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Review

Inhibiting NF-κB activation by small molecules as a therapeutic strategy

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ABSTRACT

Because nuclear factor-κB (NF-κB) is a ubiquitously expressed proinflammatory transcription factor that regulates the expression of over 500 genes involved in cellular transformation, survival, proliferation, invasion, angiogenesis, metastasis, and inflammation, the NF-κB signaling pathway has become a potential target for pharmacological intervention. A wide variety of agents can activate NF-κB through canonical and noncanonical pathways. Canonical pathway involves various steps including the phosphorylation, ubiquitination, and degradation of the inhibitor of NF-κB (IκBα), which leads to the nuclear translocation of the p50–p65 subunits of NF-κB followed by p65 phosphorylation, acetylation and methylation, DNA binding, and gene transcription. Thus, agents that can inhibit protein kinases, protein phosphatases, proteasomes, ubiquitination, acetylation, methylation, and DNA binding steps have been identified as NF-κB inhibitors. Because of the critical role of NF-κB in cancer and various chronic diseases, numerous inhibitors of NF-κB have been identified. In this review, however, we describe only small molecules that suppress NF-κB activation, and the mechanism by which they block this pathway.

1. Introduction

The nuclear factor-κB (NF-κB) signaling pathway plays a major role in the development, maintenance, and progression of most chronic diseases. NF-κB controls the expression of genes involved in a number of physiological responses, including immune inflammatory responses, acute-phase inflammatory responses, oxidative stress responses, cell adhesion, differentiation, and apoptosis [1]. Recent studies have suggested that NF-κB dysregulation is associated with many diseases including AIDS, atherosclerosis, asthma, arthritis, diabetes, inflammatory bowel disease, stroke, muscle wasting and viral infections. Mounting evidence indicates that NF-κB acts as a link between inflammation and cancer progression [2–11], making NF-κB essential to and a potential drug target in hematological malignancies and solid tumors [12,13].

NF-κB was first identified in 1986 by Sen and Baltimore [6] in the nucleus bound to an enhancer element of the immunoglobulin kappa light chain gene in B cells [6,14]. It is now known to be ubiquitous in nature present in all the cell types and is evolutionary conserved. It belongs to the family of Rel proteins that includes c-Rel, RelA (p65), RelB, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100) all of which can form hetero- or homodimers [15–17].

NF-κB activation is tightly regulated mainly through its localization. In resting cells, NF-κB proteins are kept in the cytoplasm in association with inhibitory IκB proteins including IκBα, IκBβ, and IκBε [16] among which IκBα is the most abundant. NF-κB signaling occurs through the canonical (classical) pathway initiated by NF-κB1 (p50/p105) and a noncanonical (alternative) pathway initiated by NF-κB2 (p52/p100) (Fig. 1). Before the active NF-κB is translocated into the nucleus, NF-κB1 and NF-κB2 are cleaved to the active p50 and p52 subunits, respectively. While the classical pathway depends on IKK complex consisting of IKKα, IKKβ, IKKγ and the inhibitory subunit IκBs, the alternative pathway depends on IKKα homodimers and NF-κB inducing kinase (NIK) [18–20]. During classical activation, the IKK complex specifically phosphorylates IκBs on two conserved N-terminal serine residues which target them for E2- and E3-ligase-mediated polyubiquitination and subsequent 26S proteasomal mediated degradation. This process releases and activates NF-κB which now translocates to the nucleus. The activation of alternative pathway, which is commonly associated with RelB results in regulated processing of the p100 precursor protein to p52 and subsequent translocation of p52-RelB

Abbreviations: AgR, antigen receptor; ATM, ataxia-telangiectasia mutant; BAFF, B-cell activating factor; BCL, B-cell lymphoma; BCR, B cell receptor; CARMA, CARD-containing MAGUK protein; CD40L, CD40 ligand; CK, casein kinase; DSBS, Double-stranded DNA breaks; ECSIT, evolutionary conserved signaling intermediates on Toll pathways; EGF, epidermal growth factor; EGFR, EGF receptor; ELKS, glutamate, leucine, lysine, serine-rich protein; GSK, glycogen synthase kinase; Hsp90, heat shock protein 90; IκB, inhibitor of NF-κB; IKK, IκB kinase; IRAK, IL-1R-associated kinase; LTβ, lymphotoxin β; LPS, lipopolysaccharide; MALT, mucosa-associated lymphoid tissue; MAPK, mitogen activated protein kinase; MAPK/Erk, kinase kinase; MyD88, myeloid differentiation factor; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; NEMO, NF-κB essential modulator; PDK, Phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; RANKL, receptor activator of NF-κB ligand; RIP, receptor-interacting protein; Syk, Spleen tyrosine kinase; TAB, TAK1-binding protein; TAK, transforming growth factor-β-activated kinase; TCR, T cell receptor; TLR, Toll-like receptor; TNF, tumour necrosis factor; TNFR1, TNF receptor 1; Tpl2, tumour progression locus-2; TRADD, TNF-receptor-associated death domain protein; TRAF, TNF-receptor-associated factor

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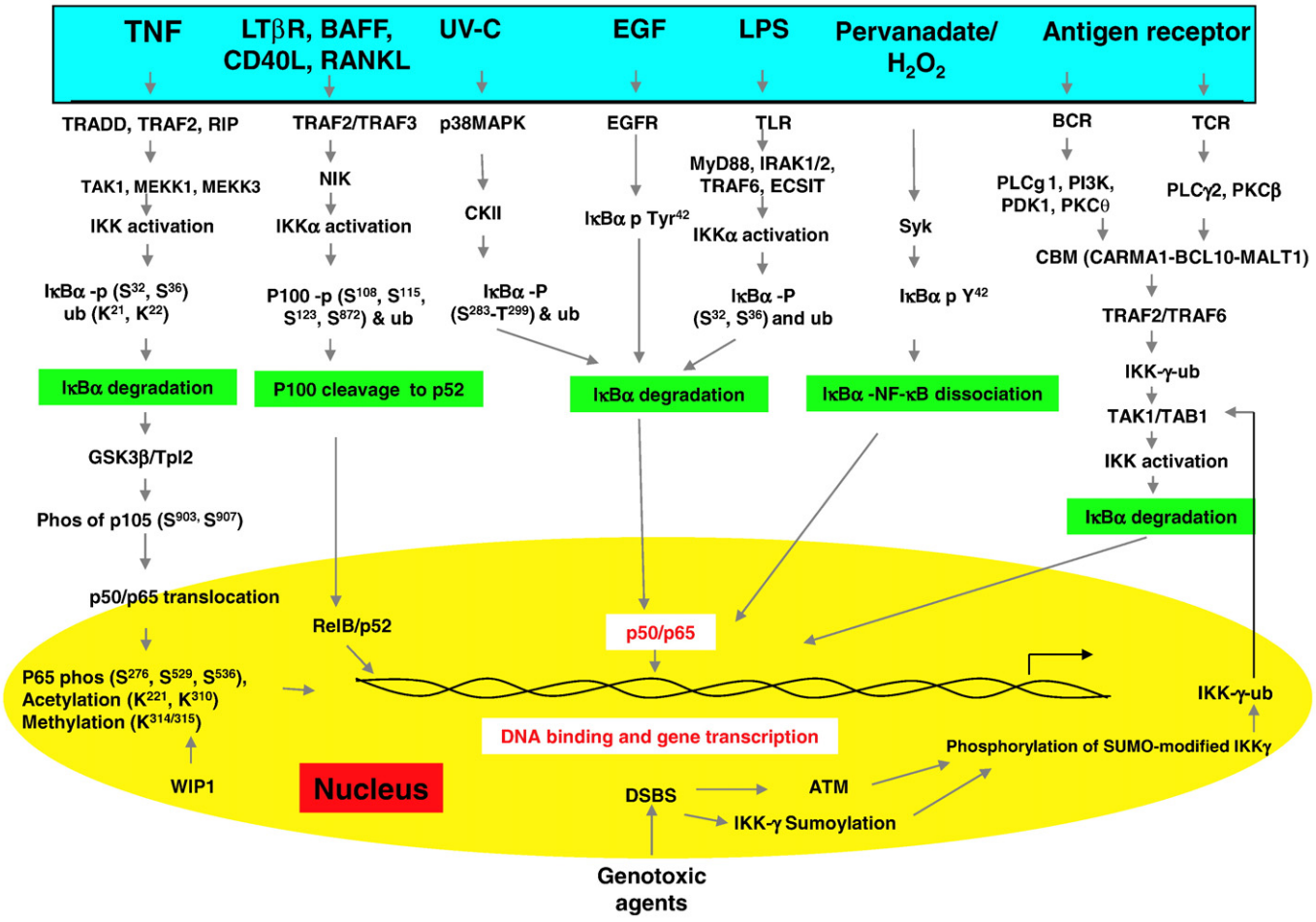

Fig. 1. Schematic representation of major NF-κB activation pathways. In the classical pathway, binding of TNFα to the receptor triggers the sequential recruitment of the adaptors TRADD, TRAF2 and RIP to the membrane. TRAF2 then recruits the IKK complex composed of IKKα, IKKβ and IKKγ (NEMO) through mediation of kinases like TAK1, MEKK1, MEKK3. Activation of the IKK complex leads to the phosphorylation and ubiquitination of IκBα at specific residues followed by its degradation via the proteasome pathway. The p105 subunit of NF-κB then undergoes GSK3β and Tpl2 mediated phosphorylation at S^{903} and S^{907} and subsequent degradation. The heterodimer p50–p65 is then released and migrates to the nucleus where it undergoes a series of posttranslational modifications including phosphorylation, acetylation and methylation and binds to specific κB sites and activates NF-κB target genes [49,209,210]. The alternative pathway is IKKγ independent and is triggered by binding of the CD40, RANK, LTβR, BAFF ligands to their receptor, leading to recruitment of TRAF proteins and the sequential activation of NIK and IKKα. Activation of IKKα then induces the processing of the inhibitory protein p100. p100 proteolysis releases p52 which then translocates to the nucleus and triggers transcription of NF-κB target genes [211]. NF-κB activation in response to UV-C does not depend on IKK activation and relies on sequential recruitment of p38MAPK and CKII. Activated CKII phosphorylates IκBα at C-terminus (S^{283}–T^{299}). The phosphorylated IκBα undergoes ubiquitination and degradation leading to release of active NF-κB in to the nucleus [212,213]. EGF induced NF-κB activation proceeds without serine phosphorylation and ubiquitination of IκBα and is IKK independent. It relies on phosphorylation of IκBα at Tyr^{42} through mediation of tyrosine kinases that triggers its proteasome mediated degradation and subsequent release of active NF-κB to the nucleus [214]. NF-κB activation in response to bacterial endotoxin LPS involves Toll like receptor and is mediated through recruitment of MyD88, TRAF6 and ECSIT. Recruitment of these adaptors leads to sequential activation of IRAK1/2 and IKK and eventual release of active NF-κB [215]. NF-κB activation by pervanadate and H_2O_2 induces phosphorylation of IκBα at Tyr^{42} by protein tyrosine kinase like Syk. The Tyr phosphorylation does not lead to IκBα degradation but makes the binding weak thereby dissociating the IκBα and releasing active NF-κB to the nucleus [216,217]. Antigen receptor viz., T-cell receptor and B-cell receptor mediated signaling to NF-κB activation depends on recruitment of a trimolecular protein complex CARMA1-BCL10-MALT1. In this pathway PKCθ (in T cells) and PKCβ (in B cells) along with other kinases act upstream to the trimolecular complex to promote IKKγ polyubiquitination and consequent IKK activation. Activation of IKK through this pathway involves mediation of TRAF2, TRAF6, TAK1 and TAB1 [218,219]. A novel pathway of NF-κB activation originating from the nucleus is associated with DNA damage. Double-stranded DNA breaks in response to genotoxic agents initiate signals that trigger SUMOylation of nuclear-localized IKKγ , preventing its nuclear export. Concomitantly, these breaks activate ATM which phosphorylates SUMO-modified IKKγ, promoting the removal of SUMO and enhancing IKKγ ubiquitination. Ubiquitinated IKKγ then translocates to the cytoplasm, where it activates IKK in cooperation with ATM and the ELKS protein, leading to IκBα phosphorylation and degradation, p65 nuclear translocation and induction of NF-κB dependent target genes [220-223]. NF-κB can also be regulated by phosphatases. WIP1, a Ser/Thr phosphatase was recently shown to negatively regulate NF-κB activation by dephosphorylating p65 at Ser^{536} [80].

heterodimers to the nucleus [19]. Although NF-κB activation occurs mainly through canonical and non-canonical pathways, during the past decade a number of pathways for NF-κB activation has been elucidated (Fig. 1).

Once in the nucleus, activated NF-κB undergoes a series of posttranslational modifications, including phosphorylation, acetylation, and methylation. These modifications regulate both the strength and duration of NF-κB activity. RelA/p65 is directly phosphorylated by cAMP-dependent protein kinase (PKA) at Ser^{276}, casein kinase II (CKII) at Ser^{529}, and IKK at Ser^{536} [21,22]. RelA dephosphorylation by protein phosphatase 2A (PP2A) has been reported to decrease NF-κB activity [23]. RelA is subject to inducible acetylation by p300/CBP, and acetylated RelA interacts weakly, if at all, with IκBα [24,25], but maintains its nuclear localization and NF-κB transcriptional response. RelA is also subject to methylation by lysine methyltransferase Set9 (also called Set7 or KMT7) at $Lys^{314/315}$ [26].

Fig. 2. A list of gene products regulated by NF-κB. These genes include transcription factors, cell-surface receptors, growth factors, immunoreceptors, acute phase proteins, enzymes, stress response genes, early response genes, viruses, apoptosis regulators, cytokines/chemokines and cell adhesion molecules. For more information, see http://www.nf-kb.org.