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REVIEW ARTICLE

The Nuclear Factor Kappa B (NF-kB) signaling in cancer development and immune diseases

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Abstract The nuclear factor kappa B (NF-kB) family of transcription factors plays an essential role as stressors in the cellular environment, and controls the expression of important regulatory genes such as immunity, inflammation, death, and cell proliferation. NF-kB protein is located in the cytoplasm, and can be activated by various cellular stimuli. There are two pathways for NF-kB activation, as the canonical and non-canonical pathways, which require complex molecular interactions with adapter proteins and phosphorylation and ubiquitinase enzymes. Accordingly, this increases NF-kB translocation in the nucleus and regulates gene expression. In this study, the concepts that emerge in different cellular systems allow the design of NF-kB function in humans. This would not only allow the development for rare diseases associated with NF-kB, but would also be used as a source of useful information to eliminate widespread consequences such as cancer or inflammatory/immune diseases.

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Introduction

The NF-κB transcription factor family consists of five distinct proteins as follows: RelA, RelB, c-Rel, p100, and p150 (Fig. 1A).\(^1\)\(^-\)\(^3\) They have rel homology domain (RHD), which is essential for dimerization, DNA binding, and interaction with IkB inhibitors. The domain is named for its sequence similarity with the v-rel oncogene from the T-strain of reticuloendotheliosis virus (REV), which causes the embryonic lymphatic tumor.\(^4\) Also, RelA, RelB, and c-Rel have transcription activation domain (TAD). Also, TADs are absent at p100 and p105, and these proteins are production Precursor of NF-κB, which after proteolysis, produces the p52 and p50 subunits.\(^5\)\(^-\)\(^7\) Therefore, NF-κB is a generic name and can be included in a family of dimeric proteins produced by different compounds.\(^8\)\(^-\)\(^10\) Moreover, all of them have the p50, p52, RelA, RelB, and c-Rel subunits. One of the widely studied NF-κB dimers is p50/RelA, which is expressed in most cells.\(^3\)

NF-κB main function

NF-κB proteins can regulate the expression of hundreds gene, which regulate important physiological processes such as inflammation, immunity, proliferation, and cell death.\(^11\) Since NF-κB activity is spontaneously regulated by a number of different stimuli, NF-κB proteins can be considered as regulators of cellular homeostasis.\(^12\)\(^-\)\(^14\)

A key function of NF-κB is controlling the immune response at various stages. Immunity is associated with inflammation, and an appropriate and integrated functioning of both processes is essential.\(^1\) The first response is innate immune response, facilitated by specific cell types such as macrophages and dendritic cells, which recognize bacteria and viruses through pathogen-associated molecular patterns (PAMPS).\(^19\) Among these receptors are members of the toll-like receptor (TLR) family like TLR4, which is involved in the induction of NF-κB protein after recognition of bacterial lipopolysaccharide (LPS).\(^20\)

One of the major activities of NF-κB proteins is controlling the inflammation process, which indicate that, they targeted the body’s complex defense mechanisms under inflammation conditions.\(^15\) This is performed by positively and negatively regulating expressions of many important genes in the essential process including chemokines and pro-inflammatory cytokines. Cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) are potent inducers of NF-κB.\(^3\)\(^,\)\(^16\) In addition, NF-κB also contributes to the resolution of inflammation, which then disrupts the function of NF-κB.\(^17\)\(^,\)\(^18\)

The second response is adaptive immune response due to cell or humoral, and allow the identification of non-self-antigen and antibody synthesis.\(^21\) Cell-mediated immunity consists of T lymphocytes that, through their T-cell receptor (TCR), are able to identify antigens processed by antigen-presenting cells (APCs).\(^3\) These cells are those that are involved in innate immune response and require the activation of NF-κB.\(^3\) Accordingly, activation of T cells via

Figure 1  Protein family members and structure. (A) NF-κB family members. Proteins are shown based on their functional domain. The two serine residues (in the phosphorylation regions) and the lysine residues (in the ubiquitination region) participate in p100 processing to produce p52. (B) IkB family members. Proteins are shown with their functional domains. ANK: ankyrin Repeats. The involvement of serine residues (phosphorylation sites) and lysine residues (ubiquitination sites) in IkB dysregulation has been demonstrated.\(^103\)
TCR enables their proliferation and differentiation to obtain the effector function required for NF-kB. Humoral-mediated immune consists of B lymphocytes that directly detect B antigens through the B-cell receptor (BCR). Thus, NF-kB plays critical roles in the immune response and provides B cell differentiation and maturation; and second, it is targeted by BCR as an important element in B-cell survival, which ultimately controls NF-kB secondary lymphoid regeneration, whose poor progression has important consequences on lymphocyte activation. Due to the multifaceted role of NF-kB in immunity, malfunctioning of this transcription factor may decrease immunogenicity or autoimmunity.

NF-kB transcription factors are also involved in cell proliferation and cell death. Regarding the case of cell proliferation, they have been reported to regulate the expression of several cell cycle regulators such as cyclin A, cyclin D1 or cyclin-dependent kinase 6 (CDK6). NF-kB activation is an essential step to protect the cell against the activation of this transcription factor may decrease immunogenicity and provide B cell differentiation and maturation; and finally, it allows: first as a regulator of B cell lymphoid tissue formation and provides B cell differentiation and maturation; and second, it is targeted by BCR as an important element in lymphocyte activation. NF-kB plays critical roles in the immune response as follows: first as a regulator of B cell lymphoid tissue formation and provides B cell differentiation and maturation; and second, it is targeted by BCR as an important element in B-cell survival, which ultimately controls NF-kB secondary lymphoid regeneration, whose poor progression has important consequences on lymphocyte activation. Due to the multifaceted role of NF-kB in immunity, malfunctioning of this transcription factor may decrease immunogenicity or autoimmunity.

Finally, out of the functions of NF-kB as discussed in the following chapters, is the central nervous system (CNS). However, there is a controversy regarding the precise role of NF-kB in CNS progression, indicating that, NF-kB regulates neuronal plasticity through response to neurotrophic factors or synaptic transmission and participation in neuronal survival.

### Standard pathway activating NF-kB

One of the major features of NF-kB transcription factors is their association with members of the IκB inhibitor family in the cell cytoplasm. This protein family consists of three members (IκBα, IκBβ, and IκBε) having very similar structures (Fig. 1B). At the N-terminus, they have a conserved sequence containing two serine residues (DSGXXXS) that can be altered by phosphorylation, in while half of their C-terminus contains a series of ankyrin repeats indicating their tendency for RHDs of NF-kB dimers.

The cytoplasmic position of the NF-kB protein is determined by stimulation of its nuclear localization signal (NLS), which is achieved from IκB ankyrin repeats. After stimulation, IκBs are phosphorylated, and this change stimulates their ubiquitination and degradation by the 26S proteasome. Then, the released NF-kB dimers can enter the nucleus and bind to conserved DNA motifs for positive and negative regulations of gene expression (5′-GGGRNYYYCC-3′), in which R purine, Y pyrimidine, and N are any nucleotide type.

It has been shown that, double-serine phosphorylation in the conserved IκB sequence is mediated by a kinase complex called IκB kinase (IKK). Also, its main components include two catalytic subunits, IKK1 and IKK2, and one regulatory subunit, as the NF-kB essential modulator. IKK activation is automatically regulated by phosphorylation. The kinase can be involved in IKK (via autophosphorylation) or the upstream TGFβ-activated kinase (TAK) complex. This complex consists of a catalytic subunit of TAK1 and two regulatory subunits as follows: TAB1 and TAB2 or TAB3. In addition to IKK activating, the TAK complex can also activate MKKs and MAP kinases such as p38 and JNK.

The domain components of IKK and TAK complexes represent their function in NF-kB signaling. Moreover, IKK1 and IKK2 are two related kinases, in which the domain kinase has phosphorylation activation sites at the N-terminus, the SSD mediates IKK1/IKK2 dimerization in the middle part, and the NEMO-binding domain (NBD) at the C-terminus. NBD is a helical dimeric protein containing the IKK interaction sequence at the N-terminus, which its first domain has a tendency toward ubiquitin in the middle part and a zinc finger (ZF) structure at the C-terminus.

NEMO ubiquitin binding (NUB) and ZF domains have been shown to contribute to ubiquitin detection. TAK1 is a MEKK family kinase containing a kinase domain at N-terminus and a TAB2/TAB3 binding region at the C-terminus. The N-terminus kinase domain interacts with TAB1, which is a regulatory/scaffold protein containing a large helix at the N-terminus and a short sequence interacting with TAK1 at the C-terminus. Finally, TAB2 and TAB3 are similar regulatory proteins that have a coupling of ubiquitin conjugation to ER degradation (CUE) domain at the N-terminus and Npl4 zinc finger (NZF) at the C-terminus, which both of them tend to ubiquitin. In addition, they have twisted domains that have mediate interaction with TAK1.

### Ubiquitination

Ubiquitination plays an important role at different stages of the NF-kB activation process. These post-translational changes are involved in protein complex (Fig. 2). Ubiquitin is a small 8-kDa protein synthesized as a precursor for a polypeptide. In the first step, ubiquitin was activated by the ubiquitin-activating enzyme E1 through the ATP-dependent reaction. Then, the activated ubiquitin was transferred to the ubiquitin conjugation enzyme E2 to form the E2-ubiquitin thioester. Finally, in the presence of ubiquitin protein ligase E3, ubiquitin was covalently bound to the target protein by the isopeptide bonding between the carboxyl ubiquitin end and the ε-amine lysine group of the protein. Also, there are two E1 enzymes, tens of E2 enzymes, and hundreds of E3 enzymes that result in specific substrate specificity.

In cells, proteins can be altered by one ubiquitin chain or several ubiquitin chains. In fact, ubiquitin contains seven lysines (K6, K11, K27, K29, K33, K48, and K63) that enable the poly-ubiquitination process. The type of chains synthesized from ubiquitin, named after the lysine residue, depends on E2 and also affects the fate of the modified protein. K48-associated polyubiquitin chains are identified by the recognition and degradation by the 26S proteasome. In the NF-kB signaling pathway, this poly-ubiquitination was observed at IκB levels after being phosphorylated by IKK, leading them to degrade. Other types of polyubiquitin chains like the K63-linked polyubiquitin chain, do not result in degradation; however, by interacting with substrates containing ubiquitin-binding domains they alter the activity of the modified substrate.
Ubiquitin-binding domains have been identified, most of which have identified a specific type or a limited number of polyubiquitin conformations.47 Accordingly, this provides another level of regulation of protein–protein interactions.3

Recently, it has been shown that, proteins can be altered by other types of polyubiquitin chains. A protein complex reported as linear ubiquitin chain assembly complex (LUBAC), results in the formation of "linear" chains or the chains associated with M1 polyubiquitin.48 In this case, diubiquitin occurs between the C-terminus (Gly76) and the N-terminus (Met1) of the remaining ubiquitin. Three different components of LUBAC include the followings: E3 ligase heme-oxidized IRP2 ubiquitin ligase-1 (HOIL-1), HOIL-1-interacting protein (HOIP) also called ring finger protein 31 (RNF31), and the SHANK associated RH domain interactor (SHARPIN) adapter.49,50 HOIL-1 contains UBL (pseudo-ubiquitin) domain at the N-terminus that interacts with several proteins, which among them HIOP, as a type of NZF (pseudo NplA zinc finger), binds to linear polyubiquitin and RBR and IBR domains.51–53 HOIP has a PUB domain that interacts with OTU deubiquitinase with linear linkage specificity (OTULIN), two NZF domains include the UBA domain responsible for interacting with HOIL-1 and the RBR catalytic domain.54,55 Sharpin contains a Pextrin homology highly twisted region at the N-terminus, which interacts with HOIP through the UBL domain. In addition, Sharpin has two NZF domains.5

As mentioned earlier, NEMO and TAB2/Tab3 have ubiquitin-binding domains. It has been shown that, the NUB domain/NEMO zinc finger segment identifies K63 polyubiquitin chains and linear chains,56 while the NZF-dependent TAB2/TAB3 chains identify K63-related chains.43,57,58

TNF-R1 signaling pathway

The first TNF-α signaling pathway requires the binding of TNF-α to TNFR1 (Fig. 3).39,60 This starts by connecting their death domain (DD) to the DD of TNFR-1, using TNF receptor-associated death domain protein (TRADD) and receptor interacting Serine/Threonine kinase 1 (RI PK-1) adapter molecules. Binding of E3 ligase of TRAF2/TRAF5 to TRADD causes two other E3s c-IAP1 and c-IAP2 to modulate.1 RIPK-1 along with K63-linked polyubiquitin chains. Then, polyubiquitinated RIPK-1 with K63 via HOIL-1 subunit absorbs LUBAC, through TAB2/3 subunits absorb TAK complex, and through NEMO, they uptake IKK. Linear poly-ubiquitination of RIPK-1 and NEMO by LUBAC is more stable; and therefore, called messenger receptor complex (TNF-RSC), termed complex 1.3,43 The masses induced by different compounds result in the phosphorylation of IKK by TAK1. The catalytic subunit of the TAK complex is involved, and IKK activation induces NF-kB release and stimulates transcription of its target genes.3,43

Ubiquitination events such as TNF-R1 signaling are regularly controlled through deubiquitinating enzymes (DUBs).

A20 is a protein with dual enzymatic activity (DUB/E3 ligase) encoded by the NF-kB induced gene TNF alpha induced protein 3 (TNFAIP3).61 A20 stops NF-kB activation by (1) deubiquitinating RIPK-1 and (2) polyubiquitinating RIP-1 to target it for degradation (proteolysis). CYLD lysine 63 deubiquitinase (CYLD) can hydrolyze the linear chain or chain associated with K63 and work on RIPK-1, NEMO, and TRAF-2.43,62–64 USP2 is another type of DUB that acts in level of RIPK-1.65 Finally, it has been shown that, OTULIN is a type of DUB tends to linear ubiquitin chains and inhibits LUBAC activity.66,67

TNF-α regulation of cell death

NF-kB activation in response to TNF-α also leads to an increase of cellular FLICE-like inhibitory protein (c-FLIP), which plays an important role in controlling the fate of cells.68 Immediately after TNF-RSC, TRADD, and RIPK-1 components were transferred to the cytoplasm via their DDs, they couple with the adapter fas-associated death domain (FADD) and caspase-8 primer, creating a new complex called complex Ia.68 In the case of NF-kB-induced c-FLIP synthesis, caspase-8/c-FLIP dimer inhibits caspase-8 activity. If NF-kB activation does not occur, the activated

![Figure 2](https://example.com/figure2.png)

The ubiquitination process. The various steps required to change the substrate by mono and poly-ubiquitination are shown.104
caspase-8 in complex Ila through apoptosis targets cell death. Thus, activation of NF-kB in cells is stimulated by TNF-α and enables cell survival.3

It has been shown that, TNF-α can induce another form of cell death,69 termed necroptosis, which is controlled by components of the NF-kB pathway. Necroptosis contains another protein of complex Ila known as receptor-interacting protein kinase 3 (RIPK3). When apoptosis occurs, RIPK3 is proteolyzed by caspase-8. Also, in case of inhibition of caspase-8 enzymatic activity or lack of FADD; RIPK1 and RIPK3 are both stabilized and form a new complex (called complex Ilib) leading to necroptosis. This state of cell death requires mixed lineage kinase domain like pseudokinase (MLKL),69 which is a substrate of RIPK3 kinase activity. After phosphorylation, MLKL induces pores in the cell membrane and releases cytoplasmic compounds into the extracellular environment, which destroys the cells.70,71 Accordingly, this release induce an inflammation reaction, which is a reaction that does not occur in apoptotic cell death. In this case, death involves nuclear accumulation and membrane organization changes, which eliminates non-leaky cell components.3

**IL-1βR/TLR4 signaling pathway**

The IL-1R and TLR signaling pathways are similar, due to the presence of a conserved domain called Toll/interleukin-1 receptor (TIR) domain that provides the conditions for creating the signaling complexes of several similar proteins in these receptors (Fig. 4).72 At the cell surface, IL-1R is associated with interleukin-1 receptor accessory protein (IL-1RaC), which is responsible for IL-1β detection and message transmission. TLR4 is selected as the TLR model linked to the MD-2 receptor, and represents the LPS (lipopolysaccharide) receptor, which is an outer wall component of gram-negative bacteria.3 Other TLRs tend to some compounds such as triacetylated lipoproteins (TLR1/TLR2); diacetylated lipoproteins; and peptidoglycans from gram-positive bacteria and lipoteichoic acid (TLR2/TLR6), dsRNA and poly: (TLR3) viral, flagellins (TLR5), single stranded RNA (TLR7 and TLR8), and bacterial and mycobacterial CpG-DNA (TLR9).3

After utilizing their ligands, IL-1R/IL-1RaC and TLR4/MD-2 are used through TIR domains. The TIR domain contains Myd88 adapter that has also DD. This DD domain is involved in activation of interleukin-1 receptor-associated kinase (IRAK4, IRAK1), and TNF receptor associated factor (TRAf6) E3 ligase. After phosphorylation, IRAK1 is ubiquitinated by IRAK4, IRAK1, and TRAF6 and is released by K63 from receptor and absorbs TAK and IKK complexes.73–75 This is performed by ubiquitin-binding subunits (TAB2/ TAB3 and NEMO, respectively) and induces phosphorylation and IKK activation. The E2 conjugation enzyme (Uev1A UbC13) is specifically involved in the production of these K63 ubiquitin chains and TRAF6-related EZ,43,67 as well as through cellular features, which leads to synthesis of K63-related chains.76 However, a number of LUBAC samples are required for IL-1/TLR signaling.77,78 The...
compounds that are changed by linear chains are less well known. Also, NEMO may be one of these linear chains reported for the TNF-R1 pathway as well as IRAK1.3,43

BCR/TCR signaling pathway

Both, the BCR and the TCR, pathway overlap in NF-κB activation (Fig. 5).79,80 About TCR, \( \alpha \) and \( \beta \) chains regarding TCR at the surface of T cells with \( \zeta \) and \( \gamma \) chains related to CD3 molecules. Also, TCR signaling is targeted by major histocompatibility complex (MHC) molecules principal adaptation complex and antigenic peptides. Antigenic peptides are produced by APCs such as dendritic cells and macrophages.3

The onset of signaling has come from the activation of members of the tyrosine kinase family Src (sarcoma), Fyn,
and Lck. Activation of these tyrosine kinases induces tyrosine phosphorylation of immune receptor tyrosine-based activation motifs (ITAMs) in CD3 and the utilization of zeta-chain-associated protein kinase-70 (ZAP70) by Src homology 2 (SH2) domains. Due to ZAP70 phosphorylation and activation, adapter proteins such as linker for activation of T cells (LAT) and SH2 domain-containing leukocyte protein of 76 kDa (SLP76) participate in phospholipase Cγ1 (PLCγ1) activation.3

BCR contains antigen-binding IgH heavy chain and antigen-binding IgL light chain and messages are transmitted by the message transfer section or CD79A/CD79B protein containing ITAMs. Tyrosine amino acid of these motifs are phosphorylated by Lyn kinase and other src kinase families such as Fyn and Blk. This allows Syk to be used by SH2 domains and activated.3 Signaling is expanded by BLNK and BTK, and activates and phosphorylates PLCγ2.

After activation of PLCγs, distal signaling is equally achieved in the TCR and BCR pathways. In fact, PLCγs hydrolyze inositol phospholipids (PIP2) to inositol triphosphate (IP3) and diacyl-glycerol (DAG). IP3 production stimulates intracellular calcium, and by DAG, maximizes protein kinase C (PKC) activation. PKCe represents the activated isoforms in the downstream section of TCR, whereas PKCb is downstream of BCR. Afterwards, PKCs act on caspase recruitment domain-containing membrane-associated guanylate kinase protein-1 (CARMA1) and muco associated lymphoid tissue lymphoma translocation protein 1 (MALT1), and cause CARMA1/BCL10/MALT1 (CBM) complex formation. In this complex, interaction of the caspase recruitment domain (CARD) part of CARMA1 with

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**Figure 6** NF-κB secondary pathway activation. CD40 signaling pathways.107
the CARD part of Bc110, causes CARMA1 to play the role of a scaffold protein, whereas its cyclic helical domain binds to MALT1. Accordingly, MALT1 interacts with TRAF6 to induce the K63-associated polyubiquitination in Bc110 and induces IKK uptake and activation through NEMO. In some specific cases, p100 polyubiquitination does not completely proteolyze it, but it rather causes p52 at the N-terminus. Since in cells, p100 tends to the RelB subunit and controls its cytoplasmic position, the production of NF-κB dimer is mediated by the activation of NF-κB and p52RelB secondary pathway.

In activating the NF-κB pathway, NIK must be stable, which is destroyed in static cells. Components that contribute to its instability include cIAP1/2, TRAF2, and TRAF3. Also, the components form the NIK ubiquitin ligase (E3) complex and TRAF3. The NIK ubiquitin ligase (E3) complex catalyzes the degradation of NIK.

Several receptors have been reported to activate the NF-κB secondary pathway. Also, CD40 ligand (CD40L) and lymphotixin β receptor (LTβR) are reported to be among these receptors (Fig. 6). After interaction of the receptors with their ligands, the NIK E3 complex is formed. This induces cIAP1/2 polyubiquitination by TRAF2 and targets the identification and modification of the TRAF3 polyubiquitin K48 chain. TRAF3 proteolysis causes NIK stability and IKK1 phosphorylation, and also induces NF-κB secondary pathway activation.

A specific collection of receptors that activate NF-κB secondary pathway act in the development and maintenance of peripheral lymph nodes (LTβR), maturation of peripheral B cells, B cell activation and differentiation, germinal center formation and antibody isotype switching, dendritic cell maturation, and antigen CD40 or osteoclastogenesis. The primary and secondary NF-κB activation methods do not independently operate; however, are often considered to have separate operation. In many cases, interactions between them have been observed, indicating the integration of these two methods. For example, receptors that activate the secondary pathway can simultaneously activate the primary pathway. As a consequence, the expression or activation of several combinations of one path is controlled by the other one. It has been shown that, NF-κB main dimers regulate the RelB transcription, which its product associates with p52. On the other hand, RelB can form dimers with p50 and can be controlled by IκBα.

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Conclusion

Although the secondary pathway has more limited functions compared to the primary NF-κB activation pathway, it plays an important role in controlling immune-related events such as B cell maturation, peripheral lymphoid development, and thymic development. In humans, mutations affect three major components of this pathway (NIK kinase initiator and its effector, p100/p52 subunits, and NF-κB RelB subunits). In each one of the cases, immunodeficiency is mainly discovered by hypogammaglobulinemia with re-infection resulting from B-cell dysfunction and specific abnormalities. Accordingly, this affects the activation of the secondary pathway in an organism and confirms previous reports on mice with defects.

Inherited diseases caused by NF-κB failure signaling lead to a wide variety of abnormalities in the immune system, vascular, skin, bone, and CNS. On the one hand, it allows the comparison of these abnormalities with those that were observed in similar components in mice, and on the other hand, assigns specific defects observed in humans to molecular abnormalities. All of this information should help in designing and approving the treatment.

It is also clear that, the identification of NF-κB-related genetic diseases can be considered as an appropriate strategy for identifying the essential signaling pathways. By completing studies on mice and validating them on humans, the concepts that arise in this or different cellular systems allow the design of NF-κB function in humans. This will not only enable the development of treatment for rare diseases associated with NF-κB, but will also be used as a source of very useful information to address widespread consequences such as cancer or inflammatory/immune diseases.
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