NF-kB Signaling and an Active Target in Cancer Therapy

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ABSTRACT

NF-kB transcription factor regulates important cellular processes ranging from establishment of the immune and inflammatory responses to regulation of cell proliferation or apoptasis, through the induction of a large array of target genes. NFkB is increasingly recognized as a crucial player in many steps of cancer initiation and progression. Numerous studies have demonstrated that inhibition of NFkB by different means increased sensitivity of cancer cells to the apoptotic action of diverses effectors such as TNF alpha or chemo or radio therapies. This review focuses on the current knowledge on NFkB regulation and discusses the therapeutic potential of targeting NFkB in cancer therapy.

Keywords: NFkB, Cancer, Apoptosis, Cancer therapy, Transcription, Caspase, Anti-inflammatory, Oncoprotein, Oncogenesis, Inflammation.

1. INTRODUCTION

NFkB (Nuclear factor kappa light chain enhancer of activated B cells) is a protein complex that controls transcription of DNA. It was discovered in 1986 as a nuclear factor that binds to the enhancer element of the immunoglobulin kappa light chain of activated B cells (there by coining the abbreviation NF-kB) (1). NFkB is found in almost all cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL and bacterial or viral antigens. NFkB represents a family structured related eukaryotic nuclear transcription factor which modulates cell growth, cell survival, development process, immune and inflammatory responses as well as apoptosis. In total, five members of this transcription factor family have been identified, designated as p65 (Rel A), Rel B, c-Rel, NFkB1 & NFkB2. All five members of this protein family form homo or hetero dimers and share some structural features, including a Rel homologous domain (RHD), which is essential for dimerisation as well as binding to cognate DNA elements(2). NFkB is a hetero dimer with two basic sub units: the p50 & the RelA or p65. The negative feedback of the NFkB action is represented by the protein activated kinase IKK which blocks the action and effects of NFkB. NFkB has emerged as a ubiquitous factor involved in the regulation of numerous important processes as diverse as immune and

ISBN: 978-93-83083-79-4

inflammatory responses, apoptosis and cell proliferation. These last two properties explain the implication of NFkB in the tumorigenic process as well as the promise of a targeted therapeutic intervention. In the present study, we described the structure, regulation and functions of NFkB in first part, and in second part the NFkB signaling pathway in cancers, mechanism of NFkB activation in cancer, NFkB in human cancers and NFkB targeted drug therapies are discussed.

2. STRUCTURE OF NFKB

NFkB is a dimeric transcription factor formed by members of a family proteins that share conserved N-terminal dimerization/DNA-binding region designated the Rel homology domain (RH) In mammalian cells, the NFkB family is composed of five members: RelA(p65), RelB, c-Rel, p50/p105(NFkB1) and p52/p100(NFkB2).(3) The p105 and p100 proteins of the NFkB class are synthesized as precursors containing several C-terminal ankyrin repeats that are eliminated during maturation to respectively generate DNA binding competent mature p50 and p52 proteins. RelA/p65, c-Rel and RelB of the Rel class are directly synthesized as mature proteins and possess, in contrast to the NFkB class, a C terminal transactivation domain. Rel/NFkB proteins associate as homo or hetero dimers, except for RelB that only forms hetero dimers. The most common and studied dimer is the combination p50/p65 RelA which is involved in regulating transcription of many kB dependent genes. By contrast, p50/p50 or p52/p52 dimers are rather inhibitory due to the lack of a transactivation domain. p50 and p52 subunits provide DNA binding specificity by forming heterodimers with other NFkB subunits and are known to contribute to gene expression. The 3D structure of a p50/p65 dimer bound to DNA showed that NFkB proteins adopt a specific and unique conformation to recognize DNA using loops from both subunits and not alpha helixes like other transcription factors.(4) Each subunits contact one half of the pseudo-symmetric kB sites. The two halves (five bases) are separated by one base.

3. REGULATION OF NFKB ACTIVITY

NFkB is sequestered inactive in the cytoplasm by interaction with an inhibitory subunit of the IKB (Inhibitor of kB) family. This family is composed of six proteins: IKB-alpha, IKB-beta, IKB-epsilon, IKB-gamma, IKB-zeta, Bcl-3 which possess 5-7 ankyrin repeats that mediate their inhibitory function. IKB masks nuclear localisation signals (NLS)located on each NFkB subunit to prevent their nuclear translocation. IKB gamma corresponds to the ankyrin repeats containing C terminal part of p105 NFkB1 that act by auto inhibition and is eliminated during maturation. Bcl-3 is a peculiar member of the IKB family because it exerts a positive effect on gene transcription by displacing inactive p50/p50 or p52/p52 homodimers from DNA or through interaction with p52. IKBa/p50/p65 RelA are the most studied complexes. The IKBa protein can be divided into three parts: an N-terminal domain (SRD) that integrates activation signal, a central part bearing the

ISBN: 978-93-83083-79-4

ankyrin repeats involved in contact with and inhibition of NFkB subunits, a C-terminal PEST region, rich in proline, serine, threonine and gluts mic acid that regulate the constitutive half life of the molecule. Importantly, IKB not only interferes with nuclear translocation of NFkB but can also displace NFkB bound to DNA. (5)

In 1999, 150 different stimuli that can activate NFkB have been listed. Most of the NFkB inducing stimuli such as TNF-alpha, IL1, LPS and ionising radiations activate NFkB by modulating the activity of the IkB kinase (IKK) complex, which is comprised of three subunits: IKK alpha,IKK beta and NFkB essential modulator (NEMO) (IKK gamma). IKK alpha and IKK beta are both catalytic kinases, whereas NEMO act as a regulatory scaffold component for the IKK complex. Upon stimulation the IKK complex phosphorylates the IkB proteins, which then undergo rapid ubiquitination and protea some mediated degradation, which culminates in the release of the NFkB complexes from their inhibitory interaction. The released complexes then accumulate in the nucleus, where they bind to target DNA sequences and regulate the expression of genes involved in the immune responses, cell growth control and the regulation of cell survival. (6)

It should be noted that in addition to the well characterized mechanism of negative and positive regulation of NFkB described above, other pathways involving different kinases, such as NFkB inducing kinase (NIK) and IKK epsilon, can lead to NFkB activation through alternative pathways. (7)

4. NFKB FUNCTIONS

Since it's discovery in1986, NFkB has primarily been known for its regulatory role for immune and inflammatory responses. Its function has now been extended to the regulation of cell proliferation and survival and NFkB is considered as both an important player I the tumorigenic process and a potential therapeutic target in cancer.

NFkB in immune and inflammatory responses

Two main pathways are known to regulate immune responses: the NFkB pathway and the glucocorticoid (GR) pathway. While NFkB is stimulatory for the immune system and has pro-inflammatory properties, GR are immunosuppressive and anti-inflammatory. NFkB induces expression of genes coding for antigen receptors on immune cells, adhesion molecules, pro-inflammatory cytokines (TNF, IL1, LPS) or chemoattractants (MCP-1) for inflammatory cells. GR acts by suppressing NFkB activation through several mechanisms such as up-regulation of IKB gene expression. (8)

ISBN: 978-93-83083-79-4

NFkB and cell cycle regulation

Cell cycle and cell proliferation are interconnected in a delicate balance. NFkB mainly acts through the induction of the genes for cyclin D1 which is involved in the G1/S transition. The cyclin A promoter that lacks a canonical KB site can be activated by NFkB through an unknown mechanism.

NFkB pathway: a target for caspases

Caspases are the executioners of the cell death responses and destroy cell structure and functions through the cleavage of strategic substrates. The NFkB survival pathway is a substrate for caspases at different levels. The p50 and p65 proteins Re cleaved by caspase 3 and c-Rel has three cleavage sites for caspase 3. IKB is also a substrate for caspases that remove its most N-terminal region containing the two IKK specific phospho-acceptor serines, changing the inhibitor into super repressors protein. Upon cell death, IKK2 is cleaved and crippled by caspase 3 therefore interrupting NFkB dependent survival signals emanating from the TNF receptors. (9)

NFkB and mechanicm associated with cancer

Cancer associated mechanisms include the following: self-sufficiency in growth and loss of growth inhibitory mechanisms; suppression of apoptotic thresholds; enhanced angiogenic properties; and the ability to invade local tissue and metastasize to distant sites. Interestingly, NFkB pathway affects all these cellular processes in different ways.

Growth and proliferation

NFkB can promote cellular proliferation through regulation of specific target genes. For example, NFkB can promote Rb hyper phosphorylation by binding and activating the cyclin D1 promoter. Additionally, the IkB homolog Bcl-3 in association with p52 homo dimers has also been found to transactivate potently cyclin D1 gene. Furthermore, IKK alpha has been proposed to play a role in cyclin D1 transcription through a T-cell factor site in the promoter, via its ability to control beta-catenin phosphorylation. Other mechanisms whereby NFkB may potentially cancer cel growth is reported requirement for the upregualtion of HIF-1alpha and it's regulation of c-myc transcription. (10)

Apoptosis evasion

NFkB directly regulates a potent antipoptotic pathway. Genes regulated by NFkB that suppress apoptosis, such as Bcl-2 and Bcl-xL are often expressed in human cancers. Given the strong association between NFkB and the regulation of apoptosis, it is not surprising that many studies suggest that NFkB controls the antipoptotic mechanisms associated with oncogenesis. For

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instance,proliferation and survival of Hodgking/Reed- Sternberg cells is blocked when NFkB is inhibited by IkB alpha expression. Moreover, inhibition of NFkB in Hodgking/Reed- Sternberg cells leads to the loss of expression of antipopotic effectors A1/Bfl-1, cellular inhibitor of apoptosis 2 (c-IAP2), tumor necrosis factor receptor associated factor 1 and Bcl xL. Finally, another mechanism whereby NFkB may block cell death is through its ability to suppress persistent c-Jun NH2- terminal kinase(JNK) activation and the generation of reactive oxygen species (11).

Angiogenesis, invasion and metastasis

NFkB appears to play a role in each of these processes. In this regard, NFkB has been reported to promote both angiogenesis and metastasis in certain tumor models, potentially through the regulation of vascular endothelial growth factor (VEGF) and MMPs. In addition, expression of the super-repressors form of IkB alpha in human melanoma and ovarian cancer cell correlates with reduced VEGF and interleukin (IL)-8 expression and blocks growth, angiogenesis and metastasis of tumor xenografts. Moreover, NFkB inhibition by over expression of IkB beta in lung cancer cells suppressed their ability to form metastasis (12).

5. MECHANISM OF NFKB ACTIVATION: NORMAL CELLS TO CANCERS

Activation by oncoproteins

Many oncoproteins can activate NFkB as measured through reporter assays or through analysis of nuclear levels of NFkB DNA-binding activity . Interestingly, in immortalized murine fibroblasts, oncogenic H-Ras requires the NFkB subunits RelA and c-Rel for efficient cellular transformation. Another oncoprotein shown to activate anNFkB dependent reporter in Bcr-Abl, the fusion protein associated with chronic myelogenous leukemia (CML) (13).

Activation of NFkB by oncogenic associated pathways

Different signaling pathways known to play a role in oncogenesis activateNFkB. For example, PI3K/AKT dependent signaling activatesNFkB, this occurs in the manner dependent on the relative levels of the IKK alpha sub unit. In addition, AKT is activated in primary acute myeloid leukemia and this is associated with cell survival andNFkB activation (14).

Activation downstream of growth factors and growth factors receptors

Growth factor plays an important role in promoting oncogenesis; thus, it is not surprising that certain growth factors or expression of growth factor receptor can activateNFkB. Foe example, in certain cell typesNFkB can be activated by EGF. EGF can induce recruitment of RelA to the excitatory amino acid transporter (EAAT) 2/glutamate transporter promoter through a mechanism independent of IkB degradation (15).

ISBN: 978-93-83083-79-4

6. NFKB AS TARGET FOR CANCER THERAPY

With its role in initiation and progression of cancer, the NFkB signaling pathway is a potent node of pharmaceutical interference in the clinics. Since NFkB is also an essential player in the immune response against cancer, there had always been a reluctance to use NFkB inhibitors in the treatment of malignancies. Extensive evidence demonstrates that compounds which block NFkB activation can serve to block cancer cell growth.

Proteasome inhibitors

The proteasome regulates the turnover of a range of different proteins within the cell by promoting degradation if ubiquitinated proteins, including IkB family members. Therefore, proteasome inhibitors, which prevent degradation of IkB proteins, have been extensively used in cancer studies to block NFkB activity. A highly specific inhibitor of the proteasome is bortezomib/PS-341/VELCADE which is currently approved for the treatment of multiple myeloma. A newly developed proteasome inhibitor, NPI-0052 with properties distinct from bortezomib, also induces the death of multiple myeloma cells (16).

Thalidomide and analogs

Thalidomideis an active anti inflammatory drug that can blockNFkB activity and has been shown to have anti oncogenic properties. Thalidomide and immuno modulators thalidomide analogs have shown activity against relapsed or refractory multiple myeloma (17).

Non-steroid anti-inflammatory drugs

It was recently shown in vitro that the Cox-2 inhibitor celecoxib inhibitsNFkB activation induced by TNF through a mechanism that involved suppression of IKK and AKT activation. Interestingly, celecoxib can induce apoptosis in variety of leukemia cell lines in a manner that is correlated with the suppression of NFkB activation (18).

Herbal remedies

Licorice root extract, scientist discovered that a major component of licorice inhibitedNFkB and protected rat liver cells from alcohol toxicity. Capsaicin induces cell death in many cancers by modulating NFkB. It inhibits the growth of adult T-cell leukemia cells by impairing NFkB activation.

Clove extract (eugenol) inhibits NFkB-mediated expression of inflammatory cytokines. Eugenol inhibits NFkB activation in stimulated macro-phage immune cells, reducing their synthesis of COX-2 and inflammatory cytokines.

ISBN: 978-93-83083-79-4

Ginger extracts exert anti-inflammatory activity and stimulate cancer cell death by inhibiting NFkB. Ginger reduces expression of the key inflammatory enzymes COX-1 and COX-2.Topical application of ginger extract inhibits skin inflammation in a mouse model by inhibiting NFkB.

Garlic has now been shown to exert its anti-inflammatory and immunomodulatory effects by inhibiting NFkB. Garlic extracts lowered NFkB activity by up to 41% in human blood and kidney cells that had been exposed to an inflammation-provoking challenge, thus reducing the expression of certain cytokines (19).

7. CONCLUSION

An enormous amount of data strongly implicates transcription factor NFkB in a variety of oncogenic mechanisms. NFkB represents a central factor in inflammation, stress response, cell differentiation or proliferation as well as cell death. Not only NFkB promotes survival of cancer cells but it also contributes to abnormal proliferation and metastasis, which provides the key target for cancer therapy. The pharmaceutical industry is producing several inhibitors of the NFkB pathway that are going to be used in association with conventional therapies. In vitro data have established this concept and clinical trials are now in sight.

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