Role of the NFκB-signaling pathway in cancer

Abstract: Cancer is a group of cells that malignantly grow and proliferate uncontrollably. At present, treatment modes for cancer mainly comprise surgery, chemotherapy, radiotherapy, molecularly targeted therapy, gene therapy, and immunotherapy. However, the curative effects of these treatments have been limited thus far by specific characteristics of tumors. Abnormal activation of signaling pathways is involved in tumor pathogenesis and plays critical roles in growth, progression, and relapse of cancers. Targeted therapies against effectors on oncogenic signaling have improved the outcomes of cancer patients. NFκB is an important signaling pathway involved in pathogenesis and treatment of cancers. Excessive activation of the NFκB-signaling pathway has been documented in various tumor tissues, and studies on this signaling pathway for targeted cancer therapy have become a hot topic. In this review, we update current understanding of the NFκB-signaling pathway in cancer.

Keywords: nuclear factor kappa-B, p65, signaling pathway, cancer, inflammation

Introduction
Malignant tumors have become one of the most deadly diseases and a prominent public health problem threatening human lives around the world. In recent years, with social and economic development, as well as aging of the population, the incidence and mortality of cancer are increasing. The overall incidence of common malignant tumors will rise from 14 million in 2012 to a predicted 19 million in 2025 and 24 million in 2035, according to the World Health Organization. Cancer occurs due to oncogene activation, tumor-suppressor gene inactivation, loss of control of the cell cycle, genomic instability, telomerase loss, and resistance of apoptosis. However, the specific pathogenesis of cancer varies with types of cancers.

The cell-signaling pathway is the process by which cells respond to stimuli of extracellular signaling molecules that bind to receptors located on the cell membrane or in the cytoplasm of cells. This binding to receptors transfers signals to the nucleus and induces corresponding gene expression, thus producing biological effects and cellular responses. Abnormal regulation and cross-talk of cell-signal-transduction pathways play a key role in cancer, and obstruction of or anomalies in signaling pathways may lead to excessive cell proliferation, apoptotic resistance, angiogenesis, invasion, and metastasis, leading to development and progression of cancer. NFκB is an important signaling pathway that is involved extensively in cancer development and progression. Through controlling the expression of target genes, such as TNFA, IL6, BCLXL, BCL2, BCLXS, XIAP, and VEGF, NFκB mediates tumor-cell proliferation, survival, and angiogenesis. This review focuses on this NFκB-signaling pathway in tumors.
Overview of NFκB-signaling pathways

Proteins and structure of NFκB family

NFκB is an important transcription-factor family of five subunits: Rel (cRel), p65 (RelA, NFκB3), RelB, p105/p50 (NFκB1), and p100/p52 (NFκB2). Among these, p65, cRel, and RelB contain an N-terminal Rel-homologous domain (RHD; about 300 amino acids) and a C-terminal transactivation domain (TAD; Figure 1), while p50 and p52 have only an RHD, but not a TAD. The C-terminal of p100 and p105 contains ankyrin repeats that function as a p52 and p50 inhibitor. The RHD is responsible for DNA binding and dimerization between different or identical family members, including the nuclear localization sequence and IκB-binding region, leading to homomeric or heteromeric binding of the subunits. Therefore, the p50–p52 homologous dimer does not activate gene transcription, but acts as an inhibitory molecule. Both p50 and p52 are usually present in cells in the form of their precursors. In these members, RelB can form dimers only with p50 or p52, but others can form either homologous or heterologous dimers. However, the most common NFκB dimer is the heterodimer of p65–p50. These homologous and/or heterologous dimers can bind to a specific sequence (ie, NFκB sites) of the target gene to regulate gene transcription. Therefore, NFκB regulates the activity of cells through the slight difference in binding of these NFκB dimers to targeted sequences.

IκBs and IKKs

IκBs and IKKs are upstream regulators of the NFκB-signaling pathway. In cells that are not stimulated, NFκB dimers are present in an inactive state by binding to three inhibitory factors (IκBα, IκBβ, and IκBε) of the NFκB in the cytoplasm, which blocks the nuclear localization sequence and prevents the NFκB from transition into the nucleus. In addition, there are also two precursor IκBs: p105/IκBγ and p100/IκBδ. IκBα specifically inhibits the p50/RelA

Figure 1 Structure of NFκB members.

Notes: The NFκB family consists of three proteins with a transactivation domain (RelA [p65], cRel, and RelB) and two proteins lacking a transactivation domain (p105/p50 and p100/p52). Similarly, only p105/p50 and p100/p52 have ankyrin repeats that function as p52 and p50 inhibitors. However, all these proteins share an Rel-homology domain, associated with DNA binding, dimerization, nuclear localization and IκB binding, and nuclear localization signal exposure, which is vital to the translocation of the dimer into the nucleus.

Abbreviation: NIK, NFκB-inducing kinase.
heterodimer, IxBβ targets the RelA/cRel heterodimer,23 and IxBε inhibits the RelA and cRel dimers.24 Although different external stimuli cause differential activation of NFκB through different IxBs,25 almost all known NFκB agonists can rapidly and directly activate NFκB by degradation of IxBs.26 The IxB proteins p105/IxBγ and p100/IxBδ play a dual role, ie, precursors of the NFκB proteins p50 and p52 and inhibitors of NFκB signaling.27 Different external stimuli have differential effects on the different subunits of IxBs, leading to differential activation of the NFκB pathway.

The IKK (IxB kinase) complex consists of the catalytic subunits IKKα and IKKβ and the regulatory subunit NFκB essential modifier (NEMO; also called IKKγ).28 The IKKs are upstream regulators of IxBs.28 Both IKKα and IKKβ subunits have about 52% of the sequence identity, but play a key but divergent role in regulation of global NFκB-signaling activity.29 NEMO contains several domains that are crucial for its function as a regulatory subunit of the canonical IKK complex. The N-terminal coiled-coil domain of NEMO interacts with IKKα and IKKβ.30 Different IKKs demonstrate differential strength and speed for different substrates. IKKα is mainly the specific upstream kinase of IxBβ and can strongly phosphorylate the Ser23 of IxBβ, but not the Ser19. This inequivalent phosphorylation of Ser23 and Ser19 in IxBβ leads to degradation of IxBβ.31 IKKβ can specifically phosphorylate the Ser sites of IxBα and IxBβ, and the intensity is 20 times that of IKKα. In addition, the phosphorylation speed of the same IKK for IxBα and IxBβ are different. IKKβ has stronger affinity for IxBα than IxBβ. Also, the IxB-kinase complex can phosphorylate the NFκB-bound IxB protein and contribute to proteasomal degradation of the IxB protein faster.32

**Activation of NFκB-signaling pathway**

In a resting status of cells, complexes formed from NFκB and IxB (NFκB–IxBα or NFκB–IxBε) shuttle between the cytoplasm and nucleus in a dynamic equilibrium.33 When cells are stimulated by extracellular signals, such as TNFα, IL1, lipopolysaccharide, viral double-stranded RNA, and ionizing radiation, NFκB is activated and enters the nucleus to bind to target genes.34–39 Upon activation, NFκB-signaling pathways are classified as canonical or noncanonical (Figure 2).40 The common regulatory step in both pathways is activation of the IKK complex. The IKK complex is phosphorylated, and in turn induces phosphorylation of IxB proteins IxBα or IxBβ, leading to ubiquitination and degradation by proteasomes. Similarly, p100 and p105 are phosphorylated and cleaved into maturated p52 and p50 upon IKK activation. Therefore, NFκB dimers are released from their inhibitors and free to translocate into the nucleus to regulate expression of their target genes.

The canonical NFκB-transcription factor is an inactive dimer composed of a p50 and RelA/p65 subunit, which largely resides in the cytoplasm as part of a latent complex

![Figure 2 Activation of NFκB cascade by the classical/canonical signaling pathway (right) and alternative/noncanonical signaling pathway (left). Abbreviations: NLS, nuclear localization sequence; RHD, Rel-homologous domain; TAD, transactivation domain.](https://example.com/figure2.png)
Ubiquitination, a PTM of addition of ubiquitin (Ub) moieties to a protein, is the primary mechanism of protein turnover in the cell, and is recognized as the “traditional” function of Ub tagging.73 Ub moieties on NFκB-signaling proteins can serve as a docking platform for other proteins with specific Ub-binding domains.72 First, the Ub moiety is activated by the E1 Ub-activating enzyme. Following activation, one of several E2 Ub-conjugating enzymes transfers Ub from E1 to several E3 enzymes (Ub ligases), to which the substrate protein is specifically bound. The Ub moiety includes seven lysine (K) residues (K6, K11, K27, K29, K33, K48, and K63) and a methionine at the N-terminus (M1), which can link another Ub to form a polyclonal chain.73

Ub signaling controls activation of NFκB and innate immunoresponses downstream of pattern-recognition receptors, such as Toll-like receptors, nucleotide-oligomerization domain-like receptors, and cytokine receptors, eg, TNFR1, in normal intestinal epithelial cells and colon cancer cells.74–76 K48-linked polyubiquitination is a key step in releasing NFκB from IκB in the canonical pathway and processing of p100/102 into p52/50 in the noncanonical pathway to activate the NFκB pathway in inflammatory diseases, autoimmune diseases, and cancers.70,77,78 The Skp1–Cullin–F-box (SCF)–βTrCP complex catalyzes the K48-linked polyubiquitination of IκBα at two N-terminal lysine residues (K21 and K22), inducing 26S proteasome-dependent degradation of IκBα and nuclear translocation of canonical NFκB. In addition, the phosphorylation of IκBα induced by NFκB-inducing kinase could cause the phosphorylation of p100 on the C-terminal region (Ser866 and Ser870) and polyubiquitination of p100 by the SCF–βTrCP–E3 ligase complex to regulate the activity of the noncanonical NFκB.79 The IKKβ subunit is also polyubiquitinated by a K63-linked chain in human cervical HeLa cells.79 Importantly, activation of IKK is essential to productive signaling and NFκB-mediated transcription, and its activation depends on the binding of Met1-Ub by the IKK subunit NEMO.80

Phosphorylation is critical for NFκB activity, including binding to and transcription of genes that contain a consensus sequence.81 Phosphorylation of key NFκB-signaling molecules often positively mediates signal transduction by inducing protein conformational changes in breast cancer cell lines.82,83 Activation of NFκB signaling is involved in a series of phosphorylation events of upstream NFκB regulators and NFκB family members. In fact, the activity of NFκB is controlled to a great extent by phosphorylation of RelA or upstream regulators in esophageal squamous-cell carcinoma, gastric cancer, and oral cancer.81,84 The main subunit RelA of

Posttranslational modifications of NFκB proteins

NFκB has hundreds of validated transcriptional targets,61 and thus NFκB-signaling activity is under stringent spatial and temporal control at the levels of nuclear translocation and posttranslational modifications (PTMs) of signaling components.51,62 There is a wide range of PTMs of NFκB subunits,63 and PTMs provide essential mechanisms differentially to regulate NFκB-signaling activity in response to the various stimuli that activate this pathway in many cancer cells.64,65 Although these modifications have a critical role in the normal and pathological functions of NFκB in vivo, the physiological significance of PTMs remains unclear in cancer cells.66 PTMs not only can contribute to the control of nuclear translocation but also have an important influence in functions of NFκB subunits, including protein degradation, DNA binding, and transcriptional activity.57–60 PTMs of NFκB include phosphorylation, ubiquitination, acetylation, and methylation.70 Herein, we focus on the ubiquitination, phosphorylation, and methylation of the functional subunits of NFκB.
NFκB is targeted for phosphorylation at many phosphoacceptor sites within both the RHD (Ser205, Ser276, Ser281, Ser311, and Thr254) and TAD (Ser468, Ser529, Ser535, Ser536, Thr435, and Thr505). For example, the phosphorylation of Ser536 induced in the cytoplasm can increase NFκB transcriptional activity, while phosphorylation of Ser529 increases DNA binding and oligomerization in laryngeal cancer cells. The phosphorylation of Ser536 results in nuclear accumulation of RelA through disruption of the cytoplasmic/nuclear shuttling of NFκB–IκBα complexes. In addition, phosphorylation of Ser276 can promote RelA interaction with the transcriptional coactivator CBP/p300. Meanwhile, p-Ser276 RelA facilitates recruitment of DNMT1/DNA (cytosine 5)–methyltransferase 1 to chromatin and subsequent BRMS1-promoter methylation and transcriptional repression in human NSCLC cells. In addition, phosphorylation of IκBα is a key step of their proteasomal degradation and the release of NFκB for nuclear translocation and activation of gene transcription. Cytokines in the tumor microenvironment, such as TNFα, could bind to the cell-surface TNF receptor, causing TNF-receptor multimerization and interacting with TRADD in the cytoplasm. TRADD recruits TRAF and kinase RIP. Then, the stimulated signals are transmitted to IKK by RIP, which can make the Ser32 and Ser36 residues in α-subunits of IκB and Ser19 and Ser23 residues in β-subunits of IκB phosphorylated. Then, IκB protein is dissociated from the p50-p65-IκB trimer and subsequently degraded by proteasomes, activating the NFκB pathway.

In recent years, accumulated evidence has suggested that histone-modifying enzymes not only modify histone proteins but also play a role in the modification of nonhistone proteins, such as NFκB. NFκB can be methylated reversibly on lysine or arginine residues by histone-modifying enzymes, including lysine and arginine methyl transferases and demethylases. The methylations of both lysine and arginine occur mainly on the p65 subunit of NFκB. The methylated K sites include K37, K218, K221, K310, K314, and K315 that are modified by different histone-modifying enzymes. Among the histone methyl transferases, SET9, SETD6, and NSD1 are capable of activating NFκB by methylating K218 and K221 of p65, which provides a potential mechanism for how NSD1 might contribute to tumor formation, as constitutive activation of NFκB is a hallmark of many cancers. Methylation of NFκB can profoundly affect the functions of NFκB by altering its stability, transactivation potency, and affinity to DNA, and thus affect the strength and duration of inducible gene expression. Meanwhile, the differential methylation of K37 and K218/221 on NFκB is able to constitute “bar codes” that guide differential activation of NFκB, binding to specific promoters.

Roles of NFκB in cancer

At present, the role of the NFκB-signaling pathway in cell biogenic activities is the hot spot of cancer research. NFκB signaling is involved in cellular immunity, inflammation, and stress, as well as regulation of cell differentiation, proliferation, and apoptosis. The NFκB pathway is often altered in both solid and hematopoietic malignancies, promoting tumor-cell proliferation and survival. However, recent evidence indicates that NFκB plays a tumor-suppressive role in certain cancers through transcriptional activation of the Fas ligand.

Pro- and anti-inflammatory effects of NFκB

The pathogenic role of inflammation in cancer has drawn intensive research and highlighted the context-dependent modulation of inflammation-associated cancer by the transcription factor NFκB. Through control of inflammatory responses, NFκB has influence in tumor development and progression by excessive innate immunity activation and abnormal cell growth. Inflammation-associated cancer can secrete various cytokines and chemokines through NFκB binding to the promoters of genes, such as IL1B, TNF, and IL6. At the same time, activation of NFκB can be regulated by the TNFα-receptor family, including RANKL.

It is well known that inflammatory gene signatures are altered in various tumor-cell lines and specimens of different histological and molecular subtypes. Researchers have found that the inflammatory genes, such as IL1, IL6, IL8, and CCL2, are also actively expressed in glioma-cell lines, playing differential and cooperative roles in promoting proliferation, invasion, angiogenesis, and macrophage polarization in vitro. Interestingly, the NFκB signaling activated by TNF can also induce proinflammatory chemokines, such as CCL20, CXCL13, and CXCL8, that are specific ligands for the chemokine receptor CXCR2 in ovarian cancer cells. It is a positive-feedback loop that high expression of proinflammatory genes in the tumor microenvironment can be increased through activation of canonical and noncanonical NFκB pathways to accelerate the development of tumors and also promote the expression of proinflammatory proteins through binding of specific dimers of activated NFκB to promoters of proinflammatory genes. For example, IL1 can induce the phosphorylation of MKK4, which is indispensable
in the processing of NFκB p100 to the p52-active form and translocation of p52 from the cytoplasm to the nucleus.\textsuperscript{10,113} Besides the proinflammatory function, NFκB has a direct anti-inflammatory effect. NFκB can inhibit the formation of inflammasomes through inhibition of inflammasome-dependent caspase 1 activation, but the mechanism is not entirely clear and is probably related to NFκB-induced expression of antiapoptotic proteins, such as PAI2 and Bcl-xL.\textsuperscript{114}

**Protumorigenic roles of NFκB**

The potential role of NFκB in oncogenesis was confirmed in the discovery of the retroviral oncogene **v-Rel**, the homologue of the gene encoding cRel, one of the NFκB subunits.\textsuperscript{11} The genes encoding NFκB subunits or IκB proteins are mutated in a variety of malignancies. Mutations and gene fusions of **IKKα**, which leads to the activation of IKKκ, were detected in breast cancer, where the activation of IKKκ can maintain the self-renewal of breast cancer progenitors and has been shown to be responsible for the tumor-promoting effects of progesterone in breast cancer.\textsuperscript{116} However, the number of tumors with persistently activated nuclear NFκB is much larger than the subfraction of malignancies with confirmed mutations in NFκB or IκB-encoding genes.\textsuperscript{105} In breast cancer, colon cancer, and lymphatic cancer, the persistent activation of the NFκB-signaling pathway leads to abnormal cell proliferation and differentiation, enhanced metastasis, and treatment resistance.\textsuperscript{102,117,118} In colitis-associated colon cancer, positive effects of NFκB have been shown by conditional silencing of IKKβ, which persistently activates NFκB in intestinal epithelial cells.\textsuperscript{119} Recent studies have found that the Epstein–Barr virus (EBV) in several T- and NK-cell neoplasms can persistently activate NFκB via the viral protein LMP1, resembling the proteins in the TNF-receptor superfamily that induce NFκB activation through interaction with TRAF and TRADD,\textsuperscript{120,121} and contribute to development of EBV-positive T- and NK-cell neoplasms.\textsuperscript{122}

Mutations in upstream NFκB effectors in tumor cells will also result in the activation of the NFκB pathway, and then the persistent activation of NFκB can specifically target the promoters of oncogenes to form a positive-feedback loop. For instance, BRCA1 silencing in breast cancer cell lines induces phosphorylation of the Ser536 site of p65 and processing of p100/p52, causing constitutive activation of the canonical NFκB pathway (p65/p50) and noncanonical NFκB pathway (p100/p52) and promoting the nuclear translocation and accumulation of p52/RelB, which can enhance proliferation of MCF1 cells.\textsuperscript{102} In glioma stem cells, MLK4 binds to and phosphorylates the NFκB regulator IKKα, leading to enhancement of the ability of NFκB binding to DNA and activation of NFκB, which can induce mesenchymal trans-differentiation and radioresistance.\textsuperscript{123} Similarly, the activation of mTORC1 induced by LMP1 is a key regulator of the NFκB pathway in NPC cells.\textsuperscript{124} With knockdown of the **MTORC1** gene, activation of NFκB induced by LMP1 and the transcription of Glut1 are markedly inhibited, negatively affecting the aerobic glycolysis in nasopharyngeal carcinoma cell line HONE1. Therefore, the activation of NFκB pathway plays an important role in regulating the energy metabolism of nasopharyngeal carcinoma cells.

**Antitumorigenic roles of NFκB**

The role of NFκB in cancer is not always positive. Researchers have found that blockade of NFκB via overexpression of IκBα promoted oncogenic Ras-induced invasive epidermal growth, resembling squamous-cell carcinoma.\textsuperscript{125} The overexpression of IκBα induced by ablation of IKKβ can enhance the stability of IκB by inhibition of the phosphorylated IκBα protein, resulting in inactivation of canonical NFκB. In addition, the high expression of IKKβ that activates classical and nonclassical NFκB can suppress the progression of hepatocellular carcinoma by preventing DEN-induced cell death.\textsuperscript{126} Meanwhile, ablation of IKKβ can enhance the activation of JNK family members, including JNK1, which contributes to hepatocellular carcinoma development.\textsuperscript{127} Functional cross-talk between Nrf2 and NFκB/RelA protects the liver from necrosis, inflammation, and fibrosis, and thus prevents development of hepatocellular adenoma.\textsuperscript{128} Transcription factors Nrf2 and NFκB regulate the cellular antioxidant defense system, which is important in cell survival.\textsuperscript{129} Recently, researchers found that **LCN2** is an upstream regulatory gene of the NFκB–Snail pathway and can inhibit the phosphorylation of p65 (p-p65) and the nuclear accumulation of p-p65 and Snail to inhibit activation of the NFκB pathway, thereby inhibiting colorectal cancer cell epithelial–mesenchymal transition and metastasis induced by the NFκB–Snail pathway.\textsuperscript{130} However, a number of studies have suggested that the NFκB pathway may upregulate the expression of **LCN2** to promote the development of many cancers.\textsuperscript{131,132} Therefore, the NFκB pathway is diversified in different tumor cells,\textsuperscript{133,134} and the complex anticancer mechanism of the NFκB pathway is still not clear. Further study is warranted.

**Prospects of NFκB inhibitors**

It is unquestionable that NFκB inhibition as a means of cancer treatment has to be prioritized. Hundreds of NFκB inhibitors have been developed.\textsuperscript{135} These inhibitors are mainly designed...
to target one of four key points in the NFκB pathway: IKKs, NFκB-subunit dimers, proteasome 26S in the case of proteasome inhibitors, and the Ub-ligase complex in the case of ubiquitination blockers. These four elements are essential to activation of the NFκB pathway. Moreover, natural products, antioxidants, nonsteroidal anti-inflammatory drugs, and glucocorticoids are capable of interfering with the NFκB-signaling cascade. As the phosphorylation step of IkBα is a common reaction for the NFκB signaling induced by diverse stimuli, IKK inhibitors are considered an interesting approach for NFκB modulation. After phosphorylation of IkBα, the polyubiquitination and proteasomal degradation of the IkBα protein will result in NFκB release for translocation to the nucleus. Therefore, ubiquitination blockers and proteasome inhibitors could also be considered as interesting modulators of the NFκB cascade.

Many NFκB inhibitors have demonstrated appreciable anticancer activity in preclinical approaches. For example, BAY11-7082 can specifically abolish the binding of p65 to targeted DNA and downregulate the expression of TNFα. As an IKKβ inhibitor, EF24 can block the NFκB-signaling pathway by inhibiting IKKβ phosphorylation, leading to cell-cycle arrest at the G1/M phase and apoptosis. The proteasome inhibitor MG132 inhibits tumor growth through downregulation of the NFκB-signaling pathway. T901 is a novel selective NFκB inhibitor functioning through binding to the NFκB complex in the cytosol, thus blocking its nuclear translocation and target-gene expression. Although many NFκB inhibitors have been developed to exert antitumor effects in a variety of experimental cancer models, ranging from lymphoma to solid tumors, no such drug has been clinically approved. Because the mechanism of the antitumor effect of NFκB inhibitors is not totally understood, many NFκB inhibitors are not effective as a single antitumor agent. The alterations of cellular signaling induced by NFκB inhibitors are generally involved in the establishment, evolution, and spread of malignant tumors. Therefore, considering the positive role of NFκB in the vast majority of cancer pathogenesis, NFκB inhibitors that are able to modulate more than one therapeutic aspect of the work.

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Author contributions
LZX and SMT contributed to drafting and editing of the manuscript. DLC and QJL designed, revised, and finalized the manuscript. HRW and LDO participated in drafting and editing of the manuscript. YIZ participated in revision and coordination. JGL, YTT, and LL contributed to the literature search. MS and HW participated in conception and coordination. All authors contributed toward data analysis and drafting and revising the paper, and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

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