the NF-κB pathway to promote invasion and metastasis of NSCLC cells [85]. In a meta-analysis, a more precise relationship between NF-κB expression and survival of NSCLC patients was attained. A total of 1159 patients from 7 studies considered to be eligible for comparison of the prognostic value of NF-κB expression levels in NSCLC were included. High levels of NF-κB were linked to shorter overall survival (OS), indicating a tumor-promotive function for NF-κB. A significant correlation between NF-κB and OS in Asians was also found in the subset analysis. NF-κB expression was associated with tumor stage, lymph node metastasis, and 5-year OS for NSCLC patients. Patients with late-stage tumors had higher NF-κB expression compared to early stage and it was more frequent in patients with lymph node metastasis. In conclusion, the expression of NF-κB was suggested to be a significantly unfavorable marker for prognosis and OS of patients with NSCLC [72].

6. Prognostic value of NF-kB family members in lung cancer based on KM Plotter database

To understand the prognostic value of NF-kB in lung cancer, results were extrapolated from the Kaplan–Meier (KM) Plotter database (https://kmplot.com/analysis/) to evaluate overall survival (OS), first progression survival (FP), and post-progression survival (PPS). In each cohort, patients were divided into high and low-expression groups based on the "auto select best cutoff" model, meaning that all possible cutoff values were computed, and the best performing threshold was used as a cutoff (Fig. 3). Lower expression levels of p100/p52 mRNA were associated with improved OS ($p = 0.00064$), FP ($p = 0.021$), and PPS ($p = 0.017$); Lower expression of p65 mRNA was associated with improved FP ($p = 0.0069$); Lower expression of RelB mRNA was associated with improved OS ($p = 2.3e-07$) and FP ($p = 5.3e-05$); Lower expression of c-Rel mRNA was associated with improved OS ($p = 0.00039$) and PPS ($p = 0.0043$). In contrast, lower p105/p50 mRNA expression was significantly associated with poorer OS ($p = 0.018$) and PPS ($p = 0.00036$); lower p65 mRNA expression was associated with poorer OS ($p = 6.8e-05$) and PPS ($p = 0.00054$); lower c-Rel mRNA expression was significantly associated with poorer FP ($p = 7.9e-05$). There was no significant correlation found between p105/p50 expression and FP in lung cancer. Similarly, there was no significant association observed between RelB expression and PPS in lung cancer. In conclusion, the analysis of the NF-kB family in lung cancer patients using the KM Plotter database revealed distinct associations between mRNA expression levels of different NF-kB family members and OS, FP and PPS. These findings emphasize the potential prognostic value of specific NF-kB family members in lung cancer, providing insight for guiding personalized treatment strategies.

7. Perspective

NF-κB, as an inflammatory transcriptional factor, induces expression of > 200 genes involved in diverse processes such as cell survival, cell adhesion, inflammation, differentiation, growth [18] and influences progression of cancer [86]. Increased NF-κB activity induces expression of responsive genes in many tumor types including lung [87,88]. Under certain circumstances, NF-κB can function as a tumor suppressor. NF-κB induced by DNA damage can suppress rather than activate gene transcription [89], perhaps involving interactions with transcriptional repressors or tumor suppressors such as p53 and ARF [90]. Thus, the effects of DNA-damaging drugs on NF-κB in can be tumor specific. With respect to NSCLC, previous reports show up-regulation of NF-κB is associated with [72] poor survival outcomes. In addition, increased NF-κB expression in patients with late-stage NSCLC and lymph node metastases implied some type of tumor promoter function in this disease. Expression of NF-κB in the nucleus (but not cytoplasm) can be associated with a worse 5-year OS for NSCLC, indicating that the relationship between NF-κB expression and 5-year OS may be linked with its subcellular localization. Classical NF-κB pathways may act in a tumor promoter fashion and perform anti-apoptotic roles, stimulating transcription of proliferation regulating genes involved in metastasis, VEGF dependent angiogenesis and cell immortality. In contrast, the non-classical NF-κB pathway may act as a tumor suppressor, inhibiting tumor growth and facilitating apoptosis. In this regard, further clarification of the prognostic value of classical and non-classical NF-κB activation pathways in NSCLC patients is required, particularly to address contradictions in the literature. Some studies show that NF-κB can have contrasting roles in different cohorts of NSCLC patients. This can be explained by the fact that NF-κB does not function alone, but is part of a network, determining the pattern of its effects on the expression of several other genes and thus, their function [91].

S-glutathionylation, has been detected not only under stress conditions, but also under physiological conditions and performs an important role in the control of numerous cellular processes. Reversible S-glutathionylation can serve as a cellular control for turning signaling pathways on and off [92,93]. GSH is the major thiol-disulfide redox buffer [94] and pools of GSH may be either free or bound to reactive cysteine thiols of various proteins [95]. NF-κB, has cysteine thiols that respond to changes in redox environment. S-glutathionylation inhibits the DNA binding activity of NF-κB. Indeed, S-glutathionylation negatively regulates NF-κB at multiple sites: (i) S-glutathionylation of cys-62 of p50 prevents p50-DNA binding; (ii) following ROS, S-glutathionylation of cys-179 of IKK-β causes inactivation; (iii) S-glutathionylation of p65 prevents p65-DNA binding and transcription;(iv) S-glutathionylation of cys-189 of IκB prevents its degradation [13–16]. In humans, cytosolic Grx1 and mitochondrial Grx2 can diminish S-glutathionylated proteins [96–98]. S-glutathionylation of NF-κB was

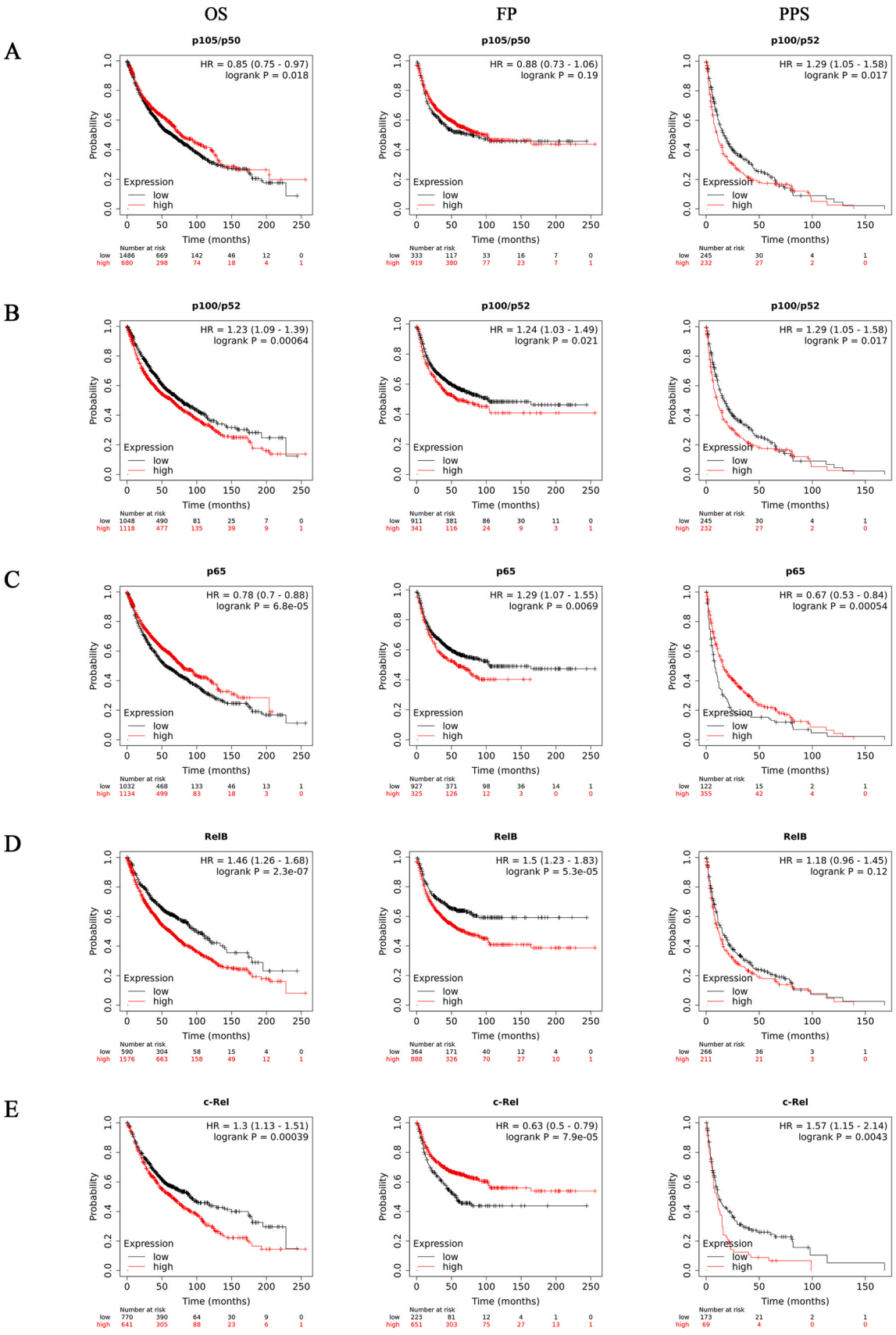

Fig. 3. OS, FP, and PPS survival curves of patients with NSCLC by expression of NF-kB family members (Kaplan–Meier Plotter). The threshold of significance was a p-value of < 0.05. The association between prognostic value and (A) p105/p50, (B) p100/p52, (C) p65, (D) RelB, (E) c-Rel. Red: high expression; Black: low expression. OS, overall survival; FP, first progression survival; PPS, post-progression survival. HR, hazard ratio (with 95% confidence interval).

observed in the lungs of Grx knockout mice, enhancing lung inflammation in response to oxidants [99].

It is perhaps not surprising that redox balance will have such a significant impact on lung cells and that S-glutathionylation of Nf-kB would be symptomatic of NSCLC. Future directions could help to identify redox-sensitive components of the canonical and non-canonical Nf-kB pathways that can be used for prognostic applications or targeted therapeutically. Technological advances in high-throughput sequencing and proteomics will help with the identification of biomarkers and the creation of individualized medicinal approaches.

CRediT authorship contribution statement

Leilei Zhang (investigation, data curation, writing); **Claudia M. Ludden** (Investigation, data curation, formal analysis)**; Alexander J. Cullen** (Investigation, data curation, formal analysis). **Kenneth D. Tew** (revision writing and restructuring), **André Luís Branco de Barros (**formal analysis, writing, visualization**) Danyelle M. Townsend** (Conceptualization, Project administration, funding acquisition, formal analysis, writing).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Danyelle M. Townsend reports financial support was provided by Medical University of South Carolina. DMT and ADB serve as Editors for Biomedicine and Pharmacotherapy.

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