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NF-kappaB in lung cancer, a carcinogenesis mediator and a prevention and therapy target

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1. ABSTRACT

Lung cancer ranks as the first malignant tumor killer worldwide. Despite the knowledge that carcinogens from tobacco smoke and the environment constitute the main causes of lung cancer, the mechanisms for lung carcinogenesis are still elusive. Cancer development and progression depend on the balance between cell survival and death signals. Common cell survival signaling pathways are activated by carcinogens as well as by inflammatory cytokines, which contribute substantially to cancer development. As a major cell survival signal, nuclear factor-kappaB (NF-kappaB) is involved in multiple steps in carcinogenesis and in cancer cell's resistance to chemo- and radio-therapy. Recent studies with animal models and cell culture systems have established the links between NF-kappaB and lung carcinogenesis, highlighting the significance of targeting NF-kappa signaling pathway for lung cancer treatment and chemoprevention. In this review, we summarize progresses in understanding the NF-kappaB pathway in lung cancer development as well as in modulating NF-kappaB for lung cancer prevention and therapy.

2. INTRODUCTION

Lung cancer is the leading cause of cancer-related death, which afflicts approximately 170,000 people each year in the United States (1). A large number of lung cancers are associated with cigarette smoke, although other factors such as environmental influences like radon or nutrition may be also involved (2). Many lung cancer patients are diagnosed at late stages of the disease when surgery is not applicable. Chemotherapy and radiation therapy, as well as a combination of both therapies, are used in an attempt to reduce tumor mass and halt disease progression. However, because such therapies are usually ineffective for lung cancer, the prognosis of the patients is

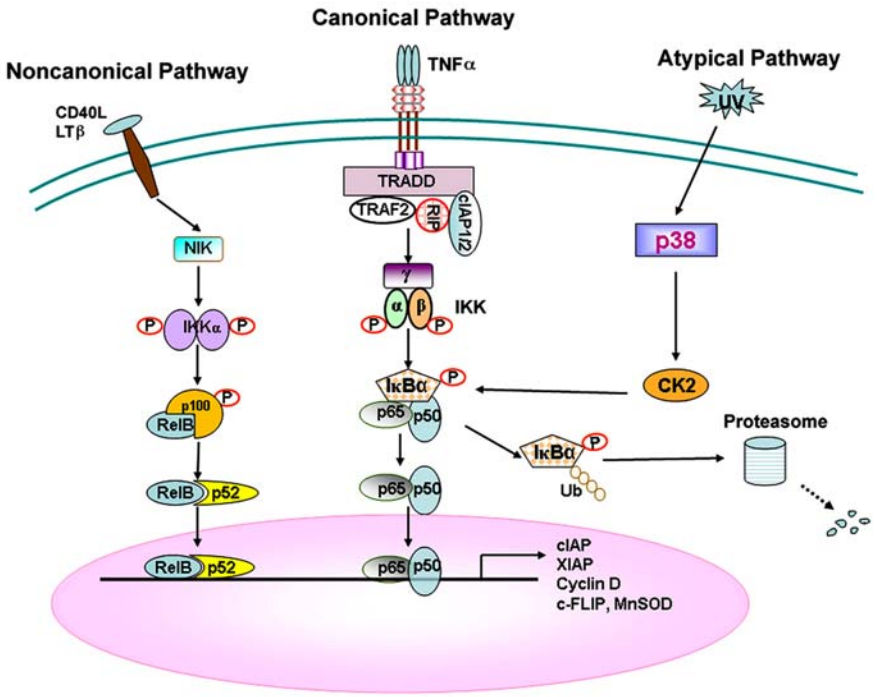

Figure 1. Pathways for NF-kappaB activation. The canonical pathway is activated by cytokines such as TNFalpha. When TNFalpha binds to its receptor 1 (TNFR1), a signaling complex is formed to recruit and activate IKK, which leads to phosphorylation on IkappaB. IkappaB is subsequently ubiquitinated and degradated in the proteasome, resulting in NF-kappaB complex (p65/p50) translocation to the nucleus and activation of gene transcription. The noncanonical pathway is activated by cytokines such as CD40L and lymphotoxin beta. This pathway involves NIK-mediated IKKalpha activation and cleavage of p100 to create p52. The NF-kappaB complex (p52/RelB) moves to the nucleus to activate gene transcription. The atypical pathway is activated by a variety of stimuli such as UV. In this pathway, IKK-independent mechanisms are involved in IkappaB phosphorylation. Representative antiapoptotic factors induced by NF-kappaB are shown.

usually very poor (3). Therefore, development of effective prevention and therapy approaches against lung cancer is critical for reducing mortality.

Cancer cells, including lung cancer cells, have acquired numerous characteristic alterations facilitating their oncogenic growth. Accumulating evidence suggests that lung cancer cells use multiple and perhaps redundant pathways to maintain survival (2). Common signal transduction pathways for cell survival and proliferation include mitogen-activated protein kinases (MAPK), Akt and NF-kappaB. In lung cancer cells, multiple mechanisms are used to override or "hijack" the signal transduction pathways to facilitate their own survival and proliferation (4). In this review, we will summarize the recent reports on NF-kappaB in lung cancer biology and discuss the preventive and therapeutic potential of targeting NF-kappaB against lung cancer.

3. NF-KAPPAB ACTIVATION PATHWAYS

3.1. Protein components in the NF-kappaB family

In mammalian cells, five NF-kappaB family members are found: p65 (RelA), RelB, c-Rel, p50/p105 (NF-kappaB1) and p52/p100 (NF-kappaB2). These proteins share a unique N-terminal Rel homology domain (RHD) for forming hetero- or homodimer dimmers and binding DNA. Having a C-terminal transactivation domain (TAD) p65, RelB, and c-Rel function as transactivators when associated with p50 or p52, while p50 and p52 lack TADs, and their homodimers serve as transcription repressors that provide a threshold for NF-kappaB activation (5). The most common form of NF-kappaB is a heterodimer consisting of p65 and p50. In most quiescent normal cells the NF-kappaB dimers are bound with and kept in the cytoplasm by inhibitor of kappaBs (IkappaBs) that mask the nuclear localization sequence (NLS) in the NF-kappaB proteins. Five members of the IkappaB protein family have been identified so far: IkappaBalpha, IkappaBbeta, IkappaBgamma, IkappaBepsilon and BCL-3. The high affinity of IkappaB proteins in binding NF-kappaB ensures the activation of this pathway in a tight check. The precursor proteins p105 and p100 function similarly as the IkappaB proteins to squelch NF-kappaB in the cytoplasm (5).

3.2. The pathways leading to NF-kappaB activation

As a multifunctional transcription factor, NF-kappaB is activated by numerous extracellular stimuli including cytokines, growth factors, carcinogens and tumor promoters and intracellular cues ignited by genotoxic or endoreticulum stress (ER stress). There are three pathways leading to NF-kappaB activation and ultimately to expression of distinct sets of target genes for diverse biological functions (Figure 1) (6).,

Being the major NF-kappaB activation pathway in most cell types, the canonical pathway involves dimers composed of p50 and p65 or c-Rel (5) and is often activated by microbial infections, growth factors and proinflammatory cytokines including TNFalpha. TNFalpha engagement induces trimerization of TNFalpha receptor 1 (TNFR1) and recruitment of multiple adaptor proteins and kinases, resulting in the phosphorylation and activation of IkappaB kinase (IKK) complex. IKKs consist of catalytic subunits IKKalpha/IKK1, IKKbeta/IKK2, and an essential regulatory subunit IKKgamma/nuclear factor-kappaB essential modulator (NEMO). The IKK activity is solely dependent on IKKbeta for the canonical pathway, and the IKK activating kinase is thought to be mitogen activated kinase 3 (MEKK3) or TGFbeta-activated kinase 1 (TAK1) (7-9). The activated IKK is switched from the TNFR1 signaling complex to the NF-kappaB/IkappaB complex, where IKKbeta phosphorylates the serine 32 and 36 in IkappaB, which is then polyubiquitinated followed by rapid degradation in the proteasome. When NF-kappaB is freed up from IkappaB, the NLS signal on p65 and p50 is exposed, leading to nuclear translocation of NF-kappaB. In the nucleus, NF-kappaB is subjected to further modifications including phosphorylation and acetylation on the p65 subunit, which impact the locating in the compartments in the nucleus (10), binding to DNA or interaction with transcriptional co-activators such as cAMP response element-binding protein (CBP)/p300 (5).

DNA damage induced by anticancer genotoxic agents and ionizing radiation activates the IKK-IkappaB-NF-kappaB cascade, which is also regarded as a canonical pathway. DNA damage rapidly induces the ataxia telangiectasia mutated (ATM) kinase, which phosphorylates the IKK subunit NEMO/IKKgamma in a complex in the nucleus called PIDDsome, consisting of RIP1, p53-induced death domain (PIDD) and NEMO (11). After a serial modification including phosphorylation and sumolysation, NEMO migrates from the nucleus to the cytoplasm to bind and activate IKKbeta. Then IKKbeta phosphorylates IkappaB and turns on the canonical NF-kappaB activation pathway (12). IKKepsilon is involved in modification of p65 in the nucleus to modulate the DNA damage-induced NF-kappaB activation (13).

The noncanonical pathway involves the non-death receptor members of the TNF receptor family such as CD40, lymphotoxin beta (LTbeta) and B-cell-activating factor (BAF). When these receptors are activated by their cognate ligands, NF-kappaB inducing kinase (NIK) is stabilized and activated, presumably by auto-phosphorylation, which mediates IKKalpha phosphorylation. Subsequently, IKKalpha triggers conformation change in p100, which is then cleaved to generate p52. A functional NF-kappaB heterodimer containing p52 and RelB is formed and translocated to the nucleus to turn on gene expression (5). The c-IAP proteins that are required for activating the canonical pathways, suppress the noncanonical pathway through NIK ubiquitination and degradation (6, 14). Therefore, the canonical and noncanonical pathways are coordinated under some circumstances, which may provide a delicate control of the overall NF-kappaB activity in the cell. The non-canonical NF-kappaB pathway is activated by K-Ras^{G12D} through TANK-binding kinase 1 (TBK1), contributing to oncogenic K-Ras-mediated lung carcinogenesis (15).

The atypical pathways lead to NF-kappaB activation by distinct mechanisms. For example, casein kinase 2 (CK2) rather than IKK is required for short wavelength ultraviolet (UV) light -induced NF-kappaB activation. In this pathway, calpain rather than proteasome is involved in IkappaB degradation (16). Also, it is reported that phosphorylation of IkappaB at Tyr42 by c-Src or Syk kinases underlies the mechanism for hydrogen peroxide-induced NF-kappaB activation (17).

4. NF-KAPPAB'S CELLULAR FUNCTIONS

4.1. NF-kappaB and transcription

NF-kappaB is a transcription factor that induces expression of more than 200 genes involved in diverse process such as cell survival, cell adhesion, inflammation, differentiation and growth (5). Activation of NF-kappaB up-regulates expression of its responsive genes in cancer cells including lung cancer cells (18, 19). However, under certain conditions, NF-kappaB can function as a transcriptional suppressor. For example, DNA-damage-induced NF-kappaB suppresses rather than activates gene transcription (20), which may involve interactions with transcriptional repressors or tumor suppressors such as p53 and ARF (21). Thus, it is important to elucidate the transcriptional functions of DNA damaging anticancer drugs-induced NF-kappaB activation in different cancer cells before applying NF-kappaB manipulating approaches for sensitizing anticancer chemotherapy.

4.2. NF-kappaB and cell proliferation

NF-kappaB is a positive mediator of cell growth and proliferation. NF-kappaB increases the expression of several factors involved in cell cycle progression such as cyclins D and E. Up-regulation of cyclin D1 expression by NF-kappaB is associated with enhanced transition from G1 to S phase. Furthermore, NF-kappaB negatively regulates expression of growth arrest and DNA damage-inducible protein 45 (GADD45), a cell cycle checkpoint protein that keeps cell at the G2/M phase transition (22). Additionally, the mutual interplay between NF-kappaB and proinflammatory cytokines such as TNFalpha and IL1beta is also involved in stimulating cancer cell proliferation, particularly during chronic inflammation (23). However, a potential proliferation suppressing function of NF-kappaB is also proposed, because it suppresses c-Jun N-terminal kinase (JNK), an important proliferation signal in some cell types, and triggers expression of the G1 arrest factor p21/WAF1 (6).

4.3. NF-kappaB and apoptosis

NF-kappaB plays a critical role in blocking apoptosis through various mechanisms, of which induction of antiapoptotic protein expression is regarded as the major one. The NF-kappaB-responsive anti-apoptotic genes include Bcl-XL, cIAP1, cIAP2, XIAP, A20, TRAF-2 and c-FLIP, which promote cell survival by desensitizing the

cells to apoptosis induced by a variety of stimuli such as cytokines and chemotherapeutics (24). Second, NF-kappaB can suppress cellular stress-mediated apoptosis through removal of reactive oxygen species (ROS) via increasing expression of manganese superoxide dismutase (MnSOD) (25, 26). Thus, NF-kappaB inhibits both the mitochondrial (intrinsic) and death receptor (extrinsic) pathways. Third, NF-kappaB also negatively regulates the apoptotic JNK activation (27). In addition, NF-kappaB suppresses apoptosis through antagonizing p53, possibly through competition for transcriptional co-activators (28). Finally, NF-kappaB down-regulates the expression of phosphatase and tensin homolog (PTEN) to activate Akt to promote cell survival and proliferation (29).

5. NF-KAPPAB IN LUNG CARCINOGENESIS

5.1. NF-kappaB activation in lung cancer

There is considerable evidence that NF-kappaB is constitutively activated in a variety of solid tumors, including prostate, breast, cervical, pancreatic and lung cancer (30, 31). Although lung tumors are histologically heterogenic, tumor samples obtained from lung cancer patients showed high levels of NF-kappaB activation in both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), and is significantly associated with disease advancement in TNM stages and poor prognosis in lung cancer patients (31, 32). Inhibiting NF-kappaB with different approaches such as siRNA, IKK inhibitors and IkappaB super suppressor inhibited lung cancer cell's survival and proliferation (18, 33, 34).

5.1.1. Oncogene-mediated NF-kappaB activation

The contributions of NF-kappaB to lung cancer development are complex, underlying mechanisms of which have not been fully understood. The findings that NF-kappaB activation is associated with K-Ras mutation led to the hypothesis that activation of NF-kappaB is the result of oncogene activation (15, 35, 36). Notably, K-Ras accounts for 90% of Ras mutation in lung cancer. Lose of p53 function and constitutively active $K\text{-}Ras^{G12D}$ collaboratively activate NF-kappaB in lung cancer cells, which is demonstrated in a mouse lung cancer model and in human lung cancer tissues and cell lines (37). $K\text{-}Ras^{G12D}$ also activates the non-canonical NF-kappaB activation pathway through TBK1, and suppression of TBK1 induced apoptosis specifically in human cancer cell lines that have oncogenic K-Ras expression (15).

5.1.2. Inflammation-associated NF-kappaB activation

A large body of evidence suggests that inflammation plays an important role in lung cancer development (38). In addition to pulmonary infection and allergies, cigarette smoke (CS) is a common cause of chronic lung inflammation (38, 39). Myeloid cells (mainly macrophages) are the major source of inflammatory cytokines for cancer promotion and progression. NF-kappaB is a major signal in mediating cytokine synthesis and secretion from myeloid cells. Thus, it is suggested that NF-kappaB in myeloid cells promotes lung cancer mainly through mediating inflammatory cytokines secretion to establish a cancer-prone inflammatory microenvironment (40). Indeed, blocking NF-kappaB in myeloid cells significantly reduced CS-induced pulmonary cytokines and chemokines such as TNFalpha, CCL2, CCL3 and IL-6, and inflammatory cell infiltration, which is associated with reduction of lung tumor multiplicity and tumor size (35, 37). In addition, NF-kappaB in epithelial cells also plays a lung cancer-promoting role (35).

5.1.3. Carcinogen-induced NF-kappaB activation

NF-kappaB can be activated by cigarette smoke (CS) and its components such as nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific carcinogen, in a panel of NSCLC cell lines (41). Because NF-kappaB is persistently activated by CS in the lung epithelial cells far before tumor formation, it is likely that this cell survival signal promotes mutant cells to proliferate and to escape apoptosis in the early phase of lung cancer development (35, 42). *In vitro* studies have suggested a positive role for NF-kappaB in cell transformation in prostate and colon epithelial cells, fibroblasts, and lymphocytes (6). It remains to be determined if NF-kappaB is also required for lung epithelial cell transformation.

5.1.4. Other NF-kappaB activation mechanisms

The crosstalk between NF-kappaB and the PI3K-Akt-mTOR pathway may also contribute to lung cancer cell survival and proliferation (41). It was shown that PI3K/Akt activates NF-kappaB activity in an IKKalpha-dependent manner (43). Fas-associated death domain protein (FADD) phosphorylation induces an IKK-mediated NF-kappaB activation and proliferation in lung adenocarcinoma, which is significantly associated with poor survival of the patients (44). Furthermore, some growth factor receptors can mediate atypical NF-kappaB activation. Epidermal growth factor (EGF) induces IKK-independent NF-kappaB activation through phosphorylation of the tyrosine residue at the position of 42 in IkappaBalpha in non-small cell lung adenocarcinoma cells (45).

5.2. A lung tumor-promoting role of NF-kappaB in animal models

Due to the complexity of carcinogenesis and the roles of NF-kappaB in different cell types, i.e., immune and parenchymal cells, NF-kappaB exerts controversial effects in different tumor models, strongly suggesting that NF-kappaB's roles in carcinogenesis are cell-, tissue-, or carcinogen-specific (46, 47). For example, opposite roles of NF-kappaB, an anti-tumor role in hepatocytes (parenchymal cells) while a pro-tumor role in Kupffer cells (myeloid cells in liver), were observed in hepatocellular carcinoma development(47). Recent studies with lung cancer mouse models have established NF-kappaB's tumor promoting role in lung carcinogenesis.

In an ethyl carbamate (urethane)-induced prototypical mouse model of multistage lung carcinogenesis in FVB or BALB/c mice, early NF-kappaB activation in airway epithelium, type II alveolar epithelial cells and macrophages was observed. When NF-kappaB was specifically blocked in airway epithelial cells,

urethane-induced lung inflammation was inhibited and tumor formation was significantly reduced, which was associated with marked reduction of Bcl-2 expression and increased apoptosis in airway epithelial cells (48).

Appealing evidence showing NF-kappaB's tumor promoting role in lung carcinogenesis was recently reported with a repetitive exposure to CS that promotes tumor development both in carcinogen-treated mice and in transgenic mice undergoing sporadic K-ras activation in lung epithelial cells. Blocking NF-kappaB in either myeloid or epithelial cells by deletion of IKKbeta dramatically inhibited CS-induced pulmonary inflammation and lung tumor multiplicity, suggesting NF-kappaB in either myeloid or epithelial cells promotes lung cancer (35, 40). Consistent with these observations, concomitant loss of p53 and expression of oncogenic K-Ras^{G12D} caused NF-kappaB activation in primary mouse embryonic fibroblasts, and inhibition of NF-kappaB significantly reduced lung tumor development caused by these genetic mutations (37). Another mouse model with knockout of tumor suppressor gene Gpre5a also showed that NF-kappaB in lung epithelial cells contributes to lung tumor formation (49). These experiments with genetically engineered mouse models demonstrate NF-kappaB's lung tumor-promoting role, pointing to novel approaches for lung cancer therapy and chemoprevention by targeting NF-kappaB (40).

5.3. NF-kappaB in lung tumor angiogenesis and metastasis

Angiogenesis is a process of new blood vessel formation, which is closely associated with cancer development and metastasis. NF-kappaB in inflammatory cells activates secretion of a variety of angiogenesis factors such as vascular endothelial growth factor (VEGF), TNFalpha, IL-8, IL-6, monocyte chemotactic protein-1 (MCP-1), and matrix metalloproteinases (MMPs) (6, 50, 51). NF-kappaB also induces stromal cell-derived factor 1 alpha (SDF-1alpha) to enhance tumor angiogenesis (52). NF-kappaB is involved in beta-arrestin-2-mediated angiogenesis through activation of CXCR2 (53). However, there is evidence showing an inhibitory role of NF-kappaB in tumor angiogenesis (54). Since inhibition of angiogenesis is one important approach in cancer therapy, such findings suggest that caution should be taken when blocking NF-kappaB is used for sensitizing cancer chemotherapy.

Through regulating factors involved in cell migration and adhesion, NF-kappaB stimulates cancer cell metastasis. These factors include MMPs, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), chemokine receptor CXCR4, and serine protease urokinase-type plasminogen activator (uPA) (6, 51). NF-kappaB is involved in upregulation of Twist-1-mediated epithelial-mesenchymal transition (EMT) that is critical for cancer cell invasion and metastasis (55). TGF-beta activates PI3K/Akt-mediated NF-kappaB activation, contributing to the migration of human lung cancer cells. Blocking NF-kappaB activity down-regulates MMP-2 and MMP-9 expressions, resulting in suppression of lung cancer invasion (56). SDF-1 was found to increase invasiveness of A549 cells through activation of ERK/NF-kappaB signaling, which is responsible for the increase of MMP-9 expression of the cells (57).

6. TARGETING NF-KAPPAB SIGNALING FOR LUNG CANCER THERAPY

Systemic chemotherapy, alone or combined with radiation, is used to treat advanced and metastatic lung cancer to improve the survival rate and quality of life of patients. The standard regimen is platinum-based doublets (cisplatin or carboplatin plus other cytotoxic agents). Unfortunately, the prognosis of advanced lung cancer is dismal, partially due to the fact that current chemotherapy for lung cancer has reached an efficacy plateau (58). Thus, new approaches are urgently needed to improve the outcome of treatment.

Besides NF-kappaB is constitutively activated in a variety of cancers, both chemotherapeutics and radiation induce NF-kappaB activation in cancer cells, which contributes to resistance to these therapies (59). For example, commonly used cytotoxic agents such as gemcitabline, paclitaxel, vinblastine and adriamycin, are NF-kappaB inducers (60). Thus, it is assumed that blockage of NF-kappaB will increase the efficacy of anticancer therapeutics. Indeed, inhibition of NF-kappaB signaling with various approaches has been shown to augment the efficacy of chemotherapeutics and radiation in killing cancer cells *in vitro* and *in vivo*(61, 62).

6. 1. Agents that inhibit NF-kappaB activation in lung cancer cells

NF-kappaB-inhibiting compounds suppress this pathway directly or indirectly. The direct inhibition involves targeting various components/steps in the NF-kappaB activation pathways such as activation of IKK, degradation of IkappaBalpha, nuclear translocation and DNA binding of NF-kappaB (6, 62). Among these targets, IKK was thought to be the most effective and selective drug target (63). Indirect NF-kappaB inhibition blocks proteins that are not components of, but can activate, NF-kappaB activation pathways.

6.1.1. Proteosome inhibitors

These include bortezomib and other proteosome inhibitors such as MG132 and proteosome inhibitor 1 (PS1) (64, 65). Bortezomib is the first drug in this category approved by FDA for cancer therapy. Proteosome inhibitors block the NF-kappaB pathway through suppression of proteosomal degradation of IkappaB. Prereatment of lung cancer cells with proteasome inhibitors suppressed TNF, TRAIL or irradiation-induced NF-kB activation (101, 103). Randomized phase I and phase II trials in advanced NSCLC show that Bortezomib as a monotherapy has limited activity in lung cancer. Combined with cytotoxic agents or other targeted agents such as erlotinib, bortezomib was well tolerated, but had modest or insufficient activity (66-69). Therefore, the future of bortezomib in treating lung cancer is still uncertain. It should be pointed out that proteasome inhibitors are not

NF-kB-specific and anti-cancer effects other than inhibition of NF-kB can also be involved.

6.1.2. Non-steroidal anti-inflammatory drugs (NSAID) and other approved drugs

NSAIDs are anti-inflammatory drugs act through inhibition of COX-1 and -2. Aspirin and sodium salicylate are able to inhibit NF-kappaB activation by suppressing IKKbeta activity (70). Sulindac, a NSAID that has preventive activity against colon cancer, is also an IKKbeta inhibitor that enhances TNF-induced apoptosis in lung cancer cells by inhibiting NF-kappaB nuclear translocation and DNA binding(71). The selective COX-2 inhibitor Celecoxib suppresses cigarette-smoke condensate (CSC)-induced NF-kappaB activation through blocking phosphorylation and degradation of IkappaBalpha and phosphorylation and nuclear translocation of NF-kappaB, resulting in abrogation of CSC's up-regulation of NF-kappaB target genes, Cyclin D1, COX-2 and MMP-9 (72). Sulfasalazine has been shown to inhibit TNFalpha- or 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced and IKK-mediated NF-kappaB activation (73). Sulfasalazine reduces NF-kappaB activity and cell invasion, and enhance doxorubicin-induced apoptosis in a series of cell lines from a NSCLC patient (74). Thalidomide inhibits TNF-induced IKK phosphorylation, p65 phosphorylation and nuclear translocation and binding to the ICAM-1 promoter, resulting in down-regulation of ICAM-1 expression and subsequent inhibition of cancer cell's invasiveness *in vitro* and metastasis *in vivo* (75). Unfortunately, Phase II and III trials failed to show any survival advantage using thalidomide in combination of standard chemotherapy in treatment of stage III and IV lung cancer (58). Nifedipine, a calcium channel blocker commonly used to treat hypertension, inhibits NF-kappaB in lung cancer cells and macrophages induced by IL-1beta, TNFalpha and TPA (76). Nifedipine exerts its NF-kappaB inhibiting activity by suppressing IKK-mediated IkappaBalpha degradation, subsequently suppressing MMP-9 expression and activity (77, 78).

6.1.3. Natural products and their synthetic derivatives

Dietary components from fruits and vegetables such as polyphenols, terpene, alkaloids and phenolics have proapoptotic, anti-proliferation, anti-angeogenic and anti-metastatic functions. Typically, these compounds show inhibitory activity on multiple signal pathways. NF-kappaB is one of the most frequent targets of these compounds (79). Dietary compounds reported to have NF-kappaB suppression function in lung cancer cells include resveratrol and its analogs, curcumin and its derivative EF24 (3,5-bis(2-flurobenzylidene)piperidin-4-one), (-)-epigallocatechin-3-gallate, genistein, luteolin, silibinin, deguelin, gallic acid, parthenolide, flavopiridol□anthocyanin, quinoclamine and dehydroxymethylepoxyquinomicin (80, 81). Studies have found that crude extracts from strawberry, deerberry, pomegranate fruit and potato sprouts have NF-kappaB suppression activity (82, 83).

Another group of natural products shown to inhibit NF-kappaB activation in lung cancer cells comes from medicinal plants. These include Triptolide purified from Chinese herb Tripterygium wilfordii (84), zyflamend from a polyherbal preparation (85), herbal mixture PS-SPES (86), coix seed extract (87), usolic acid from medical plant (88), embelia from Embelia ribes plant (89), methysticin from Kava (90), phenanthrene-based tylophorine (from Tylophora genus) synthetic derivatives (91). Nature products generally have low toxicity to normal tissues and can simultaneously block several pathways that promote carcinogenesis, making them potential agents for lung cancer therapy and prevention.

6.1.4. Other NF-kappaB inhibitors

The lipid peroxidation product 4-hydroxy-2-nonenal inhibits IKK by covalent modification of the enzymes and subsequently suppresses phosphorylation and degradation of IkappaBalpha in H1299 lung cancer cells (92). The synthetic retinoid N-(4-Hydroxyphenyl) retinamide suppresses TNF-induced NF-kappaB activation and invasion in H1299 cells (93). The histone deacetylase inhibitor suberorylanilide hydroxamic acid (SAHA) was shown to suppress NF-kappaB activation in NSCLC cells and to inhibit growth of A549 xerografts in athymic nude mice through down-regulating TNFR1 protein expression (94).

Other NF-kappaB inhibitors in development include RNA aptamer that targets NF-kappaB (p50) using adevnovirus delivery system. This aptamer was shown to inhibit NF-kappaB activation in A549 cells *in vitro* and the A549-derived xenografted tumor growth *in vivo*, and sensitize the cells to doxorubicin's killing (95).

6.1.5. Indirect inhibition of NF-kappaB activation

The tumor suppressor p53 and NF-kappaB have reciprocal inhibitory activity (62). Antagonizing p53 function by NF-kappaB contributes to chemoresistance in cancer cells (96). In A549 cells, the potent MDM2 inhibitor nutlin-3 simultaneously increases the expression of p53 and represses the expression of TNF-induced NF-kappaB target genes ICAM-1 and MCP-1 in a promoter specific manner. Consequently, combined treatment of nultin-3 and TNF reduced cell viability. The mechanism is not clear, because the processes of NF-kappaB signaling are not affected by nultin-3 (97).

Aurora kinases (aurora-A, B and C) are serine/threonine kinases that function in mitosis. Aurora-A is overexpressed in several types of cancer, and has been linked to breast and ovarian tumorigenesis (98, 99). Aurora-A activates NF-kappaB through phosphorylation of IkappaBalpha. Inhibiting Aurora-A suppressed NF-kappaB activity in A549 cells and sensitized the cells to cytotoxicity induced by cisplatin, adriamycin and epotoside (100).

Heat shock protein 90 (Hsp90) is a chaperone that stabilizes a variety of proteins including RIP1 and IKKbeta, two key components of TNFalpha-induced NF-kappaB activation pathway. The Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) targeted RIP1 and IKKbeta for degradation and thus suppressed

Table 1. NF-kappaB inhibitors that can sensitize lung cancer cells to chemotherapy and ionizing radiation

Agent	Sensitizer	Reference
Cisplatin	1) Genistein 2) Expression of IkappaBalpha mutant	(101, 102)
Docetaxel	Genistein	(102)
Gemcitabline	Expression of IkappaBalpha mutant	(103)
Paclitaxel	1) Embelin 2) Expression of IkappaBalpha	(89, 104)
Vinorelbine	Curcumin	(105)
Adriamycin	1) Expression of IkappaBalpha mutant 2) Knockdown of Ikkbeta or p65 with siRNA	(18, 101)
Etoposide	Expression of IkappaBalpha mutant	(101)
Ionizing radiation	1) Beta-lapachone 2) Nimesulide and MG132 4) Resveratrol 5) Knockdown of TRAF2 with siRNA	(106) (107, 108) (109) (110)

TNFalpha-induced NF-kappaB activation. Pretreatment with 17-AAG augmented TNFalpha and TNF-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity in lung cancer cells (33).

6.2. Hope and possible benefits of inhibiting NF-kappaB in lung cancer therapy

Since the discovery of inhibition of NF-kappaB can sensitize anticancer chemotherapy, tremendous efforts have been made to develop NF-kappaB inhibitors with a high expectation that these inhibitors will be eventually used as single or adjuvant agent for cancer treatment. Below is a summary, with focus on lung cancer, of the major goals that can be achieved by inhibiting NF-kappaB pathway.

6.2.1. Sensitization of cancer cells to apoptosis inducing therapeutic agents

Some of the NF-kappaB inhibitors that enhance lung cancer cell death induced by chemotherapeutics and ionizing radiation are listed in Table 1.

6.2.2. Inhibition of cancer invasion and metastasis

Blockage of NF-kappaB activation by a dominant negative IkappaBalpha mutant resulted in suppression of tumor cell intravasation *in vivo* and lung metastasis in a mouse model (111). Of note, chemoresistance of lung cancer cells is associated with metastatic capability. For example, overexpression of AXL, a receptor tyrosine kinase that activates NF-kappaB, induces invasiveness and resistance to doxorubicin. Inhibition of NF-kappaB signaling suppressed tumor cell invasion while synergized the cells to doxorubicin (74). Further, adhesion of SCLC cells to stromal cells protects the cells from etoposide-induced apoptosis (112). Cyanidin-3-glucoside, an anthocyanin isolated from blackberry that is an antioxidant and acts as an inhibitor of several pathways including NF-kappaB, suppresses migration and invasion of A549 cells *in vitro* and *in vivo* (113). Inhibition of the NF-kappaB/MMP-9 cascade by (-)-epigallocatechin-3-gallate also suppresses invasion in a highly metastatic human lung cancer cell line *in vitro* (114). Radiotherapy-induced MMP-9 expression and lung metastasis of Lewis lung cancer cells was also blocked by the NF-kappaB inhibitor arenic trioxide, (115). These studies emphasize the importance of NF-kappaB activation in lung cancer cell metastasis.

6.2.3. Amelioration of malignant pleural effusion

Malignant pleural effusion (MPE) is a severe complication of cancer. Lung cancer is the most frequent cause of MPE, which results in a bad prognosis. Tumor-derived TNF induces NF-kappaB activation, which is well correlated with pleural effusion volume in a mouse model with injection of lung cancer cells. Suppressing NF-kappaB significant lowered pleural effusion volume (116). At a dose that blocked NF-kappaB activation, bortezomib reduced lung cancer cell-induced MPE accumulation and improved the survival of the animals (117). These studies point to a promoting role of NF-kappaB in MPE and inhibition of NF-kappaB may eliminate MPE.

7. INHIBITING NF-KAPPAB FOR LUNG CANCER CHEMOPREVENTION

Currently there is no validated effective agent for lung cancer prevention (118). Increasing evidences showing that NF-kappaB plays a critical role in lung cancer development suggest NF-kappaB as a target for lung cancer chemoprevention. Interestingly, some agents that have lung cancer preventive potential, including NSAIDs and dietary compounds, possess inhibitory activity on NF-kappaB (119). Oral administration of pomegranate fruit extract, which inhibits NF-kappaB, significantly reduced multiplicity of lung tumor induced by benzo(a)pyrene and N-nitroso-tris-chloroethylurea (120, 121). Feeding mice with zerumbone, a tropical ginger sespuiterpene that can repress NF-kappaB, does-dependently inhibited multiplicity of lung adenoma induced by NNK.

Chemoprevention involves prolonged use of preventive agents. The long-time use of the NF-kappaB inhibitors or anti-inflammatory drugs is likely to result in un-tolerable side-effects (122). Thus, dedicated single NF-kappaB inhibitors are unlikely to be use as chemoprevention agents (119). It has been proposed that logically constructed mixtures of agents or combination treatments are a better choice for lung cancer chemoprevention (123, 124). This strategy would improve the efficacy of cancer prevention while eliminate the possible side effects.

The critical question unanswered is whether NF-kappaB inhibition can reduce human lung cancer incidence *in vivo*. The drugs used in experiments, as mentioned above, block several pathways. Though these drugs could be good candidates for chemoprevention, it is unclear that how much their NF-kappaB inhibiting activity contributes to the reduced tumor burden in animals and in humans.

8. CONCERNS ABOUT INHIBITING NF-kappaB IN LUNG CANCER THERAPY AND PREVENTION

Along with the rapid development of NF-kappaB inhibitors, general safety and efficacy regarding NF-kappaB inhibition in cancer therapy have been concerned over years. The first concern is that NF-kappaB inhibition may compromise immunity. Due to its pivotal roles in both innate and adaptive immunity, NF-kappaB is important for humans to defend themselves from environmental assaults such as microbe, physical and chemical damages (125). Thus, systemic administration of NF-kappaB inhibitors may impair immune response. Further, NF-kappaB inhibitors may also blunt anticancer immunity (59, 126). It remains to be determined whether NF-kappaB inhibition attenuates or potentiates the efficacy of anti-tumor agents *in vivo*. Careful clinical evaluation of a NF-kappaB inhibitor in individual cancer patient is crucial. In this regard, the doses of NF-kappaB inhibitors and administrating schedule are critical (59, 122, 127).

The second concern comes from the rather complex and even opposite functions of NF-kappaB in different cells and tissues (46, 122). Although NF-kappaB is shown to promote lung carcinogenesis, it may function as a tumor suppressor in other organs. The contradictory effects may occur at different carcinogenic stages and are likely associated with different carcinogens and different genetic backgrounds of the patients.

The third concern is the potential off-target effect of the NF-kappaB inhibitors. The function of bortezomib is not restricted to NF-kappaB (128), because it inhibits proteosome that would cause accumulation of other proteins that are degraded via proteosome. For example, bortezomib promotes proliferation of prostate cells by stabilizing steroid receptor coactivator-3 (129), raising the concern that bortezomib may increase the risk of prostate cancer. The widely used NF-kappaB inhibition approach with IkappaB SR overexpression also has potential off-target effects that blunt p53 or interfere expression of unrelated genes (18, 130).

9. SUMMARY AND PERSPECTIVE

Although there is no compelling evidence showing that lung cancer cells are addicted to NF-kappaB for survival, NF-kappaB inhibition has been demonstrated to be a promising adjuvant treatment in improving the efficacy of chemotherapy and radiation. Because both the constitutive and therapeutic-induced NF-kappaB activations blunt the cancer cell-killing effects of the therapy, blocking NF-kappaB may potentiate anticancer activity. With piled data showing NF-kappaB is activated in early phase of lung cancer development and growing knowledge on NF-kappaB's functions in tumorigenesis, it is reasonable to hypothesize that NF-kappaB could be a target for lung cancer chemoprevention. However, due to NF-kappaB's pivotal physiological functions in normal cells, particularly in immune cells, sustained and systemic NF-kappaB inhibition may have severe adverse effect. To develop approaches delivering NF-kappaB inhibition more specifically into transformed cells and immune cells residing in tumor-prone microenvironments would be a good direction in solving this problem. Naturally occurring compounds with NF-kappaB suppressing properties are of great interest in relieving inflammation and preventing lung cancer (80). Because TNFalpha is involved in inflammation-associated lung carcinogenesis and blocking NF-kappaB promotes TNFalpha-induced apoptosis in lung cancer cells, NF-kappaB blockage may convert TNFalpha from a tumor promoter to a tumor suppressor (26, 50, 80), making NF-kappaB suppressing compounds potential chemopreventive agents against lung cancer (80). Along with the progress in elucidating NF-kappaB activation mechanisms in tumors, identifying biomarkers for indications for NF-kappaB inhibitor application, and developing malignant cell-specific NF-kappaB inhibition approaches, NF-kappaB inhibition in chemoprevention and chemotherapy against lung cancer would be more clinically relevant in near future.

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