Lesson 9: **INFLAMMATION – MOLECULAR MECHANISM OF NF-κB ACTIVATION;**

**NF-κB AS THERAPEUTIC TARGET**

(P. Vandenabeele)

- The NF-κB family members
- The NF-κB inhibitor family members
- Structure and function of ankyrin repeats
- The canonical versus the proteolytic NF-κB activation
- Multiple ways to activate the IKK complex
- Regulation of IKK complex by Hsp90
- Transcriptional regulation of NF-κB
- Role of NF-κB in inflammatory diseases
- Role of NF-κB in tumorigenesis, lymphoid malignancies and carcinomas
- NF-κB pathways as therapeutic targets

**Article 11:**
NF-κB regulation in the immune system. Qiutang Li and Inder M. Verma. Nature Reviews Immunology 2, 725, 2002 *(activation of NF-κB)*

**Article 12:**
NF-κB in cancer: from innocent bystander to major culprit. Michael Karin, Yixue Cao, Florian Greeten and Zhi-Wei Li. Nature Reviews Cancer 2, 301, 2002 *(NF-κB and cancer)*

**Article 13:**
NF-κB REGULATION IN THE IMMUNE SYSTEM

Qiutang Li and Inder M. Verma

The nuclear factor-κB (NF-κB)/REL family of transcription factors has a central role in coordinating the expression of a wide variety of genes that control immune responses. There has been intense scientific activity in the NF-κB field owing to the involvement of these factors in the activation and regulation of key molecules that are associated with diseases ranging from inflammation to cancer. In this review, we focus on our current understanding of NF-κB regulation and its role in the immune system and inflammatory diseases. We also discuss the role of NF-κB proteins as potential therapeutic targets in clinical applications.

The nuclear factor-κB (NF-κB) family is a key player in controlling both innate and adaptive immunity. NF-κB proteins are present in the cytoplasm in association with inhibitory proteins that are known as inhibitors of NF-κB (IκBs). After activation by a large number of inducers, the IκB proteins become phosphorylated, ubiquitylated and, subsequently, degraded by the proteasome. The degradation of IκB allows NF-κB proteins to translocate to the nucleus and bind their cognate DNA binding sites to regulate the transcription of a large number of genes, including antimicrobial peptides, cytokines, chemokines, stress-response proteins and anti-apoptotic proteins. NF-κB activity is essential for lymphocyte survival and activation, and for mounting normal immune responses. The constitutive activation of NF-κB pathways is often associated with inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis (MS) and asthma. A better understanding of the regulation of NF-κB pathways will provide a platform for developing specific therapeutics for inflammatory diseases.

NF-κB proteins

As for most transcription-factor families, the mammalian NF-κB family has many members. They include RELA (p65), NF-κB1 (p50; p105), NF-κB2 (p52; p100), c-REL and RELB. These proteins have a structurally conserved amino-terminal 300-amino-acid region, which contains the dimerization, nuclear-localization and DNA-binding domains (FIG. 1). The c-REL, RELB and RELA proteins also have a carboxy-terminal non-homologous transactivation domain, which strongly activates transcription from NF-κB-binding sites in target genes. The other REL proteins, such as p50 homodimers, lack the transactivation domain, but they still bind to NF-κB consensus sites in DNA and, therefore, function as transcriptional repressors. The p50 and p52 proteins are generated by proteolytic processing of precursor p105 and p100 proteins, respectively. Each member of the NF-κB family, except for RELB, can form homodimers, as well as heterodimers with one another. The main activated form of NF-κB is a heterodimer of the p65 subunit associated with either a p50 or p52 subunit. Whereas p50 and p65 are expressed widely in various cell types, the expression of RELB is restricted to specific regions of the thymus, lymph nodes and Peyer’s patches. The expression of c-REL is confined to haematopoietic cells and lymphocytes. The transcription of RELB, c-REL and p105 is regulated by NF-κB.

Genes that encode all five members of the NF-κB family have been deleted by homologous recombination in mice (TABLE 1). These gene-knockout animal models indicate the distinct roles of the NF-κB proteins in the regulation of innate and adaptive immune responses, lymphocyte function and cell survival. Lack of the p65 subunit is embryonic lethal owing to liver degeneration; by contrast, mice that lack each of the other four members are immunodeficient without
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Figure 1 | Mammalian NF-κB- and IκB-family members. a | The nuclear factor-κB (NF-κB) family comprises five members: RELA (p65), c-REL, RELB, p105/p50 (NF-κB1) and p100/p52 (NF-κB2). They have a structurally conserved amino-terminal Rel-homology domain (RHD), which contains the dimerization, nuclear-localization (N) and DNA-binding domains. c-REL, RELB and RELA proteins also have a carboxy-terminal non-homologous transactivation domain (TD). RELB has an additional leucine-zipper motif (LZ). b | The inhibitor of NF-κB (IκB) family contains IκBα, IκBβ, IκBε (two transcripts) and BCL-3, and it is identified by the presence of many ankyrin (ANK) repeats. The amino-acid sequences of the sites of induced phosphorylation of IκBα, IκBβ and IκBε for their degradation are shown (DSGLDS, DSGLGS and DSGLEE, respectively). p105 and p100 contain RHDs at the amino terminus and ANK repeats at the carboxy terminus. Proteolytic processing of p105 and p100 at residues 435 and 405 (as indicated by arrows), respectively, generates the p50 and p52 NF-κB proteins. The glycine-rich region (GRR) and the carboxy-terminal sites of inducible phosphorylation (in the DS/V/CDS and EV/KED/SAYGS sequences for p105 and p100, respectively) are required for processing. Phosphorylation of RELA at Ser276, Ser529 and Ser536 is important for its transactivation activity. The size of each human protein is shown on the right (number of amino acids).

devolutional defects. Mice that lack more than one member of the NF-κB family — such as p50−/− RelB−/− and p50−/−p52−/− mice — have more severe phenotypes, which indicates that there is functional redundancy between the NF-κB-family members.

IκB proteins

NF-κB proteins exist in the cytoplasm in an inactive form, as a result of their association with the IκB proteins, of which the most common are IκBα, IκBβ and IκBε. These regulatory proteins are identified by the presence of many ankyrin repeats, a 33-amino-acid motif that mediates protein–protein interactions. Importantly, p100 and p105 NF-κB proteins contain similar ankyrin repeats and can function as IκB-like proteins. In these precursor proteins, the domain that contains the ankyrin repeats can be proteolytically cleaved and degraded. Another unusual member of the IκB family is BCL-3, which interacts specifically with p50 and p52 homodimers and can induce the expression of NF-κB-regulated genes. This is in contrast to the inhibitory function of the other IκB proteins.

The prevailing view is that IκB proteins retain NF-κB in the cytoplasm by masking nuclear-localization sequences (NLSs) on NF-κB subunits. However, recent studies have indicated that the cytoplasmic localization of the inactive NF-κB complexes is actually achieved by balancing continuous movement between the nuclear and cytoplasmic compartments. Structural and biochemical experiments have shown that only one of the two NLSs in an NF-κB dimer is masked by IκBα in an NF-κB–IκBα complex, which allows the complex to shuttle to the nucleus. At the same time, the nuclear-export signal (NES) that is located at the amino terminus of the IκBα protein is again recognized by the NES signal recognition receptor (NsrR) to expel the NF-κB–IκBα complex from the nucleus. As the export process is more efficient than the import process, nuclear localization of inactive NF-κB–IκBα complexes can be detected only when nuclear export is blocked by the inhibitor Leptomycin B. As for IκBε, NF-κB–IκBε complexes also shuttle actively between the nucleus and cytoplasm. The advantages of maintaining inactive NF-κB complexes in the cytoplasm by this energy-consuming shuttling process are currently unclear and require further investigation. By contrast, NF-κB–IκBβ complexes are retained in the cytoplasm owing to the masking of both NLSs on the NF-κB dimer by IκBβ. Furthermore, only IκBα, and not IκBβ, contains a functional NES at its amino terminus, which is essential for shuttling the NF-κB–IκBα complex out of the nucleus. The biological implications of the constant shuttling of NF-κB–IκBα complexes between cytoplasm and nucleus have not been determined yet.

It has been well documented that IκBε regulates transient NF-κB activation and that IκBβ maintains persistent NF-κB activation. Accordingly, IκBε is degraded rapidly in response to stimuli and quickly resynthesized, owing to the presence of an NF-κB response element in its promoter. The newly synthesized IκBε has an intrinsic NLS and, so, can enter the nucleus and displace NF-κB from its DNA binding sites and transport NF-κB back to the cytoplasm, thereby carrying out a post-induction repression of NF-κB function. By contrast, IκBβ is less sensitive to stimulus-induced degradation than IκBε. It has been proposed that the selective interaction between endogenous κB–Ras and IκBβ is crucial for inhibiting IκBβ degradation during NF-κB activation. Unlike IκBε, IκBβ does not have a functional NES and is not NF-κB inducible. Because resynthesized IκBε can interact with NF-κB complexes that are bound to the target promoters but, unlike IκBε, does not displace them, the outcome is a sustained NF-κB response.
### Table 1 | Phenotype of knockout mice for NF-κB signalling components

<table>
<thead>
<tr>
<th>Mutated gene product</th>
<th>Phenotype in knockout mice</th>
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<tbody>
<tr>
<td><strong>NF-κB family</strong></td>
<td></td>
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<tr>
<td>p65 (RelA)</td>
<td>Die at E15.5–E16.5; TNF-dependent liver apoptosis; defect in lymphocyte activation</td>
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<tr>
<td>NF-κB1 (both p105 and p65)</td>
<td>Survival to adult; defect in lymphocyte activation</td>
</tr>
<tr>
<td>NF-κB2 (both p100 and p62)</td>
<td>Survival to adult; no mature B cells and defect in lymphocyte activation; disruption of splenic and lymph-node architecture</td>
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<tr>
<td>RelB</td>
<td>Die postnatally from multi-organ inflammation; required for dendritic-cell development</td>
</tr>
<tr>
<td>c-Rel</td>
<td>No developmental defects; defects in lymphocyte and macrophage functions</td>
</tr>
<tr>
<td>p65 and NF-κB1</td>
<td>Die at E13.5–E14.5</td>
</tr>
<tr>
<td>NF-κB1 and NF-κB2</td>
<td>Die postnatally; lack mature B cells and osteoblasts</td>
</tr>
<tr>
<td>NF-κB1 and RelB</td>
<td>Die postnatally owing to immune deficiency</td>
</tr>
<tr>
<td><strong>IκB family</strong></td>
<td></td>
</tr>
<tr>
<td>IκBα</td>
<td>Die postnatally owing to immune deficiency, inflammatory dermatitis and granulocytosis; constitutive NF-κB activity increased in lymphocytes, but not in MEFs</td>
</tr>
<tr>
<td>IκBα and NF-κB1</td>
<td>Attenuated phenotype of IκBα-knockout mice</td>
</tr>
<tr>
<td>IκBβ Knock-in to IκBα knockout</td>
<td>Rescues the defects of IκBα-null mice</td>
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<tr>
<td>IκBε</td>
<td>No defect in NF-κB activation; lack severe immune defects</td>
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<tr>
<td>Bcl-3</td>
<td>Disrupted splenic architecture</td>
</tr>
<tr>
<td>NF-κB1ΔC (p105)</td>
<td>Die postnatally owing to immune deficiency</td>
</tr>
<tr>
<td>NF-κB2ΔC (p100)</td>
<td>Die postnatally owing to immune deficiency</td>
</tr>
<tr>
<td><strong>IKK complexes</strong></td>
<td></td>
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<tr>
<td>IKK1 (IKKα)</td>
<td>Defects in keratinocyte differentiation, bone and limb development and mammary epithelial proliferation; no mature B cells; impaired RANKL-induced NF-κB activation and NIK-induced p100 processing</td>
</tr>
<tr>
<td>IKK2 (IKKβ)</td>
<td>Die at E13.5–E14.5 owing to TNF-dependent liver apoptosis; impaired NF-κB activation by IL-1, TNF and LPS</td>
</tr>
<tr>
<td>IKK1 and IKK2</td>
<td>Die at E11.5–E12.5 owing to TNF-dependent liver apoptosis; no induced NF-κB activation in MEFs</td>
</tr>
<tr>
<td>NEMO (IKKγ)</td>
<td>Die at E11.5–E12.5 owing to TNF-dependent liver apoptosis; no induced NF-κB activation in MEFs</td>
</tr>
<tr>
<td><strong>Signalling components</strong></td>
<td></td>
</tr>
<tr>
<td>IKK-1</td>
<td>Impaired NF-κB activation induced by LPS, but not by TNF</td>
</tr>
<tr>
<td>TBK (NAK, T2K)</td>
<td>Die at E14.5 owing to TNF-dependent liver apoptosis; defective cytokine-induced expression of certain NF-κB target genes, but not IKK activation and IκB degradation</td>
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<tr>
<td>TRAF2</td>
<td>Die postnatally; mild effect on TNF-induced NF-κB activation</td>
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<tr>
<td>TRAF6</td>
<td>Die postnatally; osteoporosis and defective IL-1-, CD40- and LPS-mediated NF-κB activation</td>
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<tr>
<td>MEKK3</td>
<td>Die at E10.5–E11.0; required for IKK and NF-κB activation by TNF and functioning downstream of RIP and TRAF2</td>
</tr>
<tr>
<td>NIK</td>
<td>Survival to adult; lack lymph nodes and Peyer's patches; abnormal architecture of spleen and thymus; required for LTβ-induced processing of p100 and NF-κB activation</td>
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<tr>
<td>RIP</td>
<td>Die postnatally owing to immune deficiency; no TNF-induced NF-κB activation</td>
</tr>
<tr>
<td>IRAK1</td>
<td>Survival to adult; defect in NF-κB activation by IL-1 and TIR signalling</td>
</tr>
<tr>
<td>GSK3β</td>
<td>Die at E13.5–E14.5 with liver apoptosis; defect in NF-κB activation, but not IKK activation and IκB degradation</td>
</tr>
<tr>
<td>PKCζ</td>
<td>Survival to adult; defect in secondary lymphoid organs; defect in NF-κB activation, but not IKK activation and IκB degradation in MEFs</td>
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<tr>
<td>PKCθ</td>
<td>Survival to adult; reduced proliferation of peripheral T cells; impaired TCR-induced NF-κB activity in mature T cells</td>
</tr>
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AC, carboxy-terminal deletion; E, embryonic day; GSK3β, glycogen synthase kinase 3β; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL-1, interleukin-1; IRAK, IL-1-receptor-associated kinase; LPS, lipopolysaccharide; LTβ, lymphotoxin-β; MEF, mouse embryonic fibroblast; MEKK3, MAP/ERK kinase kinase 3; NAK, NF-κB activating kinase; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; PKC, protein kinase C; RANKL, receptor activator of NF-κB ligand; RIP, receptor-interacting serine/threonine kinase; TBK, TANK-binding kinase; TCR, T-cell receptor; TIR, Toll/IL-1 receptor; TNF, tumour-necrosis factor; TRAF, TNF-receptor-associated factor.
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Figure 2 | NF-κB activation pathways. Nuclear factor-κB (NF-κB) activity is stimulated by many pathways, including lipopolysaccharide (LPS), tumour-necrosis factor (TNF) and T-cell receptor (TCR) signalling. The inhibitor of NF-κB (IκB) kinase (IKK) complex — composed of the kinases IKK1 and IKK2 (also known as IκKα and IκKβ, respectively) and the regulatory subunit NF-κB essential modulator (NEMO; also known as IκKγ) — is a point of convergence for all three signalling pathways. a | LPS binding to Toll-like receptor 4 (TLR4)—CD14–MD-2 complexes activates an intracellular signalling cascade that involves the recruitment of MYD88 (myeloid differentiation primary response gene 88) and IRAK (interleukin-1 receptor-associated kinase). Activation of IRAK results in the phosphorylation of TNF-receptor-associated factor 6 (TRAF6), which might relay signals through the TAK1–TAB1–TAB2 complex to IKK complexes to activate the NF-κB pathway. b | TNF receptor 1 (TNFR1) engagement by TNF results in receptor trimerization and recruitment of the adaptor protein TRADD, which, in turn, interacts with the carboxyl terminus of TRAF2, an adaptor protein that has additional affinity for various downstream signalling proteins. MAP/ERK kinase kinase 3 (MEKK3) and receptor-interacting serine/threonine kinase (RIP) likely link TNF signalling to IKK activation. c | T-cell stimulation, in response to antigen-presenting cells or anti-TCR-CD3 antibodies, results in the rapid translocation of protein kinase Cθ (PKCθ) to the plasma membrane. The components that connect PKCθ to the IKK complex are poorly defined, but trimeric complexes of membrane-associated guanylate kinase homologue (MAGUK) and the mucosal-associated lymphoid tissue (MALT)–lymphoma-associated proteins BCL-10 and MALT1 have been implicated. ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; TAB1, TAB1-binding protein; TRADD, TNF receptor associated via death domain; β-TRCP, β-transducin repeat-containing protein; ZAP70, ζ-chain-associated protein kinase, 70 kDa.

Genetic studies have shown both distinct and redundant functions of IκBα, -β and -ε for controlling NF-κB activation (Table 1). A lack of IκBα resulted in elevated NF-κB activity in haematopoietic tissues, but not in mouse embryonic fibroblasts (MEFs)26. However, IκBε is still required for the post-induction repression of NF-κB activity in fibroblasts. Interestingly, replacement of IκBα with IκBβ restores the wild-type phenotype in IκBε-deficient mice, which indicates that IκBε and IκBβ have marked similarities in their biochemical activities27. Detailed studies of the kinetics of NF-κB activation in the absence of either individual or combined IκB proteins will determine the functions of each IκB more precisely. Before describing the mechanisms of degradation of IκB proteins in response to phosphorylation by IκB kinases (IKKs), we first describe the signalling pathways that lead to NF-κB activity.

NF-κB signalling pathways

NF-κB regulates both innate and adaptive immune responses. It is activated rapidly in response to a wide range of stimuli, including pathogens, stress signals and pro-inflammatory cytokines, such as tumour-necrosis factor (TNF) and interleukin-1 (IL-1). Many pathogens are recognized by specific pattern-recognition receptors (PRRs) that have evolved to recognize pathogen-derived substances, such as lipopolysaccharide (LPS), peptidoglycans, lipoproteins, unmethylated bacterial DNA and double-stranded RNA28. The best known PRRs are the Toll-like receptors (TLRs), a group of transmembrane proteins that mediate the activation of intracellular signalling pathways after recognizing extracellular pathogens directly or indirectly. So far, ten members (TLR1–TLR10) of the TLR family have been reported in mammalian cells. Two of these, TLR2 and TLR4, have been shown to be essential for the recognition of distinct bacterial cell-wall components29,31. TLR2 recognizes peptidoglycan, lipoprotein, lipolipidmannnan and zymosan, whereas TLR4 recognizes LPS and lipoteichoic acid32. Biochemical and genetic data show that LPS binds to TLR4, CD14 and MD-2 complexes on the cell surface and activates an intracellular signalling cascade through the TLR cytoplasmic Toll/IL-1 receptor (TIR)-homology domain. The binding of LPS to TLRs results in the recruitment of MYD88 (myeloid differentiation primary response gene 88) through the homophilic interaction of TIR-homology domains in TLR4 and MYD88, whereas the amino-terminal death domain of MYD88 interacts with IL-1 receptor-associated kinase (IRAK) (Fig. 2). Subsequent to the activation of IRAK, another adaptor protein — TNF-receptor-associated factor 6 (TRAF6) — is phosphorylated and recruited to IRAK to activate the NF-κB pathway. The complex of transforming-growth-factor-β-activated kinase 1 (TAK1), TAK1-binding protein 1 (TAB1) and TAB2 was thought to be an integral component that relays the signals to IKK complexes downstream of TRAF6 (Ref. 20), but recent results from TAK1- and TAB2-deficient mice do not support a role for these proteins in NF-κB signalling (S. Akira, personal communication).
NF-κB activation by cytokines. Another important class of NF-κB inducers comprises various cytokines, such as TNF and IL-1. These cytokines are produced mainly by activated macrophages and monocytes, and they participate in lymphocyte and leukocyte activation. TNF and IL-1 activate signalling cascades that lead to the activation of activator protein 1 (AP1) and NF-κB transcription factors, which regulate the transcription of pro-inflammatory cytokine genes. IL-1 activates NF-κB in a similar manner to LPS because of homology between the cytoplasmic signalling domains of the IL-1 receptor (IL-1R) and TLRs (Fig. 2). TNF receptors are present on the surface of a wide range of cells. Receptor engagement by TNF results in receptor trimerization and recruitment of the adaptor protein TRADD (TNF receptor associated death domain) to the cytoplasmic receptor tail. In turn, TRADD interacts with the carboxyl terminus of TRAF2, an adaptor protein that has affinity for various downstream signalling proteins. Mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase kinase 3 (MEKK3) and receptor-interacting serine/threonine kinase (RIP) are likely to have a key role in linking TNF to the activation of IKKs.

NF-κB induction by TCR activation. T-cell receptor (TCR)-induced activation of NF-κB in peripheral T cells requires a co-stimulatory signal delivered from CD28 in addition to TCR signalling, and it involves the activation of protein kinase C (PKC) and IKK complexes (IKK1 and IKK2) (Fig. 2). PKC translocates rapidly to the plasma membrane of T cells in response to stimulation by antigen-presenting cells or anti-TCR-CD3 antibodies. The essential role of PKC in TCR-induced NF-κB and AP1 activation is indicated by PKC-null knockout mice. In mature T cells from Pkcb−/− mice, neither AP1 nor NF-κB is activated through the TCR, which results in a deficiency in IL-2 production. So far, the signalling components that connect PKC and IKK2 have been poorly defined. Interestingly, trimeric complexes of membrane-associated guanylate kinase homologue (MAGUK) and the mucosal-associated lymphoid tissue (MALT)-lymphoma-associated proteins BCL-10 and MALT1 have been implicated in signalling from PKC0 to IKK complexes.

Regulation of NF-κB activation by IκB and IKKs. For most known stimuli, except ultraviolet radiation and hydrogen peroxide, the degradation of IκB is an essential step for releasing NF-κB and its subsequent activation. A crucial regulatory step in this process is the signal-induced phosphorylation of IκB at specific amino-terminal serine residues (Ser32 and Ser36 for IκBα), which is mediated by the IKK complex. The phosphorylated IκBα is then ubiquitylated at Lys21 and Lys22 by β-TRCP (β-transducin repeat-containing protein) — an F-box WD-repeat protein that is the receptor subunit of SCF-β-TRCP, a RING E3 protein — which targets it for degradation by the 26S proteasome, thereby releasing NF-κB dimers from the cytoplasmic NF-κB–IκB complex and allowing them to translocate to the nucleus. In the past few years, ubiquitylation has attracted increasing attention in the NF-κB field as a result of its common involvement in the regulation of signalling pathways. Proteolysis-associated ubiquitylation is required not only for IκB degradation, but also for the processing of p100 and p105 NF-κB precursors. In addition, proteolysis-uncoupled ubiquitylation has been shown to be involved in the modification of signalling factors that might lie upstream of NF-κB, such as TRAF6 and TAK1–TAB1–TAB2 complexes. However, the importance of TAK1 ubiquitylation for TNF and IL-1 signalling is not supported by gene-targeting studies, because the loss of TAK1 or TAB2 has no effect on the TNF- and IL-1-induced activation of IKK and degradation of IκB (S. Akira, personal communication). The importance of proteolysis-uncoupled ubiquitylation in NF-κB signalling needs to be investigated further.

IκB kinases. The seminal event in the activation of NF-κB is the phosphorylation of IκB, which is mediated by IKKs. The 700–900 kDa IKK complex consists of several proteins — the main ones being IKK1 (IKKe), IKK2 and the regulatory subunit NF-κB essential modulator (NEMO; also known as IKKγ), which has no known intrinsic kinase activity but contains the helix-loop-helix and leucine-zipper motifs that are known to be involved in protein–protein interactions. The IKK complex is a converging point for the activation of NF-κB by a large number of stimuli. The importance of this complex in NF-κB activation is supported further by gene-targeting analysis. Without two IKKs or NEMO in MEFs, NF-κB activation is blocked after induction with various stimuli. IKK1 and IKK2 can phosphorylate all three known IκBs — IκBα, IκBβ and IκBγ — in vitro. Although the biochemical functions of IKK1 and IKK2 in vitro seem to be very similar, genetic analysis has shown that they have distinct in vivo functions. Similar to p65-knockout mice, IKK2-mutated mice die from liver apoptosis by day 13–14 post gestation. The Ikkα−/− phenotype is rescued by crossing with TNF receptor 1 (Tnfpr1)−/− mice, which indicates that the liver apoptosis is probably induced by TNF. The lack of IKK2 results in a marked decrease in IκB degradation and NF-κB activation. Surprisingly, keratinocyte differentiation is defective in IKK1-deficient mice, which is independent of both the kinase activity of IKK1 and NF-κB activity. In Ikk1−/− MEFs, IκBα phosphorylation and degradation induced by TNF and IL-1 is similar to that in wild-type MEFs. However, the DNA-binding activity of NF-κB and the induction of expression of certain NF-κB target genes by TNF are impaired. This indicates that IKK1 has a role in enhancing the transactivation function of NF-κB that is independent of IκB degradation. Recently, it was shown that IKK1 also functions as an IκB kinase and is essential for the degradation of IκBα and activation of NF-κB in RANKL (receptor activator of NF-κB ligand) signalling. So, IKK1 is a crucial signalling component of the NF-κB pathway in response to certain stimuli. Furthermore, IKK1 kinase activity is required for p100 precursor processing induced by NIK, which indicates that IKK1 has specificity for substrates other than IκBα.
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NEMO. As mentioned above, NEMO is an important member of the IKK complex. Mutation of NEMO is embryonic lethal in mice owing to massive hepatic apoptosis50. NEMO-deficient cells have no NF-κB activity downstream of various stimuli. NEMO is located on the X-chromosome, and mutations of this gene are associated with two human disorders — INCONTINENTIA PIGMENTI (IP) and ANHIDROTIC ECTODERMAL DYSPLASIA WITH IMMUNODEFICIENCY58,59. Similarly, heterozygous NEMO-mutant female mice develop granulocytic infiltration of the skin and hyperproliferative keratinocytes with increased apoptosis — the symptoms that are often present in IP patients60,61. At present, the mechanism by which NEMO regulates the NF-κB pathway remains poorly understood. It has been proposed that NEMO activates the IKK complex by recruiting it to the vicinity of other proteins, thereby allowing upstream components to modulate IKK function62. Alternatively, after interacting with components of upstream signal-transduction molecules such as RIP, NEMO might undergo oligomerization, thereby activating IKK63. Enforced oligomerization of NEMO can activate IKK64. In addition, NEMO might also be regulated by phosphorylation; for example, mutations of Ser85 and Ser141 of NEMO lead to attenuation of the phosphorylation of IKK2 and IkBα in response to TNF, IL-1 or LPS65,66.

Recently, two additional components of the IKK complex — cell-division cycle 37 (CDC37) and heat-shock protein 90 (HSP90) — have been identified47. Disruption of the interaction between CDC37/HSP90 and IKK complexes using geldanamycin impairs the activation of IKK and NF-κB. It alters the assembly of IKK complexes and prevents the recruitment of IKK complexes to TNFR147. It is not known whether CDC37 and HSP90 have any role in signalling in addition to their assembly function.

IKK homologues. Two IKK homologues — IKK-i (also known as IκKβ) and TBK1 (also known as T2K or NAK) — have been identified recently. Although they have some homology to IKK2, IKK-i and TBK1 apparently have distinct functions and are not components of IKK complexes68. IKK-i mediates LPS signalling, but not TNF signalling, in immune cells, as shown in gene-knockout animals (F. Mercurio, personal communications), which indicates that IKK-i has a role in a subset of the NF-κB pathways in certain cells. Like many NF-κB-pathway-mutant mice, TBK1-deficient mice die (at embryonic day 12.5) owing to liver apoptosis69. Although IKK activation and IkB degradation are normal, the induced expression of certain NF-κB target genes by TNF and IL-1β is inhibited in Tbk1−/− MEFs, which indicates that TBK1 regulates NF-κB activation independently of IkB degradation68.

IKK activation. The molecular mechanism by which the IKK complex is activated is still poorly defined. One possibility is that an upstream IKK kinase is activated by receptor activation, which, in turn, results in phosphorylation of IKK. Alternatively, a scaffolding protein might bring the IKK complex close to the receptor, resulting in a conformational change that induces IKK autophosphorylation.

Several potential upstream IKK kinases have been proposed, including MEKK1, MEKK2, MEKK3, NIK, TBK1, TPL2, TAK1 and PKCζ (an atypical PKC). They have all been shown to activate IKK, and some of them can phosphorylate the serine residues in the activation loop of IKK directly in vitro. However, the role of these proteins in NF-κB signalling requires further clarification, because knocking out MEKK1 (REFS 51,52), NIK63, TBK1 (REF 54), TPL2 (REF 55), TAK1 (S. Akira, personal communication) or PKCζ66 in vivo does not seem to alter the activation of IKK during TNF and IL-1 signalling in MEFs. Genetic studies have confirmed a requirement for MEKK3 for TNF-induced IKK activation and NF-κB activation67. Also, distinct from its role in MEFs, PKCζ is required for IKK activation in the lungs, where it is expressed abundantly68. The diverse nature of NF-κB inducers indicates the presence of different upstream activators and mechanisms of IKK activation.

Regulation of transcriptional activity of NF-κB

Traditionally, NF-κB activity has been considered to be regulated by signal-induced IkB degradation leading to NF-κB activation. However, accumulated evidence indicates that NF-κB activity is also regulated by the direct modification of NF-κB proteins through phosphorylation and, perhaps, acetylation (FIG. 3). Several investigators have shown that IL-1 and TNF induce phosphorylation and activation of the p65 NF-κB subunit by pathways that are distinct from those that lead to IkB degradation and NF-κB nuclear translocation. For example, phosphorylation of p65 Ser276 by the PKA catalytic subunit (PKAc) after IkBα degradation is essential for the efficient binding of p65 to the transcription co-activator CREB-binding protein (CBP)69. Similarly, signal-dependent phosphorylation of p65 Ser529 by casein kinase II (CKII) enhances its transcriptional activity70. Furthermore, phosphorylation of Ser529 and Ser536 of p65 by IKK2 has been shown to be required for the transcription function of p65 (REF 59).

In addition, genetic studies have shown that glycogen synthase kinase 3β (GSK3β)71,72, TBK1 (REF 50), IKK1 (REFS 33,34) and PKCζ66 are important for the control of NF-κB transcriptional activity — presumably by phosphorylation — but are not essential for IKK activation and IkB degradation. The loss of phosphorylation of p65 influences both its DNA-binding and transcriptional activities. Indeed, phosphorylation of the NF-κB p50 subunit in response to IL-1-stimulated phosphatidylinositol 3-kinase (PI3K)/AKT increases the DNA-binding activity of the NF-κB complex73. It is, therefore, becoming apparent that signal-induced phosphorylation of NF-κB is crucial for its function and might provide an additional means of regulation in response to diverse signals.

A main mechanism by which transcription factors regulate gene expression is to bring histone acetyltransferases (HATs) and histone deacetylases (HDACs) to
NF-κB phosphorylated, which is essential for its binding to CREB-binding protein (CBP) and replacing necrosis factor (TNF). p50 therefore repress transcription, whereas p50

IgM to IgG. another — for example, from

When B cells change their class

β

deacetylating p65 and enhancing the binding affinity between p65

activity and activate transcription. Furthermore, HDAC3 might help to switch off NF-

Ikk complexes phosphorylate IκBα, which leads to its degradation and allows nuclear factor-κB (NF-κB) dimers to enter the nucleus. During or after IκB degradation, p65 is phosphorylated, which is essential for its binding to CREB-binding protein (CBP) and replacing the p50–p50–HDAC1 complex and activating the transcription of target genes, such as IκBα, interleukin-2 (IL-2), granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumour-necrosis factor (TNF). p50–p50–HDAC1 complexes have histone-deacetylase activity and therefore repress transcription, whereas p50–p65–CBP complexes have histone-acetylase activity and activate transcription. Furthermore, HDAC3 might help to switch off NF-κB activity by deacetylating p65 and enhancing the binding affinity between p65–p50 and IκBα. Kinases such as protein kinase A catalytic subunit (PKA), casein kinase II (CKII), glycogen synthase kinase 3β (GSK3β), TBK1, IKK1/2, PKCζ and NIK might be involved in p65 phosphorylation. HDAC, histone deacetylase, IκBα, inhibitor of NF-κB; IκB, IκBα kinase; NEMO, NF-κB essential modulator; β-TRCP, β-transducin repeat-containing protein.

Figure 3 | A model of how NF-κB phosphorylation regulates its transactivation function. After induction, IκK complexes phosphorylate IκBα, which leads to its degradation and allows nuclear factor-κB (NF-κB) dimers to enter the nucleus. During or after IκBα degradation, p65 is phosphorylated, which is essential for its binding to CREB-binding protein (CBP) and replacing the p50–p50–HDAC1 complex and activating the transcription of target genes, such as IκBα, interleukin-2 (IL-2), granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumour-necrosis factor (TNF). p50–p50–HDAC1 complexes have histone-deacetylase activity and therefore repress transcription, whereas p50–p65–CBP complexes have histone-acetylase activity and activate transcription. Furthermore, HDAC3 might help to switch off NF-κB activity by deacetylating p65 and enhancing the binding affinity between p65–p50 and IκBα. Kinases such as protein kinase A catalytic subunit (PKA), casein kinase II (CKII), glycogen synthase kinase 3β (GSK3β), TBK1, IKK1/2, PKCζ and NIK might be involved in p65 phosphorylation. HDAC, histone deacetylase, IκBα, inhibitor of NF-κB; IκB, IκBα kinase; NEMO, NF-κB essential modulator; β-TRCP, β-transducin repeat-containing protein.

**NF-κB and adaptive immunity**

Clearly, NF-κB function is required for the rapid induction of expression of acute-phase antimicrobial defence genes in response to invading pathogens. Often, aberrant NF-κB activity in mice and humans is associated with susceptibility to microbial infection. But, NF-κB also has an important role in the development of adaptive immunity. Mice that lack individual NF-κB proteins have defects in B- and T-cell proliferation, activation, cytokine production and isotype switching (for B cells), although no important defects in B- and T-cell development are observed, probably owing to the functional redundancy between the family members. The elimination of both p65 and p50 has indicated the function of NF-κB proteins in lymphopoiesis. Similar observations have been made recently in Ikk2−/− reconstituted chimaeras. Interestingly, the blockade of lymphopoiesis in Ikk2−/−mutant mice has been shown to be TNFR1-dependent, which indicates that the anti-apoptotic effect of NF-κB is required during early lymphocyte development.

The use of different genetic/experimental approaches has revealed an important role for NF-κB in T-cell development and function. For example, T cells from transgenic mice that express IκBαM (a mutant IκBα that cannot be degraded) under the control of a T-cell-specific promoter have markedly impaired proliferative responses. Although the precise mechanism of these proliferative defects is not known, recent studies have shown that T cells that express IκBαM cannot activate signal transducer and activator of transcription 5a (STAT5a), a transcription factor that is required for T-cell proliferation induced by IL-2 and IL-4 (REF. 76).
In addition, the development of CD8+ T-cell responses requires p65 (REF 77). Similarly, inhibitors of NF-κB activation have been shown to block the maturation of dendritic cells32. The expression of B7-H, a new costimulatory homologue of B7-1 (CD80) and B7-2 (CD86), also requires NF-κB.

T-cell responses can be divided broadly into T helper 1 (Th1) and T helper 2 (Th2) responses (associated with cellular immunity) and Tγ2 responses (associated with humoral immunity). NF-κB is involved in the production of IL-18 and interferon-γ (IFN-γ), which are required for the function of Th1 cells33. T cells that express IκBxM have reduced production of IFN-γ after TCR stimulation34,35. Therefore, it is clear that NF-κB activity is essential for the development of Th1-type responses. The role of NF-κB proteins in the regulation of Th2-type responses is less well explored.

Various B-cell defects that involve NF-κB proteins — ranging from a lack of immunoglobulin class switching, lack of germinal centres or disruption of splenic microarchitecture leading to B-cell abnormalities — have been described. NF-κB regulates B-cell maturation at later stages than it does T-cell maturation. For example, mice that lack both NF-κB1 and NF-κB2 do not have mature B cells36 (TABLE 1). Similarly, the deletion of NIK or IKK1 results in defects in B-cell maturation, but not in early B-cell development37,38,39. So, NF-κB has diverse roles in regulating lymphocyte development. The current consensus is that NF-κB proteins regulate lymphocyte development through effects on proliferation, protection from TNF-induced apoptosis and the regulation of expression of anti-apoptotic genes74,75.

**NF-κB and inflammatory diseases**

NF-κB is one of the pivotal regulators of pro-inflammatory gene expression and it induces the transcription of pro-inflammatory cytokines, chemokines, adhesion molecules, matrix metalloproteinases (MMPs), cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS)30,31. NF-κB is highly activated at sites of inflammation in diverse diseases, such as rheumatoid arthritis, inflammatory bowel diseases, MS, psoriasis and asthma. ELECTROPHORETIC MOBILITY-SHIFT ASSAYS and tissue-section staining with NF-κB-specific antibodies consistently detect increased NF-κB activity with nuclear localization in biopsies from these patients. These changes are accompanied by the enhanced recruitment of inflammatory cells and production of pro-inflammatory mediators, such as IL-1, IL-6, IL-8 and TNF. It is unclear whether increases in pro-inflammatory cytokine production are the cause or result of NF-κB activation. Although genetic alterations of NF-κB and IκB themselves have not yet been reported to be associated with these diseases, aberrant, constitutive NF-κB activity could be caused by defects in the regulatory mechanisms that control NF-κB activation.

The pathogenic effects of NF-κB overactivation in inflammatory diseases are indicated by studies of p50- and c-Rel-knockout mice, which do not develop eosinophilic airway inflammation when sensitized and challenged with allergen ovalbumin32,33. Specific inhibition of NF-κB activity has been shown consistently to be effective at controlling inflammatory diseases in several animal models. Blocking NF-κB activity by the overexpression of IκBx inhibits both the inflammatory response and tissue destruction in rheumatoid synovium44. Administration of NF-κB decoys seems to be effective in animal models of rheumatoid arthritis35.

**Clinical application of NF-κB inhibitors**

Several drugs that are used to treat inflammatory diseases have effects on NF-κB activity46. These range from anti-IL-1 and anti-TNF therapy to widely used anti-inflammatory drugs, such as corticosteroids, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Although these drugs do not target NF-κB specifically, at least some of their effects are due to the inhibition of NF-κB activation46.

Many pharmaceutical companies have programmes to develop selective inhibitors of NF-κB, which include directly targeting the DNA-binding activity of individual NF-κB proteins using small molecules or decoy oligonucleotides; blocking the nuclear translocation of NF-κB dimers by inhibiting the nuclear import system; stabilizing IκBx protein by developing ubiquitylation and proteasome inhibitors; and targeting signalling kinases such as IKK using small-molecule inhibitors. All of these therapeutic strategies are aimed at blocking NF-κB activity. With increasing knowledge of the signalling pathways that lead to NF-κB activation, many targets can be identified for potential interaction with small-molecule inhibitors. From upstream kinases, such as IKK1, IKK2, MEKK3 and NIK, to their downstream effector IκB ubiquitin E3 ligase47, all are attractive targets for the discovery of drugs that selectively regulate NF-κB function. Other components of the TNF and IL-1 signalling pathways — including TRADD, RIP, TRAF2, TRAF6 and IRAK, as well as PKC isoforms and PI3K — might provide additional targets for the discovery of inhibitors of NF-κB.

Although it is an attractive target for therapeutic intervention in inflammatory diseases, NF-κB is also involved in normal cellular physiology, such as mounting effective immune responses. The global inhibition of NF-κB might result in serious side effects, including hepatotoxicity, at least during embryonic development. Even if NF-κB inhibition is well tolerated in adult liver, NF-κB blockade still compromises normal host defences and leaves mice unable to clear opportunistic infections, such as with *Listeria monocytogenes*31. In addition, the involvement of NF-κB in the embryonic development of skin, limbs and bones poses potential dangers. By selectively targeting specific NF-κB subunits or signalling components that are involved in a particular disease, it might be possible to minimize systemic toxicity. Identifying individual key components for a specific disease is crucial to develop specific therapeutics. It has been shown that IKK2 is important in rheumatoid-arthritis synoviocytes, whereas p65 is associated with inflammatory bowel disease48,49. Furthermore, c-REL is required for systemic, but not local, joint disease, whereas p50 is essential for local joint inflammation and destruction50.
Conclusion

The challenge now is to understand how different signal-transduction pathways selectively activate different NF-κB complexes in a coordinated manner. Analysis of the downstream genes that are regulated by NF-κB is also likely to provide important insights into the function of this pathway and could establish connections to other human diseases. In this respect, comparisons between normal mice and NF-κB mutants using complementary-DNA microarrays or DNA-chip technology might identify differentially expressed transcripts that are relevant to NF-κB function in the various physiological and pathological processes. No doubt, a better understanding of the NF-κB signalling pathways that are involved in specific processes will be important for the development of new generations of anti-inflammatory drugs that have high efficacy, fewer side effects and lower costs.

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Nuclear factor of κB (NF-κB) is not a single protein, but a small menagerie of closely related protein dimers that bind a common sequence motif known as the κB site. The molecular identification of its p50 subunit as a member of the reticuloendotheliosis (REL) family provided the first evidence that linked NF-κB to cancer, as v-REL is an oncoprotein of the REL retrovirus (REV-T).

The REL proteins belong to two classes, which are distinguishable by their mode of synthesis and trans-activation properties. One class consists of RELA (also known as p65), RELB and c-REL — proteins that are synthesized in their mature forms. These proteins contain an amino-terminal REL homology domain (RHD) that is required for dimerization and DNA binding, and transcription-modulating domains at their carboxy terminus. The second class consists of NF-κB1 (also known as p105) and NF-κB2 (also known as p100), which are synthesized as large precursors (p105 and p100) with an N-terminal RHD and a C-terminal series of ANKyrin REPEATs. Ubiquitin-dependent proteolytic processing removes this C-terminal domain, resulting in production of the mature DNA-binding proteins (p50 and p52). The final products contain the RHD, but lack transcription-modulating domains.

These proteins form various NF-κB homo- and heterodimers, the activity of which is regulated by two main pathways (Fig. 1). The first regulatory pathway — the canonical NF-κB activation pathway — applies to dimers that are composed of RELA, c-REL and p50, which are held captive in the cytoplasm by specific inhibitors that are known as the inhibitor of κB (IκB) proteins. IκB proteins consist of an N-terminal regulatory domain followed by a series of ankyrin repeats, similar to those present within the C-terminal portions of p100 and p105. The canonical pathway is normally triggered in response to microbial and viral infections and exposure to proinflammatory cytokines, all of which activate the IκB kinase (IKK) complex.

The second pathway affects NF-κB2, which preferentially dimerizes with RELB. This processing-dependent pathway is triggered by certain members of the tumour-necrosis factor (TNF) cytokine family that selectively activate the catalytic subunit IKKα, along with another protein kinase called NIK. Together, IKKα and NIK induce the phosphorylation-dependent proteolytic removal of the IκB-like C-terminal domain of NF-κB. This allows RELB–p52 dimers to translocate to the nucleus (Fig. 1).

Once in the nucleus, the transcriptional functions of NF-κB are further modulated by phosphorylation. Although each NF-κB dimer is likely to have distinct regulatory functions, many of the target genes are common to several, if not all, NF-κB proteins. These genes fall into four broad functional categories: immunoregulatory and inflammatory genes;
Summary

- Nuclear factor of κB (NF-κB) is a transcriptional regulator that is made up of different protein dimers that bind a common sequence motif known as the κB site.
- Although NF-κB target genes have been most intensely studied for their involvement in immunity and inflammation, this transcription factor also regulates cell proliferation, apoptosis and cell migration. Therefore, it is not surprising that NF-κB has been shown to be constitutively activated in several types of cancer cell.
- NF-κB activity is tightly controlled by several regulatory proteins, and disruption of this process has been associated with various haematological malignancies, as well as epithelial tumours such as breast cancer.
- A causal connection between inflammation and cancer has been suspected for many years. Because NF-κB becomes activated in response to inflammatory stimuli and its constitutive activation has been associated with cancer, NF-κB might also serve as the missing link between these two processes. Numerous inhibitors of NF-κB are therefore under development or have been developed.
- Because of the widespread importance of this factor, it has been difficult to develop NF-κB inhibitors that act specifically in cancer cells. Learning more about the complicated process of NF-κB regulation should lead to better therapeutic approaches to target the factor in specific cell types.

A role for NF-κB in tumorigenesis

According to Hanahan and Weinberg, tumorigenesis requires six essential alterations to normal cell physiology: self-sufficiency in growth signals; insensitivity to growth inhibition; evasion of apoptosis; immortalization; sustained angiogenesis; and tissue invasion and metatasis. NF-κB is able to induce several of these cellular alterations (Fig. 2), and has been shown to be constitutively activated in some types of cancer cell. There are several mechanisms by which NF-κB transcription factors are uncoupled from their normal modes of regulation, and these have been associated with cancer. For example, the avian REV-T oncovirus produces the constitutively active v-REL oncprotein, which causes rapidly progressing lymphomas and leukaemias. The TAX oncoprotein of human T-cell leukaemia virus (HTLV)-1 has been shown to directly interact with and constitutively activate the IKK complex, which results in the activation of both NF-κB signalling pathways. Other viral oncoproteins have also been shown to activate NF-κB by means of different mechanisms.

Cancer-associated chromosomal translocations, deletions and mutations might also disrupt genes that encode NF-κB and IκB proteins, uncoupling NF-κB factors from their regulators and causing constitutive NF-κB activation. Finally, autocrine and paracrine production of proinflammatory cytokines, oncogenic activation of upstream signalling molecules and chronic infections have been shown to persistently stimulate IκK activity, which leads to constitutive NF-κB activation. Constitutively activated NF-κB transcription factors have been associated with several aspects of tumorigenesis, including promoting cancer-cell proliferation, preventing apoptosis, and increasing a tumour’s angiogenic and metastatic potential.

Stimulating cell proliferation. NF-κB controls cell proliferation by activating target genes such as interleukin (IL)-2, granulocyte-macrophage colony-stimulating factor (GM-CSF) and CD40 ligand (CD40L), which encode growth factors that stimulate the proliferation of lymphoid and myeloid cells. Constitutive production of such cytokines can chronically stimulate cell proliferation in an autocrine or paracrine fashion. In addition to this indirect mode of action, NF-κB has also been shown to directly stimulate the transcription of genes that encode G1 cyclins. A κB site is present within the cyclin D1 promoter, and there is strong evidence that NF-κB-dependent cyclin D1 induction drives the proliferation of mammary epithelial cells during pregnancy.

Inhibition of apoptosis. NF-κB is also an inhibitor of programmed cell death. This factor activates the transcription of several target genes that are known to block the induction of apoptosis by TNF-α and other pro-apoptotic members of this family. The anti-apoptotic factors that are induced by NF-κB include cellular inhibitors of apoptosis (cIAPs), caspase-8/FADD (FAS-associated death domain)-like IL-1β-converting enzyme (FLICE) inhibitory protein (c-FLIP) and members of the BCL2 family (such as A1/BFL1 and BCL-XL). NF-κB can also attenuate the apoptotic response to genotoxic anticancer drugs and ionizing radiation. Tumour cells in which NF-κB is constitutively active are highly resistant to anticancer drugs or ionizing radiation, and inhibition of NF-κB activity in these cells greatly increases their sensitivity to such treatments. In addition to conferring resistance to cancer therapies, the anti-apoptotic activity of NF-κB can also have an important role in the emergence of neoplasms, by preventing the death of cells that have undergone chromosomal rearrangements or other types of DNA damage. Such cells are normally eliminated by means of checkpoint controls, such as the p53 pathway. In fact, there is evidence for transcriptional antagonism between NF-κB and p53 (Ref. 20). Regardless of mechanism, prevention of apoptosis increases the pool of genetically altered cells, which will eventually give rise to transformed progeny.
**Box 1 | NF-κB and IκB proteins**

There are five mammalian reticuloendotheliosis family (REL)/nuclear factor of κB (NF-κB) proteins that belong to two groups those that do not require proteolytic processing and those that do require proteolytic processing. The first group consists of RELA (also known as p65), c-REL and RELB. The second group includes NF-κB1 (also known as p105) and NF-κB2 (also known as p100), which are processed to produce the mature p50 and p52 proteins, respectively. These two groups dimerize — the most commonly detected NF-κB dimer is p50–RELA. Due to the presence of a strong transcripational activation domain, RELA is responsible for most of NF-κB transcriptional activity. p50–c-REL dimers are less abundant and seem to be activated with slower kinetics. Both p50–RELA and p50–c-REL dimers are regulated by interactions with inhibitor of κB (IκB: κB1 or B2), which target them for ubiquitination, are also present within the N-terminal regulatory domain. The RHD also contains, at its carboxyl terminus, an ankyrin repeat domain (ANKR), which mediates its binding to DNA. The RHD interferes with the function of the NLS. The IκB proteins include IκBα, IκBβ and IκBε, which contain multiple ankyrin repeats, which mediate their binding to Rel dimer. All IκBα contains 6–7 ankyrin repeats, which mediate their binding to RHDs. IκBα, IκBβ and IκBε contain an amino-terminal regulatory domain, within which there are two conserved serine residues (SS). Phosphorylation at this site targets the IκB proteins to ubiquitin-dependent degradation. Lysine residues, which are targets for polyubiquitylation, are also present within the N-terminal regulatory domain. The C-terminal halves of p105 and p100 are similar in sequence, structure and function to the IκBα, and the C-terminal portions of p105 and p100 prevent nuclear entry, and are removed by ubiquitin-dependent degradation. Whereas the processing of p105 is constitutive, the processing of p100 is regulated. GRR, glycine-rich region; LZ, leucine zipper. The arrows point to the C-terminal residues of p50 and p52 (following processing of p105 and p100, respectively).

**Increased metastasis and angiogenesis.** Another important component of tumour growth is angiogenesis — a process that requires both migratory and invasive capabilities of vascular epithelial cells. Chemokines — chemotactic factors that induce cell migration — are present in many regulatory genes. Rel proteins stimulate angiogenesis, possibly by inducing expression of IL-8 and vascular endothelial growth factor (VEGF).

NF-κB and lymphoid malignancies

Leukaemia and lymphoma — cancers of the bone marrow and lymphoid nodes, respectively — are induced by uncontrolled proliferation of blood cells. Given its importance in immune-cell function, it is not all that surprising that NF-κB is involved in the development of such cancers. Initial evidence that linked NF-κB to haematopoietic malignancies came from the v-Rel oncogene, which was shown to cause aggressive lymphomas and leukaemias in chickens. The transforming activity of v-Rel is much higher than that of its cellular homologue c-REL, due to accumulation of mutations that reduce the susceptibility of v-Rel to IκB inhibition, increase v-Rel stability and alter its DNA-binding properties.

Genetic alterations that affect the activity and expression of cellular NF-κB/REL proteins have also been linked to leukaemia and lymphomas. The human c-REL locus maps to 2p14–15 — a chromosomal region that is amplified in 23% of extranodal diffuse large B-cell lymphomas (DBCLs), as well as in other non-Hodgkin’s B-cell lymphomas. This results in a 4–35-fold increase in c-REL expression. Insertion of a promoter element into the c-REL locus, which increases its expression, has been detected in a lymphoid tumour cell line. However, no direct correlation between c-REL expression levels and the progression of lymphoid tumours, or their ability to express NF-κB target genes and resist apoptosis, has been made. The only direct evidence that elevated c-REL expression can result in lymphomagenesis is derived from its ability to transform primary chicken lymphoid cells in culture. In comparison to c-REL, amplifications or chromosomal rearrangements that affect the RELA locus, which encodes the p65 subunit of NF-κB, are rare. Genetic alterations of the RELB locus have been reported.

Conversely, chromosomal rearrangements that affect the NFKB2 locus at chromosomal region q10q24 have been associated with a variety of B- and T-cell lymphomas, including chronic lymphocytic leukaemia (CLL), multiple myeloma, T-cell lymphoma and cutaneous B- and T-cell lymphomas. Although they differ molecularly, all of these rearrangements or deletions result in removal of the C-terminal IκB-like sequences of p100 and constitutive production of p52. Interestingly, a targeted 3′ deletion within the mouse Nkβ2 locus, which also causes constitutive p52 production, results in lymphoid hyperplasia, but not cancer. So far, none of the lymphoma-derived NFKB2-encoded polypeptides have been shown to have oncogenic activity in vivo.
No rearrangements, amplifications or deletions of the human NFκB1 locus, located at 4q24, have been associated with leukaemia or lymphoma. However, BCL3, a gene that is located at chromosomal region 19q13.1 and that encodes an IkB family member, was originally cloned as a rearranged locus from a B-cell lymphocytic leukaemia (B-CLL). This translocation causes over-expression of BCL3. Unlike other IkB proteins, BCL3 associates with p50 or p52 homodimers in the nucleus to function as a transcriptional co-activator. Overexpression of Bcl3 in transgenic mice is sufficient to induce T-cell leukaemogenesis, but can cause splenomegaly when overexpressed by B cells. BCL3 probably acts in conjunction with other oncogenic mutations or DNA rearrangements to promote cancer.

DBCLs can be further classified into two subtypes — germinal-centre-like and activated-B-cell-like — on the basis of their gene-expression profiles. Congruent with their putative origin, DBCLs that display the activated B-cell phenotype have elevated expression of NFκB target genes, which encode cytokines, chemokines and anti-apoptotic proteins (L. M. Staudt, personal communication). Expression of a non-phosphorylatable IkB mutant — the so-called super-repressor — in these cells inhibits their proliferation (L. M. Staudt, personal communication). By contrast, DBCLs with a germinal-centre-like gene-expression profile seem to be resistant to the IkBα super-repressor. These results indicate that the canonical NFκB signalling pathway, which depends on the IkBα catalytic subunit and IkBα degradation, is constitutively active in the activated-B-cell-like DBCL cells. As the second NFκB signalling pathway, which depends on Iκκα and NFκB2/p100 processing, is involved in germinal-center formation, it would be interesting to determine whether it is constitutively activated in germinal-center-like DBCLs.

NFκB signalling pathways also seem to be involved in RNA and DNA virus-induced leukaemias and lymphomas. TAX, the transforming protein of HTLV-1, directly interacts with and activates IKKα. In addition, TAX can divert IKKα into new complexes that stimulate the phosphorylation-dependent processing of NFκB2/p100 (Ref. 8). So, TAX is capable of activating both the canonical and the processing-based NFκB activation pathways. NFκB activation has been shown to be essential for TAX-mediated transformation,

Epstein–Barr virus (EBV) — a DNA tumour virus that has been implicated in Burkitt’s and Hodgkin’s lymphomas, as well as in B-cell lymphomas in immunocompromised hosts — is also capable of inducing persistent NFκB activation. At least two EBV-encoded proteins — EBV nuclear antigen (EBNA)1 and latent membrane protein-1 (LMP-1) — increase both NFκB DNA binding and transcriptional activities by unknown mechanisms.

BCR–ABL is another oncoprotein — aet of cellular origin — that is capable of activating NFκB44. Although the tumorigenic mechanisms of BCR–ABL, which is associated with myelogenous leukaemia, are not well understood, its ability to activate NFκB has been proposed to be an essential component of its oncogenic activity. B-cell maturation antigen (BCMA) — a member of the TNF receptor (TNFR) family — was first identified as the product of t(4;18) in T-cell lymphoma cells. BCMA has been proposed to activate IKK, leading to constitutive NFκB activation. Similarly, chronic activation of another TNFR family

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**Figure 1** The IKK complex controls two distinct NFκB activation pathways. The inhibitor of κB (IkB) kinase (IKK) complex is composed of two catalytic subunits, IKKα and IKKβ, and one regulatory subunit, IKKγ. In response to stimuli such as tumour-necrosis-factor-α (TNF-α), COX-2, interleukin-1 (IL-1) or lipopolysaccharide (LPS), the IKKβ subunit is activated, and phosphorylates the κB proteins (bound to the NFκB heterodimers) at two conserved serines. This phosphorylation event triggers the ubiquitin-dependent degradation of κB by the 26S proteasome, resulting in the nuclear translocation of RELA–p50 (or c-REL–p50) heterodimers and transcriptional activation of target genes. In response to other stimuli, such as the TNF family members lymphotixin B (LTβ) and BAFF, IKKα is activated to induce the phosphorylation of p100 (bound to RELB) at two serine residues at its carboxyl terminus. This phosphorylation event triggers the ubiquitin-dependent degradation of the carboxy-terminal half of p100, releasing its amino-terminal half, the p52 polypeptide, which together with its heterodimer partner, RELB, translocates to the nucleus to activate transcription.

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**Figure 2** NFκB contributes to the induction of four classes of genes. The genes that are induced in the response to nuclear factor of κB (NFκB) activation can be divided into four functional classes: genes that have products that are involved in negative-feedback control of NFκB activity; genes that have products that serve various immunoregulatory functions; genes that have products that inhibit caspase activation and apoptosis; and genes that promote cell proliferation. COX2, cyclooxygenase-2; FLIP, FLICE (FAS-associated death domain (FADD)-like IL-1β-converting enzyme) inhibitory protein; cIAPs, cellular inhibitors of apoptosis; IkBα, inhibitor of κBα; IkBβ, inhibitor of κBβ; IκBα, inducible nitric oxide synthase.
NF-κB regulators

Inhibitor of κB (IκB) kinase (IKK) is a protein complex that is composed of three subunits: IKKα and IKKβ are catalytic (protein kinases) and IKKγ (NEMO) is regulatory. IKKα and IKKβ have protein kinase activity towards their substrates in vitro, but in intact cells, their activity and/or activation depend on the IκKγ subunit. IKKγ is a bifunctional protein that is required for the assembly of a high-order IKK complex, in which two IKKα and IKKβ homo- or heterodimers are held together by two or four IKKγ subunits. In addition, IKKγ is required for the activation of IKKα and IKKβ, which is mediated through their phosphorylation at two conserved serine residues that are located within their activation loops. Whereas IKKβ is required for activation of the canonical nuclear factor of κB (NF-κB) pathway that is based on the phosphorylation-induced degradation of IκBα, IKKβ is required for activation of a second NF-κB pathway that is based on the phosphorylation-induced processing of p100. The exact mechanisms by which IKKα and IKKβ are activated in response to extracellular stimuli are not clear, but it seems that IKKα and IKKβ are differentially regulated. The IKKγ-mediated p100 processing pathway is activated by two specific members of the TNF family: lymphotoxin B (LTβ) and BAFF (BLYS). The IKKγ-dependent, canonical NF-κB pathway is activated by many other stimuli, including TNF-α and most members of its family, IL-1, and various innate immune stimuli, such as lipopolysaccharide (LPS) and double-stranded RNA, which activate Toll-like receptors (TLRs).

Once the IκBα are phosphorylated at their N-terminal regulatory serines by the IKK complex, they are recognized by a second protein complex, E3IκB ligase. This complex consists of at least four subunits: CULLIN, SKP1, ROD, and the F-box protein β-TrCP. The latter is responsible for the ubiquitylation of p100 and p105, which is required for their processing. It is not known whether the ubiquitylation of p100 and p105, which is required for their processing, is dependent on the E3IκB ligase complex or a different ubiquitin ligase.

Apoptosis inhibitors

CD40, by autocrine production of its ligand, CD40L, has been linked to the genesis of CLL. A particularly interesting example of how continuous IKK activation can promote lymphoma development is the case of mucosa-associated lymphoid tissue (MALT) lymphomas. These lymphomas, which are associated with t(1;14) and t(11;18), are the most common subset of extranodal non-Hodgkin’s lymphoma. The t(1;14) moves the iκBα promoter upstream of the gene that encodes BCL10, which results in overexpression of a truncated BCL10 protein. Somewhat, overexpression of truncated BCL10 leads to constitutive NF-κB activation and, eventually, lymphoma. Loss of BCL10, by contrast, as shown in knockout mice, prevents IKK and NF-κB activation in lymphocytes in response to antigen receptor stimulation. The t(1;18) results in generation of a different fusion protein — API2-MALT1 (REF. 49) — which also activates NF-κB. In fact, expression of MALT1 alone can enhance the activation of NF-κB by BCL10 (REF. 50). So, MALT1 and BCL10 seem to be components of an IKK-dependent NF-κB activation pathway that is initiated by antigen receptor activation. The chromosomal translocations that are associated with MALT lymphoma create fusion proteins that induce constitutive activation of this pathway, which results in lymphoma.

In addition to EBV, other factors are thought to be involved in the persistent activation of NF-κB and IKK in the Reed–Sternberg (HRS) cells of Hodgkin’s lymphomas. Although much remains to be learnt about the exact mechanisms of IKK and NF-κB activation in HRS cells, inhibition of NF-κB has been shown to induce apoptosis in these cells. Similarly, inhibition of NF-κB causes the spontaneous apoptosis of EBV-transformed lymphoblastoid cells, Kaposi’s sarcoma-associated herpesvirus-associated lymphomas and B-CLL. These are further indications that pathogenic viruses constitutively activate this transcription factor to prevent host-cell apoptosis, leading to oncogenesis. Different mechanisms by which NF-κB can contribute to leukaemia and lymphogenesis are illustrated in FIG. 3.

NF-κB and breast cancer

NF-κB has also been shown to be involved in the development of carcinomas — cancers of epithelial
nulliparous

A female that has never borne offspring.

origin, such as breast cancer. Numerous studies have documented elevated or constitutive NF-κB DNA-binding activity both in mammary carcinoma cell lines and primary breast cancer cells of human and rodent origin. Almost all chemically induced rat mammary carcinomas have high levels of NF-κB activity, and in human breast cancer cells, NF-κB activity was reported to correlate with oestrogen independence. Subsequent studies have shown that NF-κB is activated in most human breast cancer cells, regardless of hormone-dependency status. Elevated NF-κB DNA-binding activity was also detected in mammary glands of carcinogen-treated rats within three weeks of exposure — well before the appearance of detectable tumours. This indicates that constitutive NF-κB activation might be one of the early events in breast cancer pathogenesis.

It is not clear, however, whether there is a difference in the composition of NF-κB between normal mammary epithelial cells, which primarily use NF-κB/p50–RELA(p65) heterodimers, and mammary carcinomas. High levels of NF-κB2 expression (both p100 and p52) were observed in normal mammary carcinoma cell lines and primary tumours. Although overexpression of NF-κB2/p100 inhibits NF-κB DNA-binding activity, the presence of elevated NF-κB activity in most breast cancers indicates that either the expression levels of NF-κB2/p100 are not very high, or that most of this protein is processed to p52. NF-κB2/p100 interacts with RELB, and elevated expression of RELB has also been reported in primary breast carcinomas. However, the same study also reported increased expression of c-REL and NF-κB1/p50.

Apart from increased expression of NF-κB components, the exact cause of elevated NF-κB activity in breast cancer is not known. The mechanisms that underlie the elevated expression of NF-κB family members in some cancer cells are also not clear. It is possible that oncoproteins that are known to be activated in breast cancer cells, such as ERBB2 (HER2/neu) or HRAS, trigger signalling cascades that lead to NF-κB activation.

Another possible mechanism for NF-κB upregulation in breast cancer cells has been revealed by a recent report of the importance of IKKα and NF-κB1 in mammary-gland development. NF-κB (primarily p50–RELA heterodimers) is activated during two phases of mouse mammary-gland development — pregnancy (peaking around days 15–16 post-coitus) and involution. Involution is the stage when most of the epithelial network regresses in size by means of apoptosis, and tissue is remodelled to that resembling a nulliparous female.

The activation of NF-κB during pregnancy is driven by a member of the TNF cytokine family known as receptor activator of NF-κB (RANK) ligand (RANKL). RANKL is produced by mammary epithelial cells during pregnancy in response to hormonal stimuli and acts in an autocrine manner through its receptor RANK, which is also expressed in the mammary epithelium. A deficiency in either RANKL or RANK arrests the mammary gland at the nulliparous stage, preventing the extensive lobuloalveolar proliferation that occurs during pregnancy. Unlike TNF receptor 1 (TNFR1), which signals to NF-κB by IKKβ, RANKL signals to NF-κB by IKKα. Interference with IKKα kinase activity, or a specific inhibition of NF-κB within the mammary epithelium, results in the same lobuloalveolar proliferative defect that is caused by RANKL or RANK deficiency.

Apparently, the only function of NF-κB in mammary epithelial cells during pregnancy is to stimulate cell proliferation by increasing transcription of the cyclin D1 gene. Maintenance of normal cyclin D1 levels by expression of a mammary-specific cyclin D1 transgene obliterates the requirement for IKKα or NF-κB. These studies have established the role of the IKKα-dependent RANK–NF-κB pathway in controlling the proliferation of the mammary epithelium during pregnancy (Fig. 4). These findings also raise the possibility that deregulated production of NF-κB in normal mammary epithelial cells may contribute to the normal lobuloalveolar proliferation during pregnancy.
RANKL, or constitutive RANK or IKKα activation, might underlie the elevation in NF-κB activity in breast cancer.

Unlike other cell types that express similar levels of all three G1 cyclins, mammary epithelial cells express mostly cyclin D1, and their proliferation is therefore highly dependent on this particular protein. The cyclin D1 promoter contains binding sites for several transcription factors, the activity of which is induced in response to extracellular stimuli, including AP1 and NF-κB. Whereas the NF-κB site is likely to be responsive to the RANK-generated signal, the AP1 site seems to be responsive to signals that are generated by receptor tyrosine kinases, such as ERBB2. Importantly, cyclin D1 expression is also required for breast carcinogenesis following mammary-specific expression of the ERBB2 or HRAS oncogenes. Future studies should examine whether transmisison of these oncogenic signals to the cyclin D1 promoter depends on IKKα and NF-κB, or whether it relies solely on activation of AP1.

Support for a possible involvement of IKK in breast carcinogenesis is provided by a study that showed elevated IKK activity in both breast carcinoma cell lines and primary tumors. Overexpression of catalytically inactive IKKα or IKKβ in such cell lines resulted in inhibition of NF-κB activity, loss of tumorigenic potential, and increased sensitivity to apoptosis-inducing anticancer drugs. It is not clear whether the inhibitory effect of the catalytically inactive IKKβ reflects its direct involvement in the tumorigenic process, or whether the overexpressed IKKβ mutant somehow interferes with IKKα activity.

Inflammation and cancer

A causal connection between inflammation and cancer has been suspected for many years. However, the mechanistic link between inflammation and tumorigenesis is not well understood. Because NF-κB becomes activated in response to inflammatory stimuli and its constitutive activation has been associated with cancer, NF-κB might be the missing link between these two processes. By virtue of its anti-apoptotic activity, the persistent activation of NF-κB that occurs during chronic inflammation or infection might prevent the elimination of genetically altered, precancerous cells. In addition, by stimulating the transcription of cyclin D1 and other G1 cyclins, constitutively active NF-κB might cause enhanced cell proliferation. In inflammatory cells, continuous NF-κB activity could promote the production of reactive oxygen species, thereby damaging DNA of surrounding epithelial cells. Some of the best circumstantial evidence that supports such a role for NF-κB comes from various gastrointestinal cancers.

One of the main risk factors that is linked to gastrointestinal cancer — the second most common malignancy worldwide — is Helicobacter pylori infection. Single-nucleotide polymorphisms in the IL1 gene, which are believed to increase its expression levels, are another risk factor for gastric cancer. Both IL1 and H. pylori are potent NF-κB activators. Not only can H. pylori activate NF-κB in gastric epithelial cells, but activated NF-κB was also found in cells of gastric biopsy specimens that were infected with H. pylori. The activation of NF-κB by H. pylori requires genes in the CAG PATHOGENICITY ISLAND. Once NF-κB is activated in gastric epithelial cells, it activates the transcription of IL-1, IL-6, IL-8, TNF-α, cyclooxygenase-2 (COX2) and, probably, other mediators of inflammation.

Knockout mice that lack the inhibitory C-terminal domain of NF-κB1/p100, and therefore constitutively express NF-κB2/p52, have dramatic hyperplasia of the gastric epithelium. So, in addition to promoting inflammation, NF-κB (probably p52–RELB heterodimers) can stimulate the proliferation of the gastric epithelium, and thereby increase susceptibility to carcinogenesis by ingested environmental and naturally occurring mutagens.
NF-κB activation is also associated with colorectal cancer. Colon cancer cell lines and human tumour samples, as well as nuclei of stromal macrophages in sporadic adenomatous polyps, were found to have increased NF-κB activity77,78. Most cases of colorectal cancer are sporadic, but 10–15% of cases are caused by hereditary syndromes, such as familial adenomatous polyposis (FAP) or hereditary non-polyposis cancer (HNPCC) and colitis-associated cancer79,80. Ulcerative colitis is a chronic inflammatory bowel disease (IBD), which, together with Crohn’s disease (another type of IBD), is associated with persistent NF-κB activation in tissue macrophages and epithelial cells of the colonic mucosa81,82. Crohn’s disease also increases the risk of tissue macrophages and epithelial cells of the colonic mucosa81,82. Downregulation of NF-κB expression in such mice, by use of antisense oligonucleotides, resulted in a significant reduction of IBD symptoms83,84. NF-κB inhibitors might, therefore, be useful in treating human IBD. Most of the drugs that are commonly used for treating acute IBD and for controlling remission, including sulphasalazine, mesalamine, glucocorticoids and methotrexate, were shown to inhibit NF-κB or IKK76–88. It is notable that most of these drugs act through different mechanisms and have different molecular targets, so their common denominator is an ability to inhibit NF-κB.

NF-κB inhibitors might also prevent progression to colorectal cancer by preventing expression of COX2 — another NF-κB target gene. COX2 is responsible for inducible prostaglandin synthesis during inflammation. The link between COX2 and colorectal cancer is supported strongly by epidemiological and experimental evidence. COX2 is overexpressed in colon adenomas and carcinomas of human and mouse origin89,90, and COX2-null mice are resistant to colorectal cancer91,92. Long-term consumption of aspirin or other COX inhibitors over a period of 10–15 years has been reported to reduce the relative risk of colorectal cancer by 40–50% (REFS 93,94). Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) also reduce the incidence of colorectal cancer in animal models95,96 and the risk of gastric cancer in humans97,98. Although such drugs inhibit both COX1 and COX2, the latter is the more relevant target for suppression of colorectal cancer99.

Given the ability of both aspirin and sulindac to inhibit IKK99,100, it is possible that some of their chemopreventive activity is derived from their ability to prevent NF-κB activation. Inhibition of IKK activity using sulindac sulphide was shown to induce the apoptosis of a colorectal cancer cell line101. Curcumin — another less potent and even less specific inhibitor of IKK — is another anti-inflammatory compound102. Curcumin has been shown to reduce colon carcinogenesis in several animal models103,104 and to inhibit the proliferation of colon cancer cells105. Its extensive consumption in the Indian subcontinent has been linked to low incidence of colorectal cancer106. Despite the large body of tantalizing circumstantial evidence, some direct (preferably genetic) evidence that NF-κB and IKK are involved in colorectal and gastric cancer pathogenesis is required.

Implications and future directions

An impressive body of evidence implicates NF-κB activation in the development of lymphoid-, myeloid- and epithelial-derived malignancies. However, certain aspects of NF-κB biology indicate that it can also have a tumour-suppressor function. Given the ability of NF-κB to activate innate and adaptive immune responses, its persistent activation in tumours seems paradoxical — its ability to stimulate the production of chemokines and cytokines would lead to recruitment of immune cells to the tumour and contribute to its rejection. If NF-κB does promote tumour development, it is likely that some of its immune and inflammation-related target genes might not always be activated. If a cancer cell is able to alter its profile of NF-κB target genes, this might allow a cancer cell to suppress apoptosis without expressing the chemokines and cytokines that broadcast its presence to the immune system.

Numerous inhibitors of NF-κB are under development and have been developed. Small molecules and viral vectors that inhibit IKK, or other aspects of the NF-κB activation pathway, have been shown to induce apoptosis and inhibit the proliferation of tumours or tumour-derived cell lines. Unfortunately, none of the small molecules have proven to be completely specific for IKK or NF-κB, and viral vectors are not yet practical for clinical applications. Clearly, more specific and potent inhibitors are needed.

Given the two distinct modes of NF-κB activation by the two catalytic subunits of the IKK complex, IKKα and IKKβ, selective inhibitors that target one subunit and not the other would be of great therapeutic and basic research value. Reagents such as these would help differentiate the exact biological roles of each pathway. Based on our current knowledge, we can speculate that IKKα-specific inhibitors might offer a selective therapy for germinal-center-derived DLBCL and, possibly, breast cancer, whereas IKKβ-specific inhibitors might be useful for inducing apoptosis in all types of tumours that have constitutively active NF-κB. An IKKα inhibitor, however, would also inhibit innate and adaptive immunity, and it would increase the sensitivity of many normal cells to TNF-α-induced apoptosis.

To investigate the role of NF-κB proteins in cancer development, we also need to further analyse the large collection of genetically altered mouse strains that carry deletions or other genetic alterations in genes that encode NF-κB, IκB and IKK components. The susceptibility of such mouse strains to a variety of cancers and cancer treatments needs to be examined. The identification of NF-κB target genes in different types of normal cell and their transformed derivatives is another important area for future research. As we begin to understand which genes are activated by NF-κB under different...
conditions, and which transcription factors and signalling pathways are involved in NF-κB activation, we should be able to design new therapeutic strategies that will allow the blocking of certain NF-κB target genes and not others. Furthermore, drugs that specifically disrupt post-translational modification of NF-κB subunits, and therefore inhibit only subsets of subunit-specific target genes, seem to be another attractive therapeutic option. However, little is known so far about the pathways that are responsible for these modifications, and some of them might have other substrates.

In the future, drugs might be designed to inhibit NF-κB activation of anti-apoptotic or pro-proliferation target genes without affecting the induction of genes that are required for immunity, or for protecting normal cells from killing by members of the TNF-α family. Given the rapid progress in understanding the mechanisms that are involved in activation of NF-κB and its function, what might seem to be a pipe dream today will become a reality in the next decade — in the form of a widely used anticancer drug.


68. NF-κB is activated in a mouse model of human Crohn's disease. Blocking p65 abrogates the signs of colitis.


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