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Review Article

Role of NFkB in Various Immunological & Inflammatory Disorders

PRAJAPATI B.*[1], SINGHAL M. [1], Yashwant[1], Ganesh N. Sharma[1], GUPTA V.[2]

[1]*School of Pharmaceutical Sciences , Jaipur National University , Jaipur*
[2]*Apex Institute of Pharmaceutical Sciences , Jaipur*

ABSTRACT
As per the earlier findings, the principle of CENTRAL DOGMA clears each & every aspect of protein formation which include the process of Transcription & Translation .With the latest evolution it was studied that NF-kb is one of the eukaryotic transcription factors , which consists of a protein complex that controls the process of copying DNA. We have this factor in almost all types of animal cells & plays an important role in the cellular reponses to some stimuli such as stress , cytokines , free radical , oxidized low density lipoproteins , bacterial and viral antigens. Incorrect regulation of NF-kb may also leads to –cancer , inflammation , autoimmune disease , septic shock , viral infection , improper immune development.

Keywords: IKK (IkB Kinase) , NFkB, RHD (Rel Homology Domain) , IkB , ankyrin repeats , canonical , non canonical , TRAF

INTRODUCTION

Pharmacology texts tell us that aspirin, NSAIDs, and COX2 inhibitors act to reduce inflammation by blocking the cyclooxygenase enzyme that converts arachidonic prostaglandin E-2 (PGE2). In other words, these drugs inhibit inflammation by blocking PGE2. Corticosteroids work higher up the chain, inhibiting phospholipase A2, which inhibits arachidonic acid release from phospholipids in the cell membrane. These mechanisms have been known about for a long time. For example, in 1971, John Vane discovered that aspirin inhibits the COX enzyme.

In more recent years, the study of inflammation has gone deeper into the cell, to the point that cell-signaling molecules have been identified which stimulates genes that induce the expression of the COX enzyme. As it turns out, aspirin, NSAIDs, and corticosteroids can inhibit certain cell signaling molecules, such as nuclear factor kappa binding (NFkB) - which reduces inflammation.(1)

The eukaryotic transcription factor NFkB (Nulear Factor Kappa-Light-Chain-Enhancer of Activated B Cell) was identified as a protein that bound to a specific decameric DNA

sequence (ggg ACT TTC C), within the intronic enhancer of the immunoglobulin kappa light chain in mature B- and plasma cells but not pre B-cells.(2)

NFkB is the "big cheese" cell-signaling molecule for inflammation; its activation induces the expression of COX-2, which leads to tissue inflammation. "Intriguingly, the expression of the COX-2-encoding gene, believed to be responsible for the massive production of prostaglandins at inflammatory sites, is transcriptionally regulated by NFkB.

NFkB resides in the cytoplasm of the cell and is bound to its inhibitor. Injurious and inflammatory stimuli, such as free radicals, release NFkB from the inhibitor. NFkB moves into the nucleus and activates the genes responsible for expressing COX-2. Research has demonstrated that aspirin, NSAIDs, and corticosteroids can inhibit the activation of NFkB, which is why people derive relief from these drugs.(3)

MECHANISM of ACTION

The transcription factor NF-kB consists out of homo- or heterodimers of different subunits. The subunits are members of a family of structurally related proteins (Rel/NF-kB proteins). Five different Rel proteins (also called Rel/NF-kB proteins) have been identified so far: p50, p52, p65, RelB, and c-Rel. (4)

Here NFkB heterodimer between Rel and p50 protiens is used in order to explain the mode of its action.In its inactivated state NFkB is located in the cytosol along with the inhibitory protein IkBalpha.Due to intermediacy of integral membrane receptors a variety of extracellular signals can activate the enzyme known as IkB kinase (IKK).IKK leads to phosphorylation of IkBalpha protein , which in turns dissociates IkBalpha from NFkB and eventually degrades IkBalpha by the proteosomes. The activated NFκB is then translocated into the nucleus where it binds to specific sequences of DNA called response elements (RE). The DNA/NFκB complex then recruits other proteins such as coactivators and RNA polymerase which transcribe downstream DNA into mRNA, which, in turn, is translated into protein, which results in a change of cell function as shown in figure 1.(5.6.)

NFkB FAMILY

***Corresponding author:**
Email : manu_mpharm@yahoo.co.in
Phone no: +91-9829153193

In mammals, the NFkB family is composed of five related transcription factors: p50, p52, RelA (p65), c-Rel and RelB . These transcription factors are related through an N-terminal, 300 amino acid, DNA binding/dimerization domain, called the Rel homology domain (RHD), through which they can form homodimers and heterodimers that bind to 9-10 base pair DNA sites, known as kB sites, in the promoters and enhancer regions of genes, thereby modulating gene expression. RelA, c-Rel and RelB contain C-terminal transcriptional activation domains (TADs), which enable them to activate target gene expression. In contrast, p50 and p52 do not contain C-terminal TADs; therefore, p50 and p52 homodimers repress transcription unless they are bound to a protein containing a TAD, such as RelA, c-Rel or RelB or Bcl-3 (a related transcriptional co-activator). Unlike the other NFkB family members p50 and p52 are derived from larger precursors, p105 and p100, respectively. [7,8]

NFkB is not synthesized de novo; therefore its transcriptional activity is silenced by interactions with inhibitory IkB proteins present in the cytoplasm. There are currently seven identified IkB family members - IkBa, IkBb, Bcl-3, IkBe, IkBg and the precursor proteins p100 and p105 - which are characterized by the presence of ankyrin repeats.[9]

STRUCTURE

NFkB was first discovered in the lab of Noble Prize laureate David Baltimore via its interaction with an 11-base pair sequence in the immunoglobulin light-chain enhancer in B cell.[10]

Basically,as shown if figure 2. There are two structural classes of NFkB proteins denoted by CLASS I and CLASS II .Both of these classes of proteins contain a N-terminal DNA –binding domain(DBD), which also serves as a dimerization interface to other NFkB transcription factors in addition binding to the inhibitory IkBalpha protein. All proteins of the NFkB family share a Rel homology domain(RHD) in their N-terminus. The C-terminus of class I protein contains a number of Ankyrin repeats and has transrepression activity. In contrast , the NFkB proteins, have a transactivation domain in their C-terminus of classII protein.[11]

In contrast, the NF-κB1 and NF-κB2 proteins are synthesized as large precursors, p105, and p100, which undergo processing to generate the mature NFκB subunits, p50 and p52, respectively. The processing of p105 and p100 is mediated by the ubiquitin/proteasomes pathway and involves selective degradation of their C-terminal region containing ankyrin repeats. Whereas the generation of p52 from p100 is a tightly-regulated process, p50 is produced from constitutive processing of p105.[11,12,13]

ACTIVATION

Part of NFκB's importance in regulating cellular responses is that it belongs to the category of "rapid-

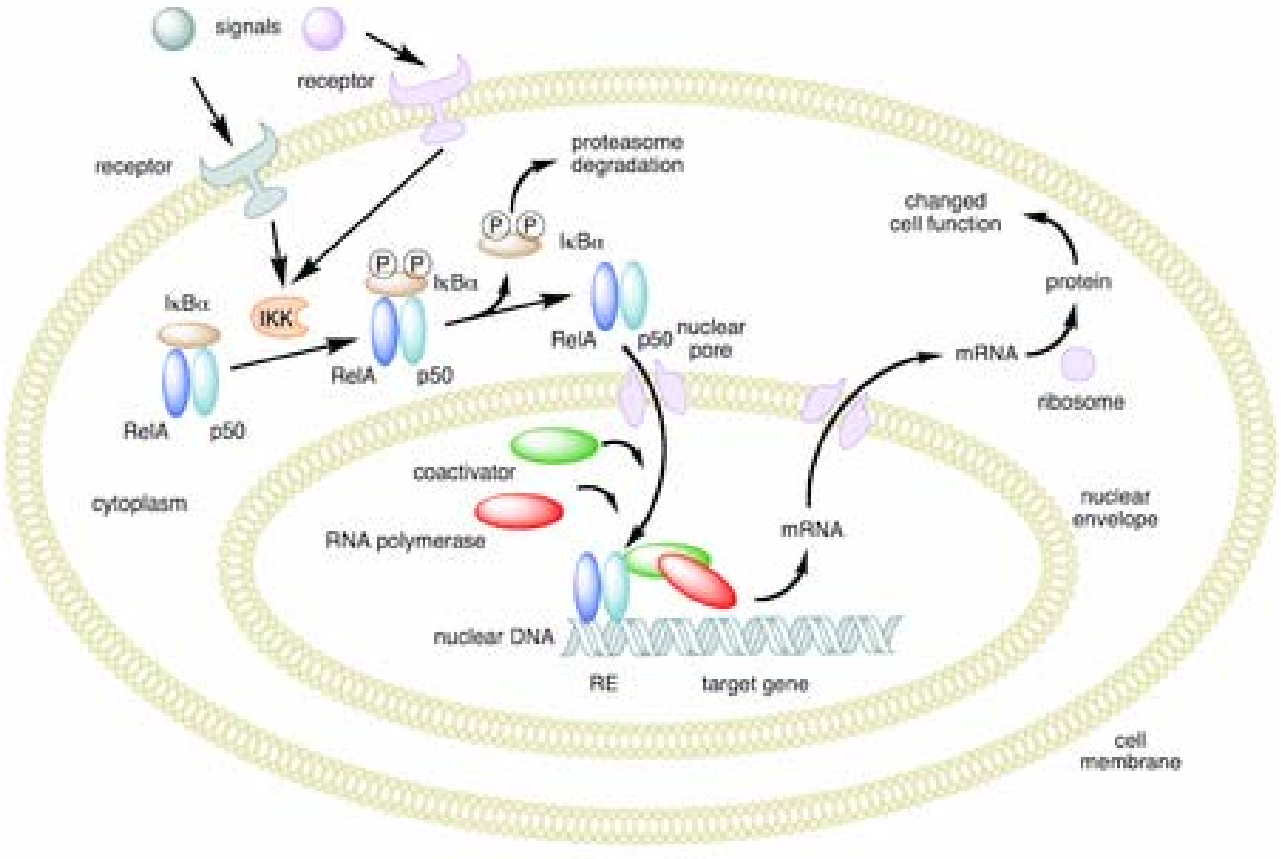

Figure 1: Mechanism of action of NFkB

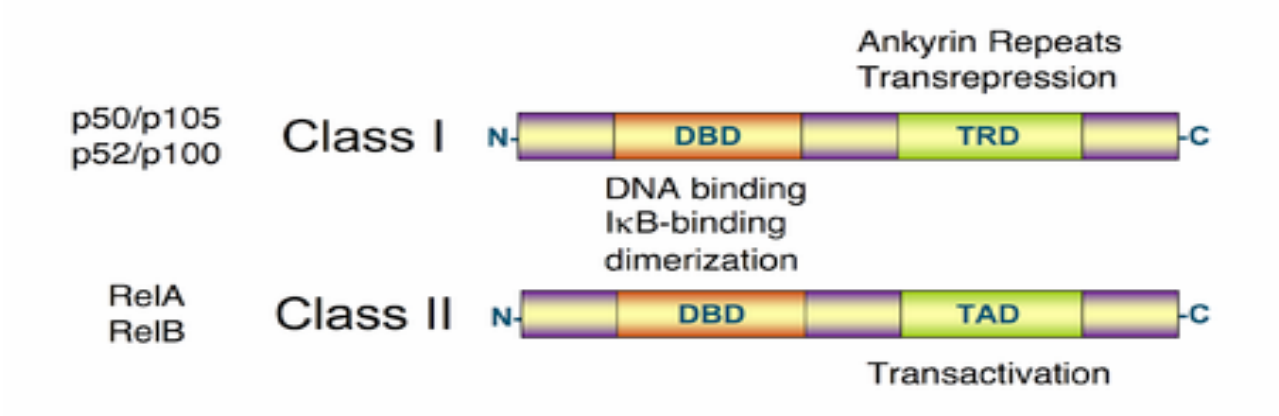

Figure 2: Structure of NFkB

acting" primary transcription factors, i.e., transcription factors that are present in cells in an inactive state and do not require new protein synthesis to be activated. This allows NFκB to act as a "first responder" to harmful cellular stimuli. Stimulation of a wide variety of cell-surface receptors such as RANK,(receptor activator of nuclear factor kB) TNFR, leads directly to NFκB activation and fairly rapid changes in gene expression. Activation of NFkB dimers is due to IKK-mediated phosphorylation-induced proteasomal degradation of the IkB inhibitor enabling the active NFkB transcription factor subunits to translocate to the nucleus and induce target gene expression.Different pathogens can activate NFkB through the stimulation of TLRs(Toll Like Receptors) which are the key regulators of both innate & adaptive immune responses.[14,15,16]

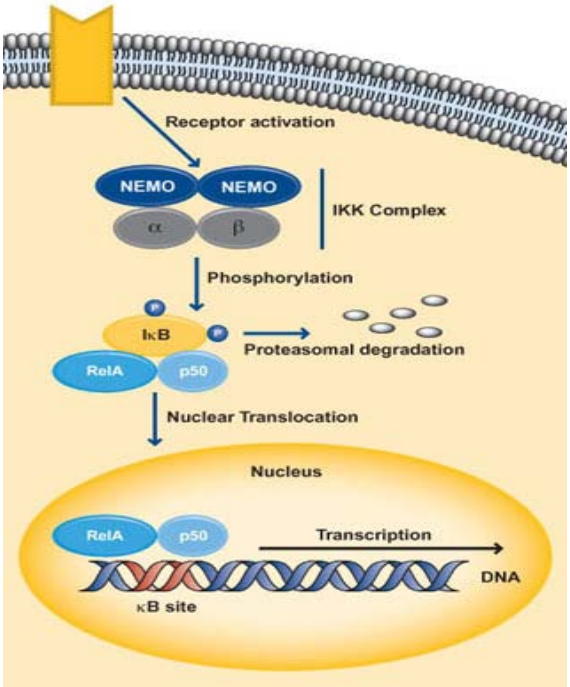

Figure 3: Classical Canonical NFkB signaling pathway

INHIBITION

In unstimulated cells, the NFkB dimers are sequestered in the cytoplasm by a family of inhibitors, called IkB_S (Inhibitor of kB), which are proteins that contain multiple copies of a sequence called ankyrin repeats. By virtue of their ankyrin repeat domains ,the protien mask the nuclear localization signals (NLS) of NFkB proteins and keep them sequestered in an inactive state in the cytoplasm.[17]

IκBs are a family of related proteins that have an N-terminal regulatory domain, followed by six or more ankyrin repeats. Although the IκB family consists of IkBalpha , IkBbeta , IκBgama , IkB_e and Bcl-3 , the best-studied and major IκB protein is IκBα. Due to the presence of ankyrin repeats in their C-terminal halves, p105 and p100 also function as IκB proteins.[18,19,20]

NFkB SIGNALING PATHWAY

There are two signaling pathways leading to the activation of NFkB known as

- The Canonical Pathway (Classical)
- The Non-Canonical Pathway(Alternative)[21,22,23]

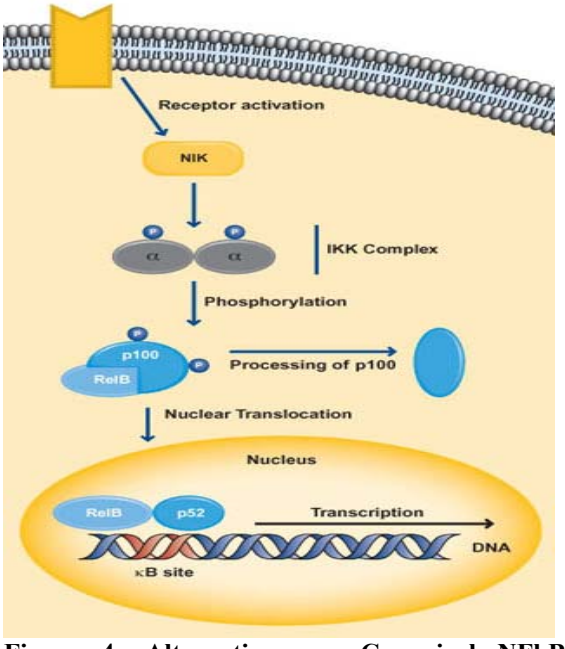

Figure 4: Alternative non Canonical NFkB signaling pathway

The Canonical Pathway

The common regulatory step in both of these cascades is activation of an IkB kinase (IKK) complex consisting of catalytic kinase subunits (IKKa and/or IKKb) and the regulatory non-enzymatic scaffold protein NEMO (NF-kappa B essential modulator also known as IKKg).

In the Canonical Signaling Pathway, binding of ligand to a cell surface receptor such as a member of the the Toll-like receptor superfamily leads to the recruitment of adaptors (such as TRAF-TNF Receptor Associated Factor) to the cytoplasmic domain of the receptor . These adaptors in turn recruit the IKK complex which leads to phosphorylation and degradation of the IkB inhibitor. The canonical pathway activates NFkB dimers comprising of RelA, c-Rel, RelB and p50.[21,22,24]

The binding of ligand to a receptor leads to the recruitment and activation of an IKK complex comprising IKK alpha and/or IKK beta catalytic subunits and two molcules of NEMO.

The IKK complex then phosphorylates IkB leading to degradation by the proteasome. NFkB then translocates to the nucleus to activate target genes regulated by kB sites as shown in figure 3.

The Non Canonical Pathway

The non-canonical pathway (Figure 4) is responsible for the activation of p100/RelB complexes and occurs during the development of lymphoid organs responsible for the generation of B and T lymphocytes. Only a small number of stimuli are known to activate NFkB via this pathway and these factors include lymphotoxin B and B cell activating factor (BAFF). This pathway utilizes an IKK complex that comprises two IKKa subunits, but not NEMO. In the non-canonical pathway, ligand induced activation results in the activation of NFkB-inducing kinase (NIK), which phosphorylates and activates the IKKa complex, which in turn phosphorylates p100 leading to the processing and liberation of the p52/RelB active heterodimer.[25 ,26,27]

Binding leads to the activation of NIK, which phosphorylates and activates an IKK alpha complex that in turn phosphorylates the IkB domain of p100 leading to the liberation of p52/RelB. This heterodimer subsequently translocates to the nucleus to activate target genes regulated by kB sites.

ROLE IN DISEASES

- NFκB is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. Active NFkB keep the cell proliferating and protect the cell from conditions that would otherwise cause it to die via apoptosis.[28] Defects in NFκB results in increased susceptibility to apoptosis leading to increased cell death. This is because NF-κB regulates anti-apoptotic genes especially the TRAF1 and TRAF2.[29] Thus, NF-κB is the subject of much active research among pharmaceutical companies as a target for anti-cancer therapy[30]
- As NFkB control many genes involved in inflammation it is found to be chronically active in many of the inflammatory diseases such as inflammatory bowel disease[31,32] , asthma[33,34],rheumatoid arthritis[35] , hepatitis , gastritis , sepsis , neurodegeneration , ischemia/reperfusion injury & many other disease including subluxation complex.[36,37]
- Many natural products , including anti oxidants that have been promoted to have anti-cancer and anti-inflammatory activity also have been shown to have inhibitory effect on NFkB.
- The patients working under the ventures such as Ariad v.Lilly , Karin[38] , Ben-Neriah[39] and others has illuminated importance of the connection between NFkB , inflammation and cancer such as leukemia, lymphoma, colon cancer and ovarian cancer, also underscored the value of therapies that regulate the activity of NFkB.[40,41]
- NFkB itself is upregulated in RA and cytokines such as TNFa that activate NFkB are elevated in the synovial fluid of patients with RA.[42]

DRUGS TARGETED TO NFkB

Intense activity of NFkB has been certified in the diseases such as cancer , inflammation etc and suppression of which have an aid in improving the status of cancer and inflammation[43,44]

There was a discovery that activated NFkB nuclear translocation can be seperated from the elevated oxidant stress which prooved to be an important hint for the development of stratergies for NFkB inhibition.[45]

A new drug called **Denusomab** acts to raise bone mineral density and reduce fracture rates in many patient sub-groups by inhibiting RANK (Receptor Activator of Nuclear Factor kB) which in turn through its receptor RANK inhibits NFkB[46] **Disulfiram , Olmesartan , Dithiocarbamate** can inhibit the NFkB signaling pathway.[47]

EFFECT OF DIET

- Antioxidant can reduce the activation of NFkB ,including green tea polyphenols; resveratrol from red wine; vitamins C and E ; curcumin ; and glutathione.[48]Supplements such as lipoic acid & coenzyme 10 maintain the glutathione in its activated state.[49]
- The anti-inflammatory omega-3 FAs reduces NFkB activity.[50,51] which means we need to reduce our grain, seed, and related oil intake.Omega-3 FAs also reduce IL-1 ,which is an activator of NFkB.[52]

CONCLUSION

With the above review we come to an conclusion that NFkB is one of the very important linking factor in the central dogma of protein synthesis which if abruptly activated may leads to a significant decrease in autolytic characteristics of the cells, that might be one of the cause in aggressive worsening of the disease such as cancer and various types of different inflammatory disorders. The diet & drugs can also be designed by keeping the target as NFkB ,as its inhibition may improve the conditions of the patients suffering from above disease.

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