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Cellular and Intercellular Signaling in Rheumatoid Arthritis – New Therapeutic Targets

Międzykomórkowe i wewnątrzkomórkowe szlaki sygnalizacyjne w reumatoidalnym zapaleniu stawów – nowe możliwości terapeutyczne

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Abstract


Słowa kluczowe: reumatoidalne zapalenie stawów, MAP-kinazy, NF-κB, przekazywanie sygnału, TNF-α, leczenie.

Streszczenie


Key words: rheumatoid arthritis, MAP-kinases, NF-κB, signal transduction, TNF-α, therapy.

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting about 1% of the population. The inflammatory process begins in the synovium and, when untreated, leads to gradual joint destruction, irreversible deformations, and a high level of disability. Since the development of so-called biologic agents, which act at the level of cytokines and cellular targets within the synovium and immune system, the prognosis for RA patients has significantly improved. The currently used anti-rheumatic drugs, especially the constantly developing group of anti-cytokine agents, affect certain cytokines and their receptors. These proteins are monoclonal antibodies or protein constructs which bind to cytokines or cytokine receptors and limit their proinflammatory potential. These substances are mainly targeted at TNF-α or various interleukins, such as interleukin 1, 6, or 15. They are regarded as very potent anti-inflammatory agents capable not only of reducing current disease activity, but also hampering cartilage and bone destruction.

The most commonly used cytokine-targeted therapeutics are TNF-α inhibitors. They include two monoclonal antibodies against TNF-α, i.e. infliximab (chimeric) and adalimumab (fully human), and the soluble receptor etanercept. Anti-TNF-α agents have comparable clinical efficacy and induce fast clinical response in the majority of patients, improving the signs, symptoms, and radiographic progression of RA. Their use is limited, however, by their side effects, in particular infections, with special emphasis on the activation of latent tuberculosis. They have been shown to induce autoimmune response in patients, but clinically overt autoimmune diseases are very rare.

Another therapeutic agent which has proved highly efficacious in rheumatoid arthritis, rituximab, acts through the selective depletion of B lymphocytes. It has been successfully used in the treatment of follicular lymphoma and has also been employed in the therapy of RA for a few years [1]. It is a chimeric monoclonal antibody selectively
targeting CD20-positive B cells. B lymphocytes are involved in the pathogenesis of RA in many ways, not only by autoantibody production (rheumatoid factors, anti-CCP antibodies, anti-RA 33 antibodies, and others), but also by proinflammatory cytokine synthesis (TNF-α, IFN-α, IL-6, IL-8, lymphotoxin), their action as antigen-presenting cells, and their contribution to T-cell responses in RA. The binding of rituximab to CD20 molecules initiates complement-mediated B-cell lysis and cell-mediated cytotoxicity via macrophages and natural killers and induces B-cell apoptosis. B-cell depletion in RA leads to long-lasting remission and its safety profile is very satisfying [2].

T lymphocytes play a major role in RA's pathogenesis. Their activation is possible only after the delivery of two stimulatory signals, one through TCR receptor binding and the other, the so-called co-stimulatory signal or second signal, by binding the CD28 receptor on the T cell to the CD80/86 receptor on the antigen-presenting cell. This co-stimulatory signal can be blocked by CTLA-4 receptor. The new anti-rheumatic drug, abatacept, exerts its therapeutic effect by blocking CD80/CD86 binding to CD28 on T cells. Abatacept is a soluble, recombinant, fully human protein that comprises the extracellular domain of CTLA-4 and the Fc portion of the modified IgG1 molecule. Abatacept treatment leads to a significant percentage of clinical responses, inhibits radiographic progression, and has an acceptable safety profile [3].

Biological agents, although very efficacious anti-rheumatic drugs, have some important drawbacks. As proteins they have to be administered intravenously or subcutaneously and can induce various adverse events, from infusion reactions to life-threatening anaphylactic reactions. They compromise patients' immunity and are associated with increased risk of infection, especially by mycobacteria, but also by such pathogens as Listeria, Histoplasma, Coccidioides, and others. Their impact on carcinogenesis and embriogenesis is not fully elucidated. The other, often most important, factor is the very high cost of the therapy.

All these factors have prompted scientists to search for a cheaper alternative in the form of orally bioavailable low-molecular-weight compounds acting downstream of cytokine receptors. The major interest is to introduce therapeutic agents capable of inhibiting proinflammatory cytokine (mainly TNF-α) synthesis and that function at the level of intracellular signaling molecules. As the blockade of protein/protein interactions by low-molecular-weight inhibitors is difficult, the enzymes involved in signal transduction appeared to be the most amenable to treatment [4]. There are many intracellular networks associated with signal transduction from cytokine receptors to the cell nucleus which influence transcription and translation processes and have impact on the synthesis of cytokines or enzymes. They comprise proinflammatory cytokines crucial for rheumatic joint inflammation and proteolytic enzymes, i.e. matrix metalloproteinases involved in the pathomechanism of cartilage and bone destruction. Signal-transducing factors in RA usually act in cascades that are under tight regulation. We shall focus here on two important signaling pathways which are potential targets of new anti-inflammatory drugs: the pathways involving MAP kinases and NF-κB.

**MAP-Kinases**

Kinases are enzymes that cause phosphorylation of serine, threonine, and tyrosine residues on structural and regulatory proteins and thus modify their structure, function, and metabolism. Stress- and mitogen-activated protein kinases (MAPK) are elements of signal-transducing cascades essential for many cellular activities and are also implicated in the inflammatory process of rheumatoid arthritis. MAP kinases can phosphorylate a variety of transcription factors and other proteins and regulate gene transcription, mRNA stability, and translation processes. Apart from their impact on the synthesis and activation of proinflammatory cytokines, MAP kinases regulate the degradation of extracellular matrix and are important factors in angiogenesis and endothelial function by enhancing adhesion molecule expression, i.e. VCAM-1 (vascular cell adhesion molecule 1) and E-selectin. MAP kinases regulate cell proliferation, apoptosis, cytokine expression, and metalloproteinase production [5].

The MAP kinase family comprises three types of kinases: extracellular signal-regulated kinases (ERKs), the c-Jun amino-terminal kinases/stress-activated protein kinases (JNKs/SAPKs), and p38 MAPK. Extracellular signal-regulated kinases have two isoforms, ERK 1 and ERK 2, and their main role is to regulate cellular proliferation and differentiation. It has also been shown that the inhibition of ERK prevented the transport of TNF-α mRNA from the nucleus to the cytoplasm but had no effect on the transcription of the TNF-α gene or the stabilization of the TNF-α mRNA. It is suggested that ERK inhibition can induce the inhibition of TNF-α production [6]. Since TNF-α is the major mediator of chronic inflammation, this may offer an interesting therapeutic strategy.

p38 MAP kinase has four isoforms and is involved in the synthesis of many proinflammatio-
ry cytokines, such as TNF-α, IL-1, IL-6, and IL-8. This is why it is particularly interesting as a target for anti-rheumatic therapies. p38 MAPK is present in both the cytoplasm and nucleus of activated inflammatory cells. p38 can induce cytokine gene expression, especially of TNF-α and IL-1, through both pre- and post-transcriptional mechanisms. The α isoform, present in macrophages, has a regulatory effect on TNF-α production, which is the main cytokine responsible for acute joint swelling. IL-1β is the dominant cartilage-destroying cytokine as it controls the synthesis of different metalloproteinases. It has been shown that p38 is involved in interleukin 6 and 8 synthesis by rheumatoid synovial fibroblasts [7]. p38 MAPK can also stabilize the mRNA of many proinflammatory cytokines, for example IL-6 mRNA. The downstream p38 substrate MAPKAP-2 mediates many regulatory effects of TNF-α [8]. The p38 MAP kinase pathway also influences the expression of adhesion molecules in endothelial cells and neoangiogenesis in rheumatoid synovium. It is also involved in the synthesis of prostaglandins and COX-2 activity [7].

Three isoforms of c-Jun-N-terminal kinase are involved in extracellular matrix regulation. Their main substrate, c-Jun, is a critical component of AP-1 transcription factor, which binds to the binding site in protease promoters and can induce metalloproteinase gene expression. Metalloproteinases are involved in the degradation of extracellular matrix. The other MAP kinases can also influence metalloproteinase production, but to a lesser extent, depending on the type of cells and stimulus [5, 9].

JNKs can also activate other transcription factors and contribute to the indication in many important proinflammatory cytokine genes, such as those encoding TNF-α, IFN-β, and IL-8 [6]. Factors initiating the transcription of c-Jun are controlled by JNK and p38 MAPK. JNK, apart from controlling the synthesis of MMP and cytokine expression, regulates T-cell differentiation into the T helper 1 (Th1) subset. As Th1 cells play an important role in RAs pathogenesis, targeting JNK can modify adaptive immune responses and suppress synovial lymphokine production in addition to blocking MMP synthesis by synoviocytes [9].

Activation of ERKs is caused by mitogens and growth factors, while JNKs and p38 MAP are activated by proinflammatory cytokines such as TNF-α and IL-1β and by stress factors such as ultraviolet light, irradiation, heat, and osmotic shock. These kinases act in pathways/cascades where phosphorylation of enzymes situated downstream results in their activation. This cascade reaction serves as an amplifying mechanism to enhance signal intensity. Acting upstream from MAP kinases are MAP kinase kinases (MAPKKs, MAPK2), which are in turn phosphorylated by MAP kinase kinase kinases (MAPKKKs, MK3). Their role, apart from amplifying the signal, is the integration of extracellular stimuli and maintaining the balance of the phosphorylation of the different kinases [5].

Proinflammatory cytokines such as TNF-α, IL-1, and IL-6 can quickly and temporarily activate p38 MAPK, JNK, and ERK, while IFNγ, IL-4, and IL-10 have no such effect [5]. Anti-inflammatory cytokines such as IL-10 suppress cytokine-mediated activation of inflammatory cells due to altered activating pathways of MAPK.

### Map Kinases in Rheumatoid Arthritis

All three families of MAP kinases are expressed in rheumatoid synovium in their active, phosphorylated forms. Although non-activated forms of ERKs, MAPKs, and JNKs are constitutively expressed in fibroblast-like synoviocytes (FLSs) in both rheumatoid arthritis and osteoarthritis, their cytokine-induced activation differs in both diseases and is more enhanced in RA. The phosphorylated form of p38 was detected mainly in the intimal layer lining the synovium, where most of the cytokines and proteases are produced. The phosphorylated isoform p38α was present in synovial macrophages and fibroblasts especially at sites of invasion into the extracellular matrix. Phosphorylated ERK has been detected in synovial blood vessels [8, 9]. The main substrate of JNK, c-jun, was detected together with AP-1 and MMP in the synovial intimal lining. It was shown that JNK isoforms are phosphorylated in RA but not in OA synovium [9]. MAPK pathways can lead to AP-1 activation and consequently to increased synthesis of matrix metalloproteinases (MMPs) and joint damage in rheumatoid arthritis. Apart from inducing MMP synthesis, kinases are important factors influencing inflammatory cell recruitment and angiogenesis via increasing the expressions of adhesion molecules [9].

### Kinase Inhibitors in Therapeutic Trials

As MAP kinases are involved in many ways in the pathogenesis of rheumatoid arthritis, they seem to be an attractive target for novel anti-rheumatic therapies. A potential target for new therapeutic strategies is p38 kinase, which plays a vital role in
the expressions of the main proinflammatory cytokines TNF-α and IL-1. This kinase has been shown to be expressed and activated in RA synovi- um. p38 inhibitors are effective in animal models of arthritis and block cytokine production by macrophages in vitro. p38 inhibitors used in animal models of arthritis, for example collagen-induced arthritis, have been shown not only to reduce inflammation by diminishing paw swelling, but also to suppress joint destruction. This is due to the combination of their indirect effect on cytokine expression and direct effect on metalloproteinase production. In humans, p38 inhibition diminished cytokine synthesis in response to endotoxin [8]. A group of orally bioavailable piridinyl imidazole compounds specifically inhibit p38 MAPK. They can competitively bind to the ATP terminal of both the active and inactive forms of the kinases. Their therapeutic function is based on suppressing the production of proinflammatory cytokines by inflammatory mononuclear cells and synovial fibroblasts. They act on a post-transcriptional level by reducing the nuclear translocation of NF-κB or cytokine mRNA. They can destabilize cytokine mRNA, which is especially the case with IL-6. In experimental models these drug compounds reduced the severity of arthritis. Their therapeutic action is achieved by reducing proinflammatory cytokine synthesis as well as angiogenesis and recruitment of proinflammatory cells to the synovium [5, 9]. The selective α/β p38 MAPK inhibitor RWJ 67657 has been shown to inhibit MMP3 and MMP1 synthesis and IL-6 and IL-8 production by rheumatoid synovial fibroblasts stimulated by TNF-α and IL-1β. This inhibition occurred at both the protein and mRNA levels. MMP3 is particularly important in RA pathogenesis as a main indicator of cartilage destruction [7].

Cartilage destruction in RA is mediated by proteolytic enzymes, including matrix metalloproteinases. Their synthesis in RA synoviocytes is regulated mainly by JNKs, which influence the MMP gene expressions. JNK2 is the main isoform constitutively expressed in RA synoviocytes. The inhibition of all three isoforms of JNK by the non-selective JNK inhibitor SP600125 in the rat adjuvant arthritis model resulted in significant protection of bone and cartilage destruction together with decreased AP-1 activation and collagenase-3 gene expression. The anti-inflammatory effect of SP600125 was relatively modest. Selective inhibition of only the JNK2 isoform was not so effective in preventing cartilage and bone damage [6, 8, 9]. The other orally bioavailable JNK inhibitor, AS601245, was efficacious in preclinical models. In collagen-induced arthritis it appeared to be not only a potent matrix-protecting agent, causing a decrease in cartilage erosions, but also a strong inhibitor of joint inflammation.

Although kinase inhibitors seem very effective in preclinical models of arthritis, their beneficial effects in humans are less comprehensive. The clinical data confirm that kinase inhibitors can reduce cytokine synthesis in humans, but their relevance in RA and other rheumatic diseases is uncertain. The results of clinical trial with a selective p38 inhibitor, VX-745, in RA showed only moderate benefit over placebo, but drug exposure was limited by significant hepatotoxicity. Another selective p38 inhibitor, VX-702, showed a modest response in ACR20%, only slightly better than placebo [9]. Its use was limited by adverse events associated with skin rash, infection, and gastrointestinal intolerance. The use of different p38 inhibitors is limited by significant preclinical and clinical toxicity. The important adverse effect is hepatotoxicity, which seems to be target based. The RWJ 67657 selective inhibitor of p38 isoforms α and β seems to be more promising, as its therapeutic effects were attained at lower concentrations and it has a better safety profile, warranting further studies [7].

There are no direct ERK inhibitors available, but there are some agents inhibiting kinases upstream or downstream from the ERK which are effective inhibitors of ERK activation. It was also shown that the activation of ERK in LPS-treated macrophages was dependent on the activation of IκB kinases (IKKs). Thus the therapeutic effect of IKK inhibitors in RA can be due to both inhibition of NFκB activation as well as ERK activation [8].

Although clinical trials with the novel MAP kinase inhibitors have not been very successful so far, it is worth mentioning that many classic anti-rheumatic drugs have been shown to influence MAP kinase pathways. Glucocorticoids repress the activity of transcription factors AP-1 and NF-κB by inhibition of the JNK signaling pathway. JNK activation by TNF-α and lipopolysaccharide (LPS) is inhibited by dexamethasone, which has no influence on the MAP p38 and ERKs pathways. Leflunomide inhibits TNF-α-induced activation of JNK and AP-1 [5].

**NF-κB Transcription Factor as a New Therapeutic Target**

The other attractive target for anti-rheumatic therapy is a signaling pathway associated with the transcription factor NF-κB. NF-κB is one of the pivotal regulators of proinflammatory gene
expression and induces the transcription of proinflammatory cytokines, chemokines, adhesion molecules, matrix metalloproteinases, cyclooxygenase 2, and inducible nitric oxide (iNOS). NF-κB is highly activated at sites of inflammation in diverse diseases, such as rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis, psoriasis, and asthma. Nuclear factor NF-κB is an essential transcription factor whose dysregulation has been linked to numerous diseases, including arthritis and cancer; the NF-κB activation pathway has thus become a major target for the development of novel therapies for these diseases. Drugs that selectively target inflammation-induced NF-κB activity while sparing the protective functions of the basal activity of NF-κB would be of greater therapeutic value [10].

NF-κB regulates a wide variety of genes, including those encoding cytokines (e.g. IL-1, IL-2, IL-6, IL-12, TNF-α, lymphotoxin α (LTα), LTβ, and granulocyte-macrophage colony-stimulating factor), chemokines (e.g. IL-8, macrophage inflammatory protein (MIP)-1α, monocyte chemoattractant protein (MCP)-1, regulated on activation normal T-cell-expressed and secreted (RANTES), and eotaxin), adhesion molecules (e.g. intercellular molecule-1, vascular cell adhesion molecule, E-selectin), acute-phase proteins (e.g. serum amyloid A), and inducible effector enzymes (e.g. inducible nitric oxide synthase and cyclooxygenase-2).

The chemokines and cytokines produced in response to NF-κB activation can stimulate the migration and maturation of lymphocytes. Furthermore, NF-κB is central to the proliferation and survival of cells mediating the immune response through its ability to activate genes coding for regulators of apoptosis and cell proliferation. This long list suggests that modulation of NF-κB activity represents an effective therapeutic strategy for combating diseases such as arthritis or other autoimmune diseases which result from dysregulation of otherwise beneficial immune responses. Consequently, there is interest in understanding the regulation of this transcriptional factor in the context of various diseases [11].

The mammalian NF-κB family has multiple members, including RelA (p65), NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), c-Rel, and RelB [10]. Of the different members of the Rel family, p65 (RelA) plays a particularly important role since the predominant form of inducible NF-κB in most cells is the p65:p50 heterodimer. As p50 does not have the ability to drive transcription, it is not surprising that most of the known regulatory events that impact NF-κB signaling actually target p65 [12]. Genes for all five members of the NF-κB family reveal distinct roles of the NF-κB proteins in the regulation of innate and adaptive immune responses, lymphocyte function, and cell survival.

NF-κB proteins are present in the cytoplasm in an inactive form through the association with inhibitory proteins called inhibitors of κB (IκBs), of which the most prominent are IκBα, IκBβ, and IκBε [13]. Cell stimulation with a variety of agonists triggers signal-transduction pathways that ultimately result in the activation of specific IκB kinases (IKKs) [11]. On activation by a plethora of inducers, the IκB proteins are phosphorylated, ubiquitinated, and subsequently degraded in the proteasomes (Fig. 1). This degradation allows the translocation of NF-κB into the nucleus and binding to its DNA binding sites, which regulates the transcription of genes, including those for antimicrobial peptides, cytokines, chemokines, stress-response proteins, and apoptotic proteins. Degradation of IκB is an essential step for the release of NF-κB and its subsequent activation.

The 700- to 900-kDa IKK complex consists of several proteins. Three of them are the kinases

![Fig. 1. Signaling pathway of NF-κB](image_url)
IKK1 and IKK2 (also called IKKα and IKKβ, respectively) and the regulatory subunit NF-κB essential modulator NEMO (also called IKKγ) [10]. IKKα and IKKβ are the catalytic subunits of the complex. The third subunit, IKKγ/NEMO, is the regulatory subunit and is not related to the catalytic subunits [11]. The IKK complex is a converging point for NF-κB activation by a large number of stimuli. IKK1 and IKK2 can phosphorylate all three known IkBs, namely IkBα, IkBβ, and IkBe. Although the biochemical functions of IKK1 and IKK2 in vitro appear very similar, genetic analysis has revealed their distinct functions in vivo.

Signaling to NF-κB in response to the ligation of surface receptors proceeds through either the “classical” or the “alternative” pathway. The classical pathway is primarily activated by inducers such as TNF-α, IL-1, and lipopolysaccharide (LPS). Activation of this pathway depends on the IKK holocomplex consisting of IKKα/IKKβ/NEMO, which phosphorylates IkBs to induce their degradation. This pathway is crucial for the activation of innate immunity and inflammation and for inhibition of apoptosis. The alternative pathway, on the other hand, is dependent on the protein kinase NF-κB-inducing kinase (NIK), which phosphorylates and activates complexes of IKKα homodimers [14]. This pathway is activated by LTβ, CD40L, and B-lymphocyte stimulator (Blys/BAFF), but not by TNF-α, IL-1, or LPS [11].

The third member of the IKK complex is NEMO, which contains no known intrinsic kinase activity but has the helix-loop-helix and leucine zipper motif known for protein-protein interaction. Mutation in genes encoding NEMO leads to mouse embryonic lethality due to massive hepatic apoptosis. Cells deficient in NEMO show no NF-κB activity in response to a variety of stimuli. The mechanism by which NEMO regulates the NF-κB pathway is poorly understood. It has been suggested that NEMO activates IKK by recruiting the complexes to the vicinity of the proteins, allowing upstream components to modulate IKK function. Alternatively, upon interacting with the components of upstream signal-transduction molecules such as receptor-interacting protein (RIP), NEMO undergoes oligomerization, which activates IKK [15]. Enforced oligomerization of NEMO can activate IKK [16, 17].

The activation of the IKK complex represents an essential regulatory step in all pathways leading to NF-κB activation. To better understand the mechanism by which the IKK complex is regulated, one group investigated the interaction between IKKβ and the NEMO subunit. These studies revealed that a very small region in the COOH-terminus of IKKα (L738-L743) and IKKβ (L370-L742) was essential for stable interaction with NEMO and for the assembly of the heteromeric IKK-NE-MO complex. This region was called the NEMO-binding domain (NBD). Sequence analysis demonstrated that the IKKβ COOH-terminus contains two segments that are homologous to IKKα: a serine-rich domain and a serine-free region. It was reasoned that the small size of the NBD might permit the design of peptides that could disrupt the interaction of NEMO with the IKKs [11, 16].

Therefore, cell-permeable NBD peptides were synthesized by fusing a peptide passing the NBD to a sequence from the Drosophila antennapedia protein that facilitates cellular uptake. Wild-type (WT) NBD peptides inhibited the interaction of IKKβ with NEMO in vitro and prevented the formation of endogenous IKK complex in HeLa cells. In contrast, mutant peptides (MUT) in which W739 and W741 were substituted with alanine were inactive. To investigate the effects of the peptides on NF-κB activation, HeLa cells were pretreated with either the wild-type or mutant peptides prior to stimulation with TNF-α. The wild-type NBD peptide inhibited NF-κB activation, whereas the mutant peptide had no effect. Interestingly, treatment with peptide alone (that is, without TNF-α) led to a modest (two- or threefold) activation of NF-κB. It is also important to note that the WT peptide did not completely inhibit NF-κB activity. This suggests that any drug developed to disrupt the interaction of NEMO and IKKβ will most likely leave residual NF-κB activity that might be sufficient to maintain normal cellular processes and prevent spontaneous apoptosis [11].

ELKS has been identified as an essential regulatory subunit of the IKK complex. Silencing ELKS expression by RNA interference blocked the induced expression of NF-κB target genes, including that of the NF-κB inhibitor IkBα and pro-inflammatory genes such as cyclooxygenase 2 and IL-8. These cells were also not protected from apoptosis in response to cytokines. ELKS recruits IkBα to the IKK complex and thus serves a regulatory function for IKK activation. Since ELKS has an essential role in the NF-κB signal-transduction cascade, it may be a suitable target for drug designs aimed at modulating NF-κB activation for disease intervention [10].

**NF-κB in Rheumatoid Arthritis**

Electrophoretic mobility shift assays and tissue-section staining with specific NF-κB antibodies consistently detect increased NF-κB activity with nuclear localization in synovial biopsies from...
patients with rheumatoid arthritis. These changes are accompanied by the enhanced recruitment of inflammatory cells and production of proinflammatory mediators such as IL-1, IL-6, IL-8, and TNF-α. Specific inhibition of NF-κB activity has consistently been shown to be effective in controlling inflammatory diseases in several animal models. Blocking NF-κB activity by overexpression of IκBα inhibits both the inflammatory response and tissue destruction in rheumatoid synovium. Administration of NF-κB decoys appears to be effective in animal models of rheumatoid arthritis [18]. Several drugs used to treat inflammatory diseases, from anti-IL-1 and anti-TNF-α therapy to widely used anti-inflammatory drugs such as corticosteroids, acetylsalicylic acid, and other non-steroidal anti-inflammatory drugs, have effects on NF-κB activity.

There have been approaches to develop selective inhibitors of NF-κB which affect different elements of the signaling pathway: directly targeting the DNA-binding activity of individual NF-κB proteins using small molecules of decoy oligonucleotides, blocking the nuclear translocation of NF-κB dimers by inhibiting the nuclear import system, stabilizing IκBα protein by developing ubiquitination and proteasome inhibitors, and targeting signaling kinases such as IKK using small-molecule inhibitors. All these therapeutic strategies are aimed at blocking NF-κB activity. With increasing knowledge of the signaling pathways leading to NF-κB activation, multiple targets can be identified for potential interaction with small molecules. From the upstream kinases, such as IKK1, IKK2, MEKK-3, and NIK, to their downstream effector IκB E3 protein, all represent attractive targets for novel drugs selectively regulating NF-κB function. Other components of the TNF-α and IL-1 signaling pathways, including TRADD, RIP, TRAF2, TRAF6, and IRAK as well as PKC isoforms and phosphoinositide 3-kinase, may provide additional targets for yet to be discovered inhibitors of NF-κB.

Although an attractive target for therapeutic intervention in inflammatory diseases, NF-κB is also involved in normal cellular physiology, such as the mounting of effective immune responses. Global inhibition may result in profound side effects. One of the most prevalent toxicities of NF-κB inhibition appears to be hepatotoxicity, at least during embryonic development. Even if NF-κB inhibition is well tolerated by adult liver, NF-κB blockade still compromises normal host defense and leaves mice unable to clear opportunistic infection such as that caused by Listeria monocytogenes. Additionally, involvement of NF-κB in the embryonic development of skin, limb, and bone poses potential dangers. By selectively targeting specific NF-κB subunits or signaling components involved in particular disease, one may minimize systemic toxicity. The identification of individual key components for a specific disease is crucial for achieving specific therapeutic aims [19].

The challenge now is to understand how different signal-transduction pathways selectively activate different NF-κB complexes in a coordinated manner. Analysis of downstream genes affected by NF-κB is also likely to provide significant insight into the functions of this pathway and could establish connections with other human diseases. In this respect, comparisons between normal mice and NF-κB mutants with the use of cDNA microarray or DNA chip technology may identify differentially expressed transcripts that are pertinent to NF-κB function in various physiological and pathological processes. A better understanding of the NF-κB signaling pathways involved in specific processes will be beneficial for the development of new generations of anti-inflammatory drugs with high efficacy, fewer side effects, and low cost [10].

References


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