

■ R E V I E W

Roles of NF- κ B in health and disease: mechanisms and therapeutic potential

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A B S T R A C T

The NF- κ B (nuclear factor κ B) family of transcription factors are involved in a myriad of activities, including the regulation of immune responses, maturation of immune cells, development of secondary lymphoid organs and osteoclastogenesis. Fine tuning by positive and negative regulators keeps the NF- κ B signalling pathway in check. Microbial products and genetic alterations in NF- κ B and other signalling pathway components can lead to deregulation of NF- κ B signalling in several human diseases, including cancers and chronic inflammatory disorders. NF- κ B-pathway-specific therapies are being actively investigated, and these hold promises as interventions of NF- κ B-related ailments.

INTRODUCTION

NF- κ B (nuclear factor κ B)/Rel is a family of ubiquitously expressed transcription factors, which bind a common DNA sequence called the κ B-binding site 5'-GGGRNWWYYCC-3' (where R is any purine, Y is any pyrimidine, N is any nucleotide, and W is adenine

or thymidine) [1]. The five members of the family can form different combinations of homo- or hetero-dimers to regulate a myriad of gene expression events. NF- κ B family members are divided into two groups based on their structural similarities and mode of inactivation. The first group consists of RelA (p65), RelB and c-Rel (REL). Proteins in this group have an N-terminal

Key words: cancer, inflammation, intracellular signalling, nuclear factor κ B (NF- κ B), Rel.

Abbreviations: ABC, activated B-cell-like; ALI, acute lung injury; API, apoptosis inhibitor; BAFF, B-cell-activating factor; BCR, B-cell receptor; CARD, caspase recruitment domain; CARMA, CARD-containing membrane-associated guanylate kinase protein; CBP, CREB (cAMP-response-element-binding protein)-binding protein; c-IAP, cellular inhibitor of apoptosis; CUEDC, CUE domain-containing protein; CYLD, cylindromatosis; DEN, diethylnitrosamine; DLBCL, diffuse large B-cell lymphoma; DUSP, dual-specificity phosphatase; ES, embryonic stem; GLUT, glucose transporter; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; IBD, inflammatory bowel disease; IKK, I κ B kinase; IL, interleukin; IL-1R, IL-1 receptor; ING, inhibitor of growth family member; IP, incontinentia pigmenti; IPD, invasive pneumococcal disease; IRAK, IL-1R-associated kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; LT- β , lymphotoxin- β ; MALT, mucosa-associated lymphatic tissue; CBM, CARMA1/Bcl-10/MALT1; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MEKK, MAPK/ERK (extracellular-signal-regulated kinase) kinase kinase; MM, multiple myeloma; MMP, matrix metalloproteinase; MSK, mitogen- and stress-activated kinase; MyD88, myeloid differentiation factor 88; NF- κ B, nuclear factor κ B; NEMO, NF- κ B essential modulator; NBD, NEMO-binding domain; I κ B, inhibitor of NF- κ B; NIK, NF- κ B-inducing kinase; oxPL, oxidized phospholipid; PKC, protein kinase C; PP1, protein phosphatase 1; PP2, protein phosphatase 2; RANK, receptor activator of NF- κ B; RANKL, RANK ligand; RHD, REL homology domain; RIP, receptor-interacting protein; RNAi, RNA interference; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; SHP, Src homology 2 domain-containing protein tyrosine phosphatase; siRNA, small interfering RNA; SNAP, soluble *N*-ethylmaleimide-sensitive fusion protein; STAT, signal transducer and activator of transcription; T2DM, Type 2 diabetes mellitus; TAK, TGF- β (transforming growth factor- β)-activated kinase; TCR, T-cell receptor; TIR, Toll/IL-1R; TLR, Toll-like receptor; TNF, tumour necrosis factor; TNFR, TNF receptor; TRAF, TNFR-associated factor; TRIF, TIR domain-containing adaptor protein inducing interferon- β ; TrCP, transducin repeat-containing protein.

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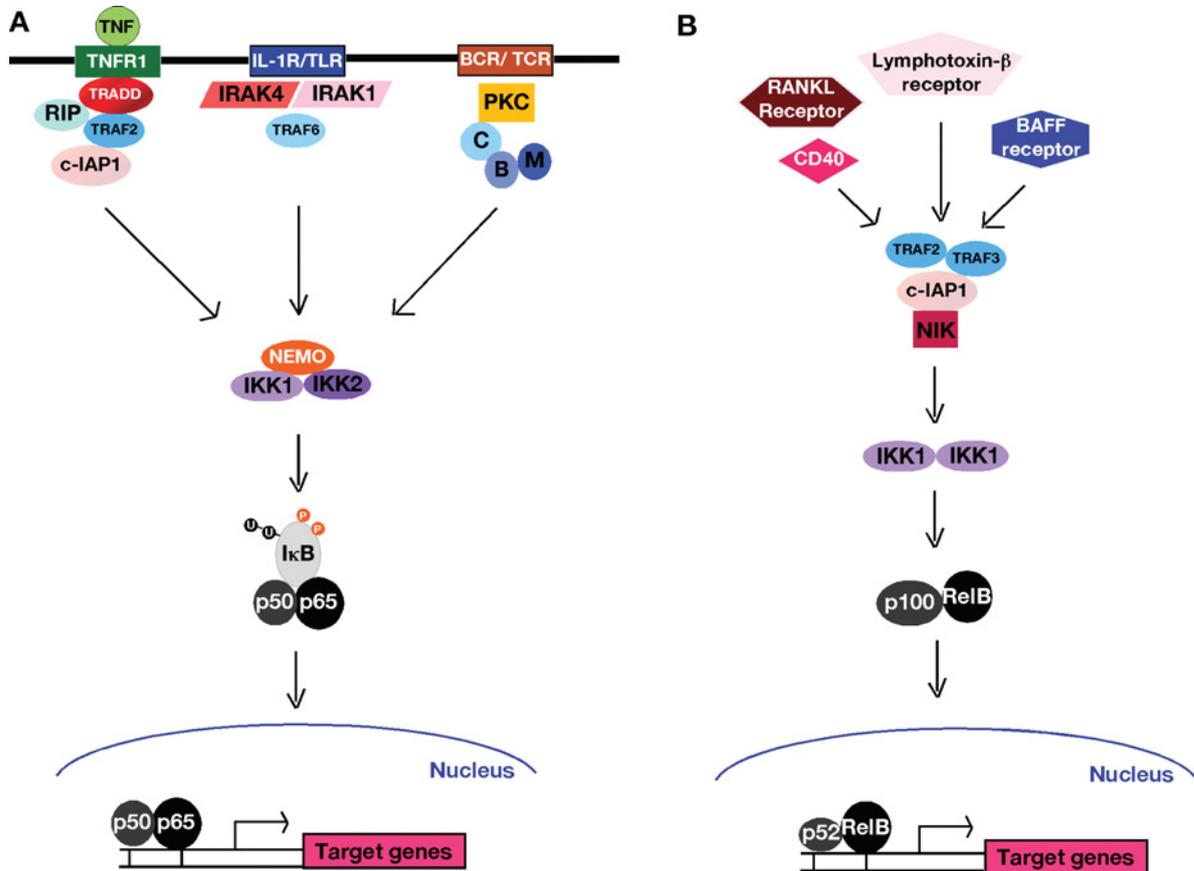


Figure 1 Canonical and non-canonical NF- κ B signalling

(A) Canonical NF- κ B signalling: acting on specific receptors, the signal is transduced through adaptor proteins and kinases to activate the IKK complex. The IKK complex phosphorylates I κ Bs at two conserved N-terminal serine residues. The phosphorylated I κ Bs are then targeted for Lys⁴⁸-linked polyubiquitination by β -TrCP-containing E3-ubiquitin ligase and subsequent degradation by the 26S proteasome. Free NF- κ B dimers can then accumulate in the nucleus to activate transcription. (B) Non-canonical signaling: receptor engagement leads to NIK activation. NIK directly phosphorylates and activates IKK1, which in turn phosphorylates NF- κ B2 (p100), leading to proteolytic cleavage of p100 to produce p52 and subsequent RelB–p52 dimer translocation into the nucleus to activate transcription.

RHD (REL homology domain) and a C-terminal transcription activation domain. The RHD is important for protein dimerization, DNA binding, interaction with I κ B (inhibitor of NF- κ B) and nuclear translocation. This group of NF- κ B proteins exhibit a steady-state cytosolic localization and a transcriptionally inactive state by binding to the I κ B proteins [2]. The typical I κ B proteins (I κ B α , I κ B β and I κ B ϵ) bind the RHD of NF- κ B through their six–seven ankyrin repeats.

The second group of NF- κ B members consists of NF- κ B1 (p105) and NF- κ B2 (p100). These are synthesized as large inactive precursors consisting of the N-terminal RHD and C-terminal ankyrin repeats. The C-terminal ankyrin domain has to be proteolytically removed before the mature form (p50 and p52 respectively) can bind DNA. The processing of p105 occurs constitutively by a co-translational mechanism, whereas that of p100 is stimulus-dependent [3]. Unlike those members in the first group, both p50 and p52 lack transactivation domains and are not capable of activating transcription

unless bound to another NF- κ B family member or an I κ B family member, BCL3 [4].

NF- κ B can be activated by a variety of stimuli, such as microbial and viral products, cytokines, DNA damage and noxious chemicals (see <http://www.NF-kB.org>). NF- κ B activation pathways may be broadly categorized into the canonical and non-canonical pathways [6] (Figure 1). Activated NF- κ B then turns on many downstream target genes to mediate innate and adaptive immune responses, development of the immune system, cell survival, cell proliferation and cell migration [7] (Table 1).

ACTIVATION AND BIOLOGICAL FUNCTIONS OF THE CANONICAL PATHWAY

The canonical NF- κ B signalling pathway in immune cells is typically activated by pro-inflammatory cytokines [such as TNF- α (tumour necrosis factor- α) and IL-1 β (interleukin-1 β)] [4], viruses, TLR (Toll-like receptor)

Table 1 Transcriptional targets of NF- κ B signalling

The biological outputs of NF- κ B signalling are mediated by the transcriptional gene products it turns on. CD40L, CD40 ligand; c-FLIP, cellular Fas-associated death domain-like IL-1 β -converting enzyme-inhibitory protein; COX-2, cyclooxygenase-2; GM-CSF, granulocyte/macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible NO synthase; IRF1, interferon regulatory factor 1; MIP-1 α , macrophage inflammatory protein-1 α ; PLA₂, phospholipase A₂; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

Function	Transcriptional target
Cytokines/chemokines	IL-8, IL-6, TNF- α , IL-1 β , GM-CSF, MIP-1 α , MCP-1 and Rantes
Antimicrobial effectors	Defensins, NO and O ₂ [•]
Enzymes	iNOS, COX-2 and PLA ₂
Immunoreceptors	B7.1 and MHC class I
Transcription factors	GATA3, STAT4 and IRF1
Growth factors/modulators	IL-2, GM-CSF, CD40L, Cyclin D1, VEGF, IL-6 and c-myc
Regulators of apoptosis	A1, c-IAPs, c-FLIP, Bcl-xl and Bcl-2
Cell adhesion molecules	ICAM-1, VCAM-1 and E-selectin
Invasion/angiogenesis	Maspin, VEGF and MMP-9

ligands [8] and engagement of the TCR (T-cell receptor) [9] (Figure 1). As a result of this signalling, the serine/threonine-specific IKK (I κ B kinase) is activated and phosphorylates I κ Bs, the cytosolic inhibitors of NF- κ B. I κ B is subsequently ubiquitinated and undergoes proteasomal degradation. Loss of I κ B binding permits nuclear accumulation of NF- κ B, as free NF- κ B binds several NF- κ B-binding sites across the genomic DNA [10].

In response to TNFR (TNF receptor) signalling, TRADD (TNFR1-associated death domain protein), TRAF2 (TNFR-associated factor 2), TRAF5 and RIP (receptor-interacting protein) are recruited to the receptor complex. Specific adaptor proteins that are recruited to the membrane proximal cytoplasmic TIR [Toll/IL-1R (IL-1 receptor)] domain of each TLR include MyD88 (myeloid differentiation factor 88), TRIF (TIR domain-containing adaptor protein inducing interferon- β), TIRAP (TIR-containing adaptor protein) and TRAM (TIR-domain-containing adaptor molecule). The adaptor proteins are capable of activating discrete downstream signalling pathways [8]. Engagement of IL-1R/TLR leads to the recruitment of TRAF6, IRAK1 (IL-1R-associated kinase 1) and IRAK4 to the receptor complex. Antigen-specific stimulation of TCR and CD28 co-receptors transduce the activating signal via PKC θ (protein kinase C θ) (T-lymphocytes) or PKC β (B-lymphocytes) to the oligomerization of the CBM [CARMA1 (caspase recruitment domain-containing membrane-associated guanylate kinase protein 1)/Bcl-10/MALT1 (mucosa-associated lymphatic tissue 1)] complex [9] and the ubiquitination of NEMO (NF- κ B essential modulator), the regulatory subunit of IKK [11].

Activation of the IKK complex requires the phosphorylation of T loop serine residues of either IKK2 or IKK1. There are two proposed mechanisms through which this phosphorylation can occur [12]. First, the induced oligomerization of TRAF, RIP, NEMO or CBM forms a multi-protein signalosome that provides a scaffold for IKK complex oligomerization, resulting in close proximity or conformational changes in IKKs that facilitates *trans*-autophosphorylation of the T loop serine residues. Secondly, the signalosome may be the platform for the putative IKK kinase to phosphorylate IKK. RIP or TRAF6 recruits the TAK1 [TGF (transforming growth factor)- β -activated kinase 1] complex through the association with TAB2 (TAK1-associated protein 2) to phosphorylate IKK2 within the activation loop, thereby activating the IKK complex [13,14]. Whether TAK1 is an IKK kinase, or acts through an intermediary kinase {e.g. MEKK3 [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase kinase 3]}, is not yet clear [15].

Activated IKK complex phosphorylates I κ B α at two conserved C-terminal serine residues (Ser³² and Ser³⁶) and I κ B β at Ser¹⁹ and Ser²³. The phosphorylated serine residues are the signals for binding by β -TrCP (transducin repeat-containing protein) containing E3 ubiquitin ligase [16,17]. An E2 ubiquitin ligase, Ubc5, is recruited to transfer Lys⁴⁸-linked ubiquitin chains from E1 ubiquitin ligase to the phosphorylated I κ Bs. Polyubiquitinated I κ Bs are then targeted for degradation by the proteasome, thus freeing NF- κ B dimers to translocate into the nucleus to activate transcription. Besides activating NF- κ B, TNFR engagement also leads to the activation of MAPK or SAPK (stress-activated protein kinase) cascades. Activation of MAPK signalling by TNFRs follows a two-stage process [18]. In stage 1, TRAF2, TRAF3, c-IAP1 (cellular inhibitor of apoptosis 1)/c-IAP2, Ubc13 (an E2 ubiquitin ligase), NEMO and MEKK1 are recruited to form a multi-protein complex at the cytosolic tail of the engaged receptor. In stage two, c-IAP1/c-IAP2 polyubiquitinates TRAF3 to promote TRAF3 degradation by the proteasome. The subsequent release of the multi-protein complex into the cytosol leads to MEKK1 activation and initiation of the MAPK/SAPK signalling cascade.

The canonical pathway plays key roles in the initiation of innate immunity and inflammation responses, as well as the development and maturation of innate and adaptive immune cells [4,19]. The canonical NF- κ B signalling pathway is highly conserved between mammals and invertebrates [20]. Two independent immune signalling pathways operate in *Drosophila* to act as defence mechanisms against microbial infections. Functional homologues of IKK, NF- κ B and I κ B can also be found in zebrafish (*Danio rerio*) to regulate vertebrate developmental processes [21,22].

In response to foreign invaders, the canonical pathway may be initiated in macrophages, granulocytes, dendritic

cells, B-cells, fibroblasts and endothelial cells. Antimicrobial products, such as defensins, RNS (reactive nitrogen species) and ROS (reactive oxygen species) produced by activated cells, have microbiocidal effects. The increased production of inflammatory cytokines, chemokines, MMPs (matrix metalloproteinases), enzymes (such as cyclo-oxygenases) and expression of cell-adhesion molecules enhanced the recruitment and activation of the recruited immune cells. Dendritic cells increase the expression of co-stimulatory molecules B7-1 (CD80) and B7-2 (CD86) to ensure the maximal activation of T-cells [23]. NF- κ B-mediated expression of anti-apoptotic genes enhances the survival of developing and mature granulocytes [24,25], and both p50 and RelA are required for the development of CD8 α^+ and CD8 α^- dendritic cells [4].

The canonical NF- κ B signalling also plays important roles in the maturation and function of lymphocytes in the adaptive immune system. Antigen-specific stimulation of TCR and CD28 co-receptors acts through NF- κ B to promote the survival and proliferation of the activated lymphocytes [19,26]. During development, NF- κ B-dependent pro-survival signals counteract the death stimulus from TNF- α [27], and p50/RelA or RelA/c-Rel are required for the early development of B- and T-cells before the pre-antigen receptor stage [4].

Although it is generally accepted that IKK2, but not IKK1, mediates the activation of canonical NF- κ B signalling, an exception occurs in the adult liver [28]. Both IKK1- and IKK2-dependent signalling are required to protect the liver from LPS (lipopolysaccharide)-induced toxicity, trigger the canonical NF- κ B signalling in response to TNF- α and maintain the integrity of the hepatic bile duct.

ACTIVATION AND BIOLOGICAL FUNCTIONS OF THE NON-CANONICAL PATHWAY

The non-canonical NF- κ B signalling pathway is activated by some members of the TNF cytokine family [LT- β (lymphotoxin- β), BAFF (B-cell-activating factor) and CD40] and RANKL [RANK (receptor activator of NF- κ B) ligand]. NIK (NF- κ B-inducing kinase) is a labile serine/threonine kinase central to the activation of non-canonical NF- κ B signalling. NIK is negatively regulated by TRAF2, c-IAP1 and c-IAP2 via ubiquitination and proteasome-dependent degradation [29–31]. Degradation of TRAF2, c-IAP1 and c-IAP2 upon receptor engagement leads to the stabilization and activation of NIK. NIK directly phosphorylates and activates IKK1, which leads to NF- κ B2 (p100) phosphorylation, proteolytic cleavage of p100 to produce p52 and subsequent RelB-p52 dimer translocation into the nucleus. The biological output by active p52 may also be the result of a hybrid pathway involving p52/RelA or p52/c-Rel [4,19,32]. The non-canonical pathway plays important roles in the de-

velopment of secondary lymphoid organs, immune cell maturation and osteoclastogenesis [33].

The secondary lymphoid organs, including the lymph nodes, Peyer's patches, MALT and spleen, are sites where organized arrangement of cells allows proximal interaction between the stromal and haemopoietic cells to promote the development and activation of lymphocytes. LT- β , RANKL and TNF- α contribute to NF- κ B activation, leading to the production of organotypic chemokines and anti-apoptotic factors to promote and maintain the architecture of these secondary lymphoid organs [33].

B-lymphocyte development is dependent on non-canonical NF- κ B signalling, which is initiated from pre-BCR (B-cell receptor) and BAFF receptors in early, late and mature B-cells. Both canonical and non-canonical NF- κ B pathways are required to promote the proliferation, survival and maturation of B-lymphocytes [4,19]. CD40 ligand, on the other hand, activates both canonical and non-canonical signalling to promote isotype switching in mature B-cells.

Osteoclastogenesis, or the formation of bone matrix, involves the deposition of organic matrix within the hollow lumen of the bone. It is the result of a balanced activity between the bone-forming osteoblasts and bone-resorption osteoclasts [33,34]. Both TNF- α - and RANKL-induced NF- κ B signalling regulates osteoclast formation and function. Osteoclasts fail to form in mice deficient in RANK, RANKL or p50/p52, resulting in enhanced bone matrix deposition in a condition called osteopetrosis [33]. p50 and p52 are likely to pair with RelB in the regulation of osteoclast differentiation *in vivo* [35].

OTHER PROPOSED FUNCTIONS OF NF- κ B AND IKK COMPLEX

Recently, the role of NF- κ B in development is extended to that of ES (embryonic stem) cells [36]. NF- κ B activity is inhibited in undifferentiated but activated in differentiating ES cells. The overexpression of NF- κ B proteins promoted differentiation, whereas suppression of NF- κ B signalling in NEMO-knockout (*ikkbkg^{-/-}*) or κ B α M-expressing ES cells enhances the expression of pluripotent markers. The roles played by NF- κ B to promote tumour growth may also be accounted for by its ability to promote aerobic glycolysis through enhancing GLUT4 (glucose transporter 4) expression [37]. RelA is activated in cells null for p53 and/or overexpressing Ras, and could account in part for GLUT4 expression and the promotion of aerobic glycolysis to provide energy for tumour growth. NF- κ B is also reported to regulate HIF-1 α (hypoxia-inducible factor-1 α) transcription and to trigger a hypoxic response in the low-oxygen environment experienced during rapid tumour growth, infection and inflammation [38].

Other than phosphorylating I κ Bs and NF- κ B subunits, more NF- κ B- and I κ B-independent targets of IKK1 and IKK2 are being reported [39], suggesting that the impact of IKK activation on cell signalling could be more widespread. Recent additions to the list of IKK2 targets include the SNARE [SNAP (soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein) receptor] family protein SNAP-23 and Aurora kinase A. IKK2 promotes pro-inflammatory cytokine production by mast cells, either by activating the transcription of pro-inflammatory cytokines through NF- κ B or by promoting mast cell degranulation through SNAP-23 phosphorylation by IKK2 in an NF- κ B-independent manner [40]. IKK2 phosphorylates Aurora kinase A to negatively regulate Aurora kinase A protein stability and kinase activity [41]. The timely inactivation of Aurora kinase A is necessary for normal mitotic progression and the maintenance of mitotic spindle polarity.

POSITIVE REGULATORS OF NF- κ B

It is of great interest as to how NF- κ B is able to activate a specific transcription programme and produce a specific biological effect in response to a myriad of stimuli. Several positive and negative mechanisms appear to operate at different levels, within specific context and cell types, to contribute to the final biological outcome. Regulators may act upstream of the IKK complex, at the IKK complex or at the NF- κ B subunits. The NF- κ B pathway does not function in isolation, but cross-talks with other pathways and co-operates with other transcription factors/co-regulators through protein-protein interactions and post-translational modifications to shape the final transcription programme [39,42,43].

The activation profiles of IKK and NF- κ B dimers differ between canonical and non-canonical pathways and between different activators of the canonical pathways. Studies from knockout mouse models indicate that NF- κ B subunits have distinct and non-overlapping functions [44]. The different combination of NF- κ B dimers that can potentially be formed adds to the diversity in transcription programmes that can be turned on [45]. Although the different dimers have broad sequence recognition specificities that largely overlap, NF- κ B-DNA interactions play an important role in determining transcriptional specificity. The affinity differences in NF- κ B- κ B site interactions may contribute to transcriptional specificity in some instances (e.g. c-Rel compared with RelA affinity for the κ B site on the IL-12 promoter) but not in many others [1]. Instead, transcriptional specificity is affected by the recruitment of different transcription factors and co-activators on to different promoters [46]. p38 MAPK is recruited to the promoters of selective cytokine and chemokine genes [e.g. IL-8 and MCP-1 (monocyte chemoattractant protein-1)] in

response to inflammatory stimuli to promote histone H3 Ser¹⁰ phosphorylation, which marks the promoter sites for NF- κ B recruitment and transactivation [47]. Histone H3 Ser¹⁰ phosphorylation and enhancement of gene expression on the I κ B α and IL-6 promoter by NF- κ B on the other hand is IKK1-dependent [48,49]. The co-operative binding by several transcriptional factors and co-activators, in combination with the induced bending and structural changes in DNA within an enhanceosome, add another level of control to the transcription [50].

Post-translational modifications of NF- κ B subunits and their regulators can influence their activity. This has been extensively reviewed recently by Perkins [42], and will only be briefly discussed here. Post-translational modifications include phosphorylation, ubiquitination, acetylation, sumoylation and nitrosylation and are reported to affect the activity of the IKK complex, I κ B proteins and NF- κ B subunits. For example, the phosphorylation of IKK2 within the activation loop at Ser¹⁷⁷ and Ser¹⁸¹ is necessary for its activation [12]. The TRAF proteins were proposed to function as ubiquitin ligases that promote Lys⁶³-linked self-ubiquitination and polyubiquitination of RIP and NEMO [51,52]. Lys⁶³-linked polyubiquitinated proteins may serve as docking sites for the assembly of signalling complexes. Phosphorylation of I κ B α at Ser³²/Ser³⁶ is a signal for Lys⁴⁸-linked ubiquitination and subsequent proteolytic degradation by the proteasome [16,17]. Phosphorylation of I κ B α at Ser²⁸³, Ser²⁸⁹, Ser²⁹³, Thr²⁹¹ and Thr²⁹⁹ within the PEST domain in response to short wavelength UV light or Her2/Neu oncogene is also a signal for protein degradation [42]. Phosphorylation of RelA at Ser²⁷⁶, Ser⁵²⁹ and Ser⁵³⁶ enhances transcriptional activation, whereas phosphorylation at Thr⁴³⁵ and Thr⁵⁰⁵ is implicated in the negative regulation of RelA transactivation [42]. Phosphorylation of RelA at Ser²⁷⁶ and Ser⁵³⁶ promotes p300/CBP [CREB (cAMP-response-element-binding protein)-binding protein] binding and RelA acetylation at Lys³¹⁰ promotes gene expression [53–56]. p300 and CBP also acetylate RelA at Lys²¹⁸ and Lys²²¹; acetylated Lys²²¹ enhances RelA DNA binding and, together with acetylated Lys²¹⁸, inhibits the association of RelA with newly synthesized I κ B α [56].

NF- κ B signalling can also be regulated by the tyrosine phosphorylation status of its regulators. For example, tyrosine phosphorylation of I κ B α leads to the release of NF- κ B from the I κ B α -NF- κ B complex to serve as a means of activating NF- κ B in response to re-oxygenation of hypoxic cells [57]. Less is known about the tyrosine kinases that are involved in NF- κ B regulation. The tyrosine phosphatase SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase-2) is reported to positively regulate NF- κ B activation [58]. SHP-2-deficient cells fail to activate NF- κ B and produce IL-6 in response to IL-1 β and TNF- α stimulation. The exact substrate of SHP-2 in this connection is not known, but

the phosphatase may function through its association with the IKK complex and IL-1R to activate NF- κ B.

CELLULAR INHIBITORS OF NF- κ B

Activation of TNF- α and TLR signalling is essential for the elimination of invading pathogens; however, excessive activation of TLR signalling and inflammation may lead to immunopathological conditions, such as sepsis and autoimmune diseases. These immune signalling pathways must be precisely controlled so that they are switched on at the right time and only for an appropriate length of time. There are several built-in negative regulators to switch NF- κ B signalling off when the stimuli are no longer present.

De-ubiquitinases

Several de-ubiquitinating enzymes can negatively regulate NF- κ B activation. The tumour suppressor the CYLD (cylindromatosis) inhibits NF- κ B and JNK (c-Jun N-terminal kinase) kinase activation by removing Lys⁶³-linked polyubiquitin chains from BCL3, RIP1, TRAF2, TRAF6 and NEMO [59–62]. The gene encoding CYLD protein is mutated in patients with familial cylindromatosis, Brooke–Spiegler syndrome and familial trichoepithelioma [63]. Mutations in the *CYLD* gene result in the loss of de-ubiquitinating activity of CYLD. Failure to remove Lys⁶³ polyubiquitin chains on TRAF2 and TRAF6 leads to increased basal and stimuli-mediated activation of NF- κ B. Patients carrying the *CYLD* mutation develop benign tumours of skin appendages called cylindromas [61,62,64]. Mice lacking the *Cyld* gene are highly susceptible to chemical-induced skin tumours [60]. These tumours have hyperproliferation, high BCL3/p50 and BCL3/p52 activity and high cyclin D1 expression. *Cyld*-deficient mice are also more susceptible to chemically induced colon inflammation and have a higher incidence of colon tumours compared with wild-type mice [65]. However, NF- κ B signalling in bone-marrow-derived macrophages appears normal in *Cyld*-knockout mice, although T-cell development and possibly TCR signalling is defective. These suggest a cell type and stimulus-specific regulatory role of CYLD [66].

Another de-ubiquitinating enzyme implicated in NF- κ B regulation is A20. Expression of A20 is induced by TNF- α stimulation and serves as a negative-feedback regulator of TNF- α - and TLR-induced NF- κ B signalling [67–69]. The activity of A20 is positively regulated by IKK2 phosphorylation [70]. A20 is an ubiquitin-editing enzyme which cleaves Lys⁶³-linked ubiquitin, but promote Lys⁴⁸-linked polyubiquitination of signalling molecules (RIP1 and TRAF6) to terminate TNF- α - and TLR-induced NF- κ B activation. A20 is part of a TAX1BP1 (TAX1-binding protein 1)–A20–Itch complex, and A20 association with Itch is required for the

recruitment and subsequent inactivation of RIP1 [71]. A20-deficient cells fail to properly terminate TNF- α - and TLR-induced NF- κ B activity, and A20-deficient mice develop chronic inflammation, cachexia and suffer from premature death [72]. IRAK1 also undergoes polyubiquitin editing, suggesting that this may be a general mechanism for attenuating innate immune signalling [73].

Two other cellular de-ubiquitinating enzymes, Cezanne and USP31 (ubiquitin-specific protease 31), have also been shown to negatively regulate NF- κ B activation [74,75], although the exact mechanism of their actions remains to be studied. A non-cellular de-ubiquitinating enzyme, YopJ, produced by the bacteria *Yersinia* can also negatively regulate NF- κ B activation as a mechanism to evade the host immune response [76].

I κ Bs

The typical I κ Bs (α , β and ϵ) are well-known negative regulators of basal NF- κ B activity. Even though these three I κ Bs are apparently dispensable for the cytoplasmic retention of NF- κ B dimers, they are essential for stimulus-dependent activation of NF- κ B [10]. The kinetics and degree of I κ B protein degradation can be affected by their differential binding to different cytosolic proteins such as κ B-Ras, G3BP2 [Ras-GTPase-activating protein Src homology 3-domain-binding protein 2], hnRNP U (heterogeneous nuclear ribonucleoprotein U) and β -arrestin 2 [77]. Hence the rate and extent of NF- κ B activation may differ between cell types depending on the different partners associated with the I κ B–NF- κ B complex. The NF- κ B dimers differ in their interaction specificity towards the I κ B proteins. Hence specific NF- κ B dimers may be activated via the degradation of specific I κ B proteins. The kinetics of the degradation and biosynthesis of different I κ B proteins differ and could set a temporal control for NF- κ B activation and inactivation. Owing to the rapid degradation and re-synthesis of I κ B α , I κ B α is a key to induce the rapid rise and decline in NF- κ B activity upon TNF- α stimulation [78]. I κ B ϵ is synthesized later upon stimulation [79] and, together with I κ B β , reduces the oscillatory potential and stabilizes NF- κ B signalling during chronic stimulation [78].

Kinases and phosphatases

Phosphorylation is perhaps the most common and key modification regulating the activity of NF- κ B and its upstream regulators. Several kinases and phosphatases are implicated in the negative regulation of NF- κ B activity, and there are potential cell-type specificities and substrate specificities in NF- κ B signalling.

Both phosphorylation and dephosphorylation events are implicated in de-activating the IKK complex. Inactivation of the IKK complex can occur via autoinhibitory phosphorylation [80]. The IKK complex is also negatively regulated through association with PP2C β (protein phosphatase 2C β) in non-stimulated cells [81]. The IKK

Table 2 Inherited human diseases with reduced NF- κ B signalling

A decrease in the ability to activate NF- κ B signalling can arise due to hyperactivated upstream negative regulators or loss-of-function in positive regulators. EDA-ID, anhidrotic ectodermal dysplasia with immunodeficiency; EGFR, epidermal growth factor receptor.

Name of disease	Symptoms	Defective protein(s)
IP	Inflammatory skin disease	NEMO
EDA-ID	Immune deficiency and abnormal development of skin adnexes	NEMO, I κ B α , EDA-A1 (ectodysplasin-A1), EDAR (ectodysplasin receptor) and EDAR-ADD (EDAR-associated death domain protein)
OL-EDA-ID	Same as EDA-ID symptoms, with osteopetrosis and lymphoedema (OL)	NEMO and V-EGFR-3
Inherited immune deficiency	Impaired TLR and IL-1R signalling, and high susceptibility to pyogenic bacterial infection	IRAK4 and MyD88
Inherited immune deficiency	Predisposed to herpes simplex encephalitis	TLR3

complex in association with PP2C β is less active, and siRNA (small interfering RNA) knockdown of PP2C β expression resulted in sustained IKK kinase activity upon cytokine stimulation. The IKK complex is also associated with another phosphatase, PP1 (protein phosphatase 1), through the adaptor protein CUEDC2 (CUE domain-containing protein 2) and GADD34 (growth-arrest and DNA-damage-inducible protein 34; a regulatory subunit of PP1) under a basal non-cytokine stimulated state [82]. Cells transfected with siRNA against CUEDC2 had higher phosphorylated IKK1/IKK2 at Ser¹⁸⁰ and Ser¹⁸¹. Another phosphatase, PP2A, was identified in an RNAi (RNA interference) screen for negative regulators of NF- κ B signalling in astrocytes [83]. The PP2A enzyme complex associates with IKK, TRAF2 and NF- κ B, and the dephosphorylation of TRAF2 at Thr¹¹⁷ and NF- κ B at Ser³³⁶ negatively regulates NF- κ B activation by TNF- α and IL-1 β . The tyrosine phosphatase SHP-1 negatively regulates NF- κ B by TLR ligands by binding to and negatively regulating IRAK1 [84]. SHP-1-deficient mice suffer from systemic autoimmune disease and severe inflammation, with hyperactivated MAPK and NF- κ B activities and excessive production of inflammatory cytokines in response to TLR stimulation.

MSK1 (mitogen- and stress-activated kinase 1) and MSK2 are involved in the negative regulation of TLR and NF- κ B signalling [85]. Negative regulation of NF- κ B by MSK is indirect, occurring through the up-regulation of the production of the anti-inflammatory cytokine IL-10 and through the inhibition of p38 MAPK via DUSP1 (dual-specificity phosphatase 1) expression. Mice deficient in MSK1 and MSK2 produce less IL-10 and DUSP1 but more inflammatory cytokines, and are hypersensitive to LPS-induced endotoxic shock.

Transcriptional repressors

The transcription functions of NF- κ B are negatively regulated by association with HDAC (histone deacetylase) and other transcriptional co-repressors. The absence of RelA phosphorylation at Ser²⁷⁶ promotes HDAC

association and gene repression near the κ B-binding sites [53]. Deacetylation of NF- κ B by HDAC also promotes I κ B α binding and nuclear export of NF- κ B [86]. ING4 (inhibitor of growth family member 4) when associated with RelA on specific promoters results in a repressed non-expressing state. Down-regulation of ING4 expression links NF- κ B activation to enhanced angiogenesis and tumour progression in gliomas [87]. Twist1/Twist2 bound on to TNF- α and IL-1 β promoters is also associated with suppression of NF- κ B-dependent transactivation of these genes [88].

HUMAN DISEASES RESULTING FROM LOSS-OF-FUNCTION IN NF- κ B SIGNALLING

The study of inherited human diseases (Table 2) resulting from mutations in the genes involved in the NF- κ B signalling pathway has provided valuable information to help understand the signalling and function of NF- κ B *in vivo*, confirm the already known functions of NF- κ B and provide new insights for the role of NF- κ B in different cell types and organ systems [89].

IP (incontinentia pigmenti) is an X-linked disorder, where chromosomal re-arrangement or mutations leads to premature termination of the *IKBKG* gene (encoding NEMO), resulting in truncated NEMO that is incapable of NF- κ B activation [90]. The disease mainly affects female heterozygotes, as homozygotes and affected males typically die *in utero*. The affected individuals have inflammatory skin phenotypes with blistering, hyperkeratinization, hyperpigmentation and dermal scarring. Owing to an imbalance in X-linked gene inactivation, the skin contains a mosaic of cells with normal or mutated NEMO. The initial trigger for this inflammatory skin disorder is not known. Conditional knockout of the *Ikkkb* gene (encoding IKK2) in the epidermis indicated that IKK2-mediated NF- κ B activity is essential for regulating mechanisms that maintain immune homeostasis in the skin [91]. A disruption of immune

homeostasis may trigger IL-1 and TNF- α production by wild-type skin cells. Wild-type skin cells undergo proliferation and trigger more inflammatory responses upon stimulation; however, due to the lack of NF- κ B activation, the *Ikkkg*-mutant cells undergo apoptosis and are cleared from the system.

Other forms of inherited *Ikkkg* gene mutation lead to smaller deletions in NEMO. The mutant NEMO retains the ability to weakly activate NF- κ B. The diseased phenotype ranges from severe immune deficiency in affected males to mild IP in females (Table 2). Other phenotypes displayed by patients with defects in NF- κ B activation point to the role of NF- κ B signalling in the proper morphogenesis of epidermis adnexes, lymphatic vessels development, osteoclastogenesis and neural function. The use of tissue-specific knockout animal models will be useful in addressing the mechanisms and roles of NF- κ B signalling in these organ systems.

Several inherited immunodeficiencies arise due to defects in TLR-induced signalling [92]. Autosomal recessive mutations in *IRAK4* resulted in specific impairment in Toll and IL-1R signalling, which result in an increased susceptibility to pyogenic bacterial infections including IPD (invasive pneumococcal disease). Mutations in *TLR3* and *UNC93B1* impair TLR3 responses and patients are predisposed to herpes simplex encephalitis. MyD88 is a key adaptor downstream of most TLRs and IL-1Rs. Patients with autosomal-recessive MyD88 deficiency suffer from life-threatening and recurrent pyogenic bacterial infections including IPD [93].

HUMAN DISEASES RESULTING FROM GAIN-OF-FUNCTION IN NF- κ B SIGNALLING

NF- κ B signalling play key roles in several cellular and developmental processes, and the timely activation and inactivation of NF- κ B signalling is essential for NF- κ B to function in a controlled manner. Uncontrolled NF- κ B signalling due to hyperactivation of NF- κ B or loss-of-function of the negative regulators has been linked to several neoplastic disorders, chronic inflammatory disorders, insulin resistance, cachexia, Alzheimer's disease, transplant intolerance and organ ischaemia/reperfusion injury. The roles of NF- κ B in cancers and chronic inflammatory disorders are briefly discussed below.

NF- κ B in cancers

Human cancer is a multi-factorial disease that could originate from aberrant or uncontrolled growth of several cell types. A normal cell needs to acquire new anomalous capabilities before it can become cancerous. These include self-sufficiency in growth, resistance to growth inhibitory and pro-apoptotic signals, angiogenic capabilities, and the ability to invade and metastasize to distant sites [94]. It has also become clear that cancer is the disease of an organ. The disturbance in the communication between tumour

cells with the cellular and non-cellular components within the microenvironment contributes to tumorigenesis and disease progression [95]. The multi-functional nature of NF- κ B signalling in both immune and non-immune cells points to the involvement of dysregulated NF- κ B signalling in multiple cell types during cancer initiation and progression. The ability of constitutive NF- κ B signalling to promote the initiation and progression of tumorigenesis can be ascribed to its ability to transcribe genes that promote cell proliferation, cell survival, angiogenesis and metastasis [96,97] (see Table 1 for target genes). Chronic NF- κ B activation can cause DNA damage and increase the probability of a cell to acquire oncogenic DNA mutations through ROS and RNS that are produced [98]. NF- κ B signalling can contribute to chemo- and radio-therapy resistance with survival and selection of clones with more aggressive cancer phenotypes by suppressing the function of tumour suppressors, such as p53 and FOXO3a (forkhead box O3a) [39,99]. *TNF* (encoding TNF- α), one of the NF- κ B target genes, can also contribute to cancer progression by impairment of immune surveillance via suppression of cytotoxic T-cell and macrophage functions [100].

Activated NF- κ B is observed in many solid tumours and haematological malignancies (Table 3). A few examples of NF- κ B activation in different cancers are described here. Familial cylindromatosis is characterized by benign tumours of the skin appendages, and tumours arise due to mutation and inactivation of the *CYLD* gene [63]. Human DLBCL (diffuse large B-cell lymphoma) is a common form of non-Hodgkin's lymphoma. In the ABC (activated B-cell-like) subtype of DLBCL, missense mutations affecting the coiled-coil domain of the CARD11 (caspase recruitment domain 11) signalling scaffold protein, or CARMA1, leads to constitutive NF- κ B activation in B-cells, which confers a pro-survival state to the malignant cells [101]. MM (multiple myeloma) is a malignant cancer of the plasma cells resulting from chronic NF- κ B activation [102,103]. Negative regulators of NF- κ B signalling that have undergone loss-of-function mutations in patients suffering from MM include TRAF2/TRAF3, c-IAP1/c-IAP2 and CYLD. Positive regulators and effectors of NF- κ B signalling that have undergone gain-of-function mutations in MM include CD40, TAC1 (transcriptional activator of CDR genes 1), LT- β R (LT- β receptor), NIK and p100/p105. MALT B-cell non-Hodgkin lymphoma arises as a result of either MALT1 overexpression or MALT1 hyperactivation through fusion with either API2 (apoptosis inhibitor 2) or c-IAP2, which results in enhanced NF- κ B activation [11,104].

Other than mutations that affect NF- κ B and its upstream regulators, cellular and viral proteins can also contribute to uncontrolled NF- κ B activation [96,97]. Cellular proteins overexpressed or hyperactivated in cancers that are reported to activate NF- κ B include ErbB2, Ras, B-raf, Vav, Pim-2, Akt, CK2, STAT3 (signal

Table 3 NF- κ B activation in various human cancers

Uncontrolled NF- κ B signalling can arise due to hyperactivated upstream activators or loss-of-function in the negative regulators. GSK3 β , glycogen synthase kinase 3 β .

Name of cancer	Type of cancer	Defective protein(s)
Familial cylindromatosis	Benign tumours of skin appendages	CYLD
MM	Cancer of the plasma cells	TRAF2/TRAF3, c-IAP1/c-IAP2, CYLD, CD40, TAC1, LT- β R, NIK and p100/p105
ABC-subtype DLBCL	Large B-cell lymphoma	CARD11
Diffuse DLBCL	Large B-cell lymphoma	High c-Rel expression
MALT lymphoma	B-cell lymphoma	Truncated BCL10 and MALT1 overexpression, MALT-AP1 and MALT-c-IAP2 fusion protein
Chronic lymphocytic leukaemia, T-cell lymphoma, and cutaneous B- and T-cell lymphoma	B- and T-cell lymphoma	Truncated p100
Breast cancer		Elevated BCL3, c-Rel, IKK ϵ , activated p50/RelA and p52
Pancreatic cancer	Ductal adenocarcinoma	Elevated Ras, Akt, Notch-1, GSK3 β , Vav1 and IL-1 α

transducer and activator of transcription 3), Pin1 and Bcr-abl. NF- κ B signalling pathways are activated in RNA and DNA virus-induced leukaemia and lymphomas. Tax protein expressed by HTLV (human T-cell leukaemia virus) directly activates the IKK complex. v-Rel produced by the avian REV-T virus (reticuloendotheliosis virus strain T) is a homologue of the c-Rel protein. EBNA2 (Epstein-Barr virus nuclear antigen 2) and LMP1 (latent membrane protein 1) activate the transcriptional function of NF- κ B by unknown mechanisms.

Chronic NF- κ B activation: linking chronic inflammation and human diseases

Chronic inflammation has been implicated in the pathogenesis of cancer, ALI (acute lung injury), muscle wasting diseases, insulin resistance and T2DM (Type 2 diabetes mellitus), neurodegenerative disorders, autoimmune diseases (e.g. rheumatoid arthritis), atherosclerosis and various symptoms of aging. Infiltrating leucocytes and activated stromal cells are the common sources for inflammatory cytokines in chronic inflammation. The cytokines in turn act in an autocrine and paracrine fashion to trigger vicious cycles of cytokine production.

Both clinical and epidemiological studies have suggested a strong link between chronic inflammation and cancer development [105–107]. Polymorphisms within the nucleotide sequences of the exons or regulatory elements of many cytokines and their receptors are linked to chronic inflammation and increased risks in cancer development. Polymorphisms in the *TNF* gene are associated with higher risks for oral cancer, gastric cancer and ulcerative colitis-associated colorectal carcinoma. Some polymorphic sequences of another pro-inflammatory cytokine, IL-1 β , are correlated with higher risks of gastric cancers, HCC (hepatocellular carcinoma) and pancreatic cancers. Sequence variants in TLR1, TLR4, TLR6 and TLR10 are associated with higher risks of prostate, nasopharyngeal and gastric cancers [108].

The stimuli for chronic inflammation may be of microbial origin, chemical in nature or of unknown cause. Infection-driven chronic inflammation accounts for 15–20% of all cancers. *Helicobacter pylori* infection and high IL-1 β are associated with gastric carcinomas, whereas flatworm *Schistosoma haematobium* infection is a high risk for bladder and colon cancer development. Chronic exposure to harmful chemicals, such as cigarette smoke, asbestos and silica, predispose individuals to liver and lung cancer development. HBV (hepatitis B virus) and HCV (hepatitis C virus) infections and chronic alcohol consumption are risk factors for HCC. Men are 3–5 times more likely to develop HCC than women [109]. The higher incidence of HCC in males is correlated with the larger amount of IL-6 production in males. Deletion of the *Il6* or *Myd88* gene appeared to abolish the gender differences in the incidence of HCC triggered by DEN (diethylnitrosamine) in mice [110]. Consistent with this notion, deletion of *Ikkkb* within the myeloid population is associated with lower incidence of cancers in the DEN-induced mouse model of HCC [111,112].

The origins of some chronic inflammatory disorders, such as IBD (inflammatory bowel disease) or Crohn's disease, is unknown, but may involve the aberrant activation of pattern recognition receptors. IBD is characterized by chronic inflammation of the intestinal mucosa and is a risk factor for the development of colon cancers. Hyperactivated NF- κ B in intestinal epithelial cells, lymphocytes and myeloid cells is accompanied by dysregulated cytokine production and colon tissue damage. The role of NF- κ B as the positive regulator of colon inflammation and colon cancer development is demonstrated using the AOM (azoxymethane)- and DSS (dextran-sulfate)-induced colitis-associated cancer model in mice. Deletion of IKK2 in enterocytes or myeloid cells promotes cell death, reduces inflammatory cytokine production and tumour incidence in mice [111].

Chronic inflammation also underlies the pathology of other diseases, of which a few will be discussed here. Chemicals, H5N1 avian flu and SARS (severe acute respiratory syndrome) viruses are the common causes for ALI in humans. The disease model for ALI in mice is characterized by local ROS production with increased oxidation and formation of oxPLs (oxidized phospholipids) [113]. Enhanced oxPL production activates NF- κ B via the TLR4–TRIF pathway within the macrophage population resulting in chronic production of inflammatory cytokines, which contributes to the pathogenesis of this inflammatory disorder.

Several inflammatory cytokines are implicated as potential mediators of muscle wasting or atrophy in aging and in various pathological conditions, such as cancer, cachexia and diabetes. NF- κ B contributes to muscle atrophy as overexpression of the activated form of IKK2 in muscle cells leads to muscle wasting, whereas pharmacological or genetic inhibition of NF- κ B signalling reversed muscle atrophy in several mouse models of muscle wasting [114].

Inflammation also underlies the metabolic disorders of insulin resistance and T2DM. Using knockout mouse models, both IKK2/NF- κ B [115–117] and JNK1 signalling [118,119] have been shown to be involved in the aetiology of insulin resistance and T2DM. Insulin responsiveness is retained in the livers of mice selectively lacking IKK2 expression in hepatocytes, but showed systemic insulin responsiveness when IKK2 expression was knocked out in myeloid cells [115]. On the other hand, the expression of constitutively active IKK2 in hepatocytes led to hepatic and systemic insulin resistance and T2DM development in mice [116].

NF- κ B signalling is crucial for neuronal survival [120,121] and regulates the growth and elaboration of neural processes [122]. NF- κ B signalling is altered in many chronic neurodegenerative disorders including Alzheimer's disease. NF- κ B exerts dual roles in the disease process [123]. It is neuroprotective through the induction of anti-apoptotic genes and antioxidants in neurons [124]. There is a strong reduction in nuclear p65 immunostaining in the cells surrounding the amyloid plaques during the late stage of Alzheimer's disease, and inhibiting NF- κ B increases amyloid- β -induced neuronal apoptosis [125]. On the other hand, NF- κ B can contribute to neurodegeneration by inducing the synthesis of inflammatory mediators in microglial cells [126,127], as inhibiting NF- κ B activity in microglial cells blocks amyloid- β -induced neurotoxicity [55].

NF- κ B SIGNALLING AS THERAPEUTIC TARGETS

As discussed above, there is ample evidence to demonstrate the roles of NF- κ B in cancers and chronic inflam-

matory disorders. Hence blocking NF- κ B signalling by specific inhibitors may be useful in the treatment of these disorders. The ability of more than 200 different stimuli to activate NF- κ B and the large number of target genes turned on by NF- κ B in many cell types make the design of pathway-specific inhibitors an arduous task. More than 700 compounds have been reported to inhibit NF- κ B activation [128]. These NF- κ B inhibitors may be classified into those that block signalling upstream of IKK, at the IKK step, at the I κ B degradation step and the nuclear function of NF- κ B subunits.

Agents that act upstream of the IKK complex include inhibitors of receptor activation by the natural ligand, inhibitors of adaptor protein recruitment to the receptor and inhibitors of IKK-activating kinases. Inhibitors of cytokine binding to its receptor are particularly relevant for certain chronic inflammatory disorders and inflammation-associated cancers. For example, the inhibition of TNF- α -induced NF- κ B activation by anti-TNF- α antibodies is used in the control of IBD and arthritis [129,130]. The restricted use of adaptor proteins and kinases by different activators of NF- κ B may provide suitable targets for pathway-specific inhibition. For example, TRAF2 is mainly used by TNFR and CD40, TRAF6 in IL-1/TLR signalling, CBM and PKC in BCR and TCR signalling, and NIK in non-canonical signalling pathways.

More than 150 compounds have been reported to inhibit NF- κ B activation at the IKK step [128,131]. The commonly used anti-inflammatory drugs aspirin and sodium salicylate exert their effects in part by acting as competitive inhibitors of the ATP-binding site of IKK2. Thalidomide and its analogues are immunomodulatory drugs that have entered clinical trials for the treatment of MM and prostate cancers [132]. Among several hypothesis, the inhibition of IKK2 has been proposed to explain the therapeutic activity of thalidomide [133]. A small-molecule inhibitor of IKK (PS-1145) is selectively toxic towards the ABC-subtype of DLBCL [134]. NEMO is also an attractive target for IKK complex inhibition. A cell-permeant ten-amino-acid peptide corresponding to the NBD (NEMO-binding domain) of IKK2 can block the binding of NEMO to IKKs and the induction of the NF- κ B canonical pathway by TNF- α [135,136]. The NBD peptide can effectively ameliorate inflammatory responses and block osteoclastogenesis in several animal models of inflammation [137].

Agents that suppress NF- κ B activation by stabilizing I κ B or blocking I κ B degradation have been reported [128]. These include blockers of proteasome function and I κ B ubiquitination. Bortezomib, a proteasome inhibitor, has entered clinical development for the treatment of MM [138]. The ubiquitination of I κ B may be blocked by YopJ, I κ B α phosphopeptides, RNAi to β -TrCP and dominant-negative mutants of β -TrCP. The efficacy of this group of NF- κ B inhibitors is unlikely

to result from the specific inhibition of NF- κ B as they also block the degradation of other polyubiquitinated proteins.

Last, but not least, the nuclear functions of NF- κ B may be blocked by the inhibition of nuclear translocation, DNA binding or transactivation function of NF- κ B dimers. Cell-permeant peptides that contain the nuclear-localizing sequence of p50 can be used to saturate the nuclear import machineries to block the nuclear uptake of p50-containing NF- κ B dimers. Inhibitors of NF- κ B DNA binding include sesquiterpene lactones and decoy oligonucleotides that contain κ B sites. Decoy oligonucleotides are not favoured for drug development due to their large size and polar nature, which are likely to hinder cellular uptake and bioavailability [131].

FUTURE DIRECTIONS IN NF- κ B-BASED THERAPEUTICS

The use of animal models of inflammatory disorders and cancers has significantly improved our knowledge concerning the role of NF- κ B in the pathogenesis of these disorders, as well as to test the efficacies of NF- κ B-inhibitory drugs in the control of disease development and progression. Genetic ablation of *IKBKG* (NEMO), *IKBKB* (IKK2) and *CHUK* (IKK1) suggested that NF- κ B plays both positive and negative roles in different cell types in the pathogenesis of inflammation-linked cancer models for different organ systems [105]. The availability of suitable cell-type/tissue-specific promoters should allow the study of the role of NF- κ B signalling in the pathogenesis of other cancers such as that of the gastrointestinal tract and lung, as well as inflammatory disorders including rheumatoid arthritis and atherosclerosis. The cell-type-specific roles played by NF- κ B also suggest that targeted delivery of NF- κ B inhibitors is required for efficient therapy of the disease.

One caveat to the chronic use of NF- κ B inhibitors is the suppression of immune activation in the normal immune cells, which can lead to severe immunodeficiency. Hence the prolonged use of NF- κ B inhibitors is not desirable in cancer prevention. Strategies directed at the initial causes of persistent NF- κ B activation (microbial, viral infections or chemicals) may be more effective. When used in cancer therapy, NF- κ B inhibitors should be used intermittently for short durations [105].

Conventional inhibitors used to block inflammation and/or NF- κ B activity, including proteasome inhibitors, glucocorticoids, NSAIDs (non-steroidal anti-inflammatory drugs) and antioxidants, are not ideal, as they target multiple pathways and produce undesirable side effects, hence highlighting the importance of pathway-specific inhibitors [77]. Even though NF- κ B can be activated

by many stimuli, there are few convergence points or nodes in the signalling cascades which are common to many upstream stimuli. Efficient targeting of proteins at these signalling nodes can be used to shut down the activity of discrete pathways while leaving others intact [139]. The distinction between the IKK2- and/or IKK1-dependent pathways had led to the design of specific IKK2 inhibitors for the treatment of chronic inflammatory diseases and cancers, such as IBD and MM, without affecting the developmental processes regulated by IKK1 [131,140]. The involvement of either MyD88- or TRIF-dependent inflammatory processes in the pathogenesis of different chronic inflammatory disorders may provide an opportunity to minimize ALI via TRIF inhibition, while leaving the MyD88-dependent innate immune functions intact [113]. Polymorphisms in certain immune signalling genes are associated with elevated risks for cancer development. Such polymorphic genes may provide promising clues and directions to pathway-, disease- and patient-specific therapy.

Cancer and chronic inflammation are complex diseases involving defects in multiple pathways. A combinatorial therapy to block several key mediators in one or more cell types is likely to be a more effective strategy. Beside NF- κ B, other signalling pathways involved in inflammatory cytokine production that are potential therapeutic targets are AP-1 (activator protein-1), STAT3 and JNK1 [106]. Similarly, the mere inhibition of NF- κ B is insufficient for a pronounced apoptotic response to induce tumour regression. Instead, NF- κ B inhibitors can be used in combination with chemotherapeutic drugs or radiation to enhance tumour cell apoptosis [105].

Other than strategies to block positive NF- κ B-activating signals, enhancing the actions of negative-acting regulators, are also useful to curb hyperactivated NF- κ B signalling. Improving the efficacies of anti-inflammatory mediators and receptors [141], immune tolerance mechanisms [142,143] and antitumour immunity [106,144] hold promises for the therapy of cancers and chronic inflammatory disorders. It could be reasonably expected that, in the years to come, there will be a steady growth in more specific and efficacious therapeutic interventions based on NF- κ B signalling.

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REFERENCES

- 1 Hoffmann, A., Natoli, G. and Ghosh, G. (2006) Transcriptional regulation via the NF- κ B signaling module. *Oncogene* **25**, 6706–6716
- 2 Ghosh, S. and Karin, M. (2002) Missing pieces in the NF- κ B puzzle. *Cell* **109** (Suppl.), S81–S96
- 3 Lin, L., DeMartino, G. N. and Greene, W. C. (1998) Cotranslational biogenesis of NF- κ B p50 by the 26S proteasome. *Cell* **92**, 819–828
- 4 Hayden, M. S., West, A. P. and Ghosh, S. (2006) NF- κ B and the immune response. *Oncogene* **25**, 6758–6780
- 5 Reference deleted
- 6 Bonizzi, G., Bebién, M., Otero, D. C. et al. (2004) Activation of IKK α target genes depends on recognition of specific κ B binding sites by RelB:p52 dimers. *EMBO J.* **23**, 4202–4210
- 7 Pahl, H. L. (1999) Activators and target genes of Rel/NF- κ B transcription factors. *Oncogene* **18**, 6853–6866
- 8 Kawai, T. and Akira, S. (2007) Signaling to NF- κ B by Toll-like receptors. *Trends Mol. Med.* **13**, 460–469
- 9 Schulze-Luehrmann, J. and Ghosh, S. (2006) Antigen-receptor signaling to nuclear factor κ B. *Immunity* **25**, 701–715
- 10 Tergaonkar, V., Correa, R. G., Ikawa, M. and Verma, I. M. (2005) Distinct roles of I κ B proteins in regulating constitutive NF- κ B activity. *Nat. Cell Biol.* **7**, 921–923
- 11 Zhou, H., Wertz, I., O'Rourke, K. et al. (2004) Bcl10 activates the NF- κ B pathway through ubiquitination of NEMO. *Nature* **427**, 167–171
- 12 Hacker, H. and Karin, M. (2006) Regulation and function of IKK and IKK-related kinases. *Sci. STKE* **2006**, re13
- 13 Kanayama, A., Seth, R. B., Sun, L. et al. (2004) TAB2 and TAB3 activate the NF- κ B pathway through binding to polyubiquitin chains. *Mol. Cell* **15**, 535–548
- 14 Wang, C., Deng, L., Hong, M., Akkaraju, G. R., Inoue, J. and Chen, Z. J. (2001) TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* **412**, 346–351
- 15 Blonska, M., Shambharkar, P. B., Kobayashi, M. et al. (2005) TAK1 is recruited to the tumor necrosis factor- α (TNF- α) receptor 1 complex in a receptor-interacting protein (RIP)-dependent manner and cooperates with MEKK3 leading to NF- κ B activation. *J. Biol. Chem.* **280**, 43056–43063
- 16 Chen, Z. J. (2005) Ubiquitin signalling in the NF- κ B pathway. *Nat. Cell Biol.* **7**, 758–765
- 17 Krappmann, D. and Scheidereit, C. (2005) A pervasive role of ubiquitin conjugation in activation and termination of I κ B kinase pathways. *EMBO Rep.* **6**, 321–326
- 18 Matsuzawa, A., Tseng, P. H., Vallabhapurapu, S. et al. (2008) Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science* **321**, 663–668
- 19 Li, Q. and Verma, I. M. (2002) NF- κ B regulation in the immune system. *Nat. Rev. Immunol.* **2**, 725–734
- 20 Silverman, N. and Maniatis, T. (2001) NF- κ B signaling pathways in mammalian and insect innate immunity. *Genes Dev.* **15**, 2321–2342
- 21 Correa, R. G., Matsui, T., Tergaonkar, V., Rodriguez-Esteban, C., Izpisua-Belmonte, J. C. and Verma, I. M. (2005) Zebrafish I κ B kinase 1 negatively regulates NF- κ B activity. *Curr. Biol.* **15**, 1291–1295
- 22 Correa, R. G., Tergaonkar, V., Ng, J. K., Dubova, I., Izpisua-Belmonte, J. C. and Verma, I. M. (2004) Characterization of NF- κ B/I κ B proteins in Zebra fish and their involvement in notochord development. *Mol. Cell. Biol.* **24**, 5257–5268
- 23 Caamano, J. and Hunter, C. A. (2002) NF- κ B family of transcription factors: central regulators of innate and adaptive immune functions. *Clin. Microbiol. Rev.* **15**, 414–429
- 24 Beg, A. A., Sha, W. C., Bronson, R. T. and Baltimore, D. (1995) Constitutive NF- κ B activation, enhanced granulopoiesis, and neonatal lethality in I κ B α -deficient mice. *Genes Dev.* **9**, 2736–2746
- 25 Francois, S., El Benna, J., Dang, P. M., Pedruzzi, E., Gougerot-Pocidallo, M. A. and Elbim, C. (2005) Inhibition of neutrophil apoptosis by TLR agonists in whole blood: involvement of the phosphoinositide 3-kinase/Akt and NF- κ B signaling pathways, leading to increased levels of Mcl-1, A1, and phosphorylated Bad. *J. Immunol.* **174**, 3633–3642
- 26 Ouaz, F., Li, M. and Beg, A. A. (1999) A critical role for the RelA subunit of nuclear factor κ B in regulation of multiple immune-response genes and in Fas-induced cell death. *J. Exp. Med.* **189**, 999–1004
- 27 Sentman, C. L., Shutter, J. R., Hockenbery, D., Kanagawa, O. and Korsmeyer, S. J. (1991) Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. *Cell* **67**, 879–888
- 28 Luedde, T., Beraza, N., Kotsikoris, V. et al. (2007) Deletion of NEMO/IKK γ in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* **11**, 119–132
- 29 Petersen, S. L., Wang, L., Yalcin-Chin, A. et al. (2007) Autocrine TNF α signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell* **12**, 445–456
- 30 Varfolomeev, E., Blankenship, J. W., Wayson, S. M. et al. (2007) IAP antagonists induce autoubiquitination of c-IAPs, NF- κ B activation, and TNF α -dependent apoptosis. *Cell* **131**, 669–681
- 31 Vince, J. E., Wong, W. W., Khan, N. et al. (2007) IAP antagonists target cIAP1 to induce TNF α -dependent apoptosis. *Cell* **131**, 682–693
- 32 Alcamo, E., Hacohen, N., Schulte, L. C., Rennert, P. D., Hynes, R. O. and Baltimore, D. (2002) Requirement for the NF- κ B family member RelA in the development of secondary lymphoid organs. *J. Exp. Med.* **195**, 233–244
- 33 Dejardin, E. (2006) The alternative NF- κ B pathway from biochemistry to biology: pitfalls and promises for future drug development. *Biochem. Pharmacol.* **72**, 1161–1179
- 34 Ware, C. F. (2005) Network communications: lymphotoxins, LIGHT, and TNF. *Annu. Rev. Immunol.* **23**, 787–819
- 35 Vaira, S., Johnson, T., Hirbe, A. C. et al. (2008) RelB is the NF- κ B subunit downstream of NIK responsible for osteoclast differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 3897–3902
- 36 Torres, J. and Watt, F. M. (2008) Nanog maintains pluripotency of mouse embryonic stem cells by inhibiting NF κ B and cooperating with Stat3. *Nat. Cell Biol.* **10**, 194–201
- 37 Kawauchi, K., Araki, K., Tobiume, K. and Tanaka, N. (2008) p53 regulates glucose metabolism through an IKK-NF- κ B pathway and inhibits cell transformation. *Nat. Cell Biol.* **10**, 611–618
- 38 Rius, J., Guma, M., Schachtrup, C. et al. (2008) NF- κ B links innate immunity to the hypoxic response through transcriptional regulation of HIF-1 α . *Nature* **453**, 807–811
- 39 Perkins, N. D. (2007) Integrating cell-signalling pathways with NF- κ B and IKK function. *Nat. Rev. Mol. Cell Biol.* **8**, 49–62
- 40 Suzuki, K. and Verma, I. M. (2008) Phosphorylation of SNAP-23 by I κ B kinase 2 regulates mast cell degranulation. *Cell* **134**, 485–495
- 41 Irelan, J. T., Murphy, T. J., DeJesus, P. D. et al. (2007) A role for I κ B kinase 2 in bipolar spindle assembly. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 16940–16945
- 42 Perkins, N. D. (2006) Post-translational modifications regulating the activity and function of the nuclear factor κ B pathway. *Oncogene* **25**, 6717–6730
- 43 Ang, H. L. and Tergaonkar, V. (2007) Notch and NF κ B signaling pathways: do they collaborate in normal vertebrate brain development and function? *BioEssays* **29**, 1039–1047
- 44 Gerondakis, S., Grossmann, M., Nakamura, Y., Pohl, T. and Grumont, R. (1999) Genetic approaches in mice to understand Rel/NF- κ B and I κ B function: transgenics and knockouts. *Oncogene* **18**, 6888–6895

- 45 Saccani, S., Pantano, S. and Natoli, G. (2003) Modulation of NF- κ B activity by exchange of dimers. *Mol. Cell* **11**, 1563–1574
- 46 Leung, T. H., Hoffmann, A. and Baltimore, D. (2004) One nucleotide in a κ B site can determine cofactor specificity for NF- κ B dimers. *Cell* **118**, 453–464
- 47 Saccani, S., Pantano, S. and Natoli, G. (2002) p38-Dependent marking of inflammatory genes for increased NF- κ B recruitment. *Nat. Immunol.* **3**, 69–75
- 48 Anest, V., Hanson, J. L., Cogswell, P. C., Steinbrecher, K. A., Strahl, B. D. and Baldwin, A. S. (2003) A nucleosomal function for I κ B kinase- α in NF- κ B-dependent gene expression. *Nature* **423**, 659–663
- 49 Yamamoto, Y., Verma, U. N., Prajapati, S., Kwak, Y. T. and Gaynor, R. B. (2003) Histone H3 phosphorylation by IKK- α is critical for cytokine-induced gene expression. *Nature* **423**, 655–659
- 50 Panne, D., Maniatis, T. and Harrison, S. C. (2007) An atomic model of the interferon- β enhanceosome. *Cell* **129**, 1111–1123
- 51 Chen, Z. J., Bhoj, V. and Seth, R. B. (2006) Ubiquitin, TAK1 and IKK: is there a connection? *Cell Death Differ.* **13**, 687–692
- 52 Johnston, J. A. and Burrows, J. F. (2006) De-ubiquitinating enzymes: intracellular signalling and disease. *Biochem. Soc. Trans.* **34**, 764–769
- 53 Dong, J., Jimi, E., Zhong, H., Hayden, M. S. and Ghosh, S. (2008) Repression of gene expression by unphosphorylated NF- κ B p65 through epigenetic mechanisms. *Genes Dev.* **22**, 1159–1173
- 54 Hoberg, J. E., Yeung, F. and Mayo, M. W. (2004) SMRT derepression by the I κ B kinase α : a prerequisite to NF- κ B transcription and survival. *Mol. Cell* **16**, 245–255
- 55 Chen, J., Zhou, Y., Mueller-Steiner, S. et al. (2005) SIRT1 protects against microglia-dependent amyloid- β toxicity through inhibiting NF- κ B signaling. *J. Biol. Chem.* **280**, 40364–40374
- 56 Chen, L. F., Mu, Y. and Greene, W. C. (2002) Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF- κ B. *EMBO J.* **21**, 6539–6548
- 57 Imbert, V., Rupec, R. A., Livolsi, A. et al. (1996) Tyrosine phosphorylation of I κ B- α activates NF- κ B without proteolytic degradation of I κ B- α . *Cell* **86**, 787–798
- 58 You, M., Flick, L. M., Yu, D. and Feng, G. S. (2001) Modulation of the nuclear factor κ B pathway by Shp-2 tyrosine phosphatase in mediating the induction of interleukin (IL)-6 by IL-1 or tumor necrosis factor. *J. Exp. Med.* **193**, 101–110
- 59 Reiley, W., Zhang, M. and Sun, S. C. (2004) Negative regulation of JNK signaling by the tumor suppressor CYLD. *J. Biol. Chem.* **279**, 55161–55167
- 60 Massoumi, R., Chmielarska, K., Hennecke, K., Pfeifer, A. and Fassler, R. (2006) Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF- κ B signaling. *Cell* **125**, 665–677
- 61 Brummelkamp, T. R., Nijman, S. M., Dirac, A. M. and Bernards, R. (2003) Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF- κ B. *Nature* **424**, 797–801
- 62 Kovalenko, A., Chable-Bessia, C., Cantarella, G., Israel, A., Wallach, D. and Courtois, G. (2003) The tumour suppressor CYLD negatively regulates NF- κ B signalling by deubiquitination. *Nature* **424**, 801–805
- 63 Sagar, S., Chernoff, K. A., Lodha, S. et al. (2008) CYLD mutations in familial skin appendage tumours. *J. Med. Genet.* **45**, 298–302
- 64 Trompouki, E., Hatzivassiliou, E., Tschritzis, T., Farmer, H., Ashworth, A. and Mosialos, G. (2003) CYLD is a deubiquitinating enzyme that negatively regulates NF- κ B activation by TNFR family members. *Nature* **424**, 793–796
- 65 Zhang, J., Stirling, B., Temmerman, S. T. et al. (2006) Impaired regulation of NF- κ B and increased susceptibility to colitis-associated tumorigenesis in CYLD-deficient mice. *J. Clin. Invest.* **116**, 3042–3049
- 66 Reiley, W. W., Zhang, M., Jin, W. et al. (2006) Regulation of T cell development by the deubiquitinating enzyme CYLD. *Nat. Immunol.* **7**, 411–417
- 67 Opiari, Jr, A. W., Boguski, M. S. and Dixit, V. M. (1990) The A20 cDNA induced by tumor necrosis factor α encodes a novel type of zinc finger protein. *J. Biol. Chem.* **265**, 14705–14708
- 68 Krikos, A., Laherty, C. D. and Dixit, V. M. (1992) Transcriptional activation of the tumor necrosis factor α -inducible zinc finger protein, A20, is mediated by κ B elements. *J. Biol. Chem.* **267**, 17971–17976
- 69 Boone, D. L., Turer, E. E., Lee, E. G. et al. (2004) The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat. Immunol.* **5**, 1052–1060
- 70 Hutti, J. E., Turk, B. E., Asara, J. M., Ma, A., Cantley, L. C. and Abbott, D. W. (2007) I κ B kinase β phosphorylates the K63 deubiquitinase A20 to cause feedback inhibition of the NF- κ B pathway. *Mol. Cell Biol.* **27**, 7451–7461
- 71 Shembade, N., Harhaj, N. S., Parvatiyar, K. et al. (2008) The E3 ligase Itch negatively regulates inflammatory signaling pathways by controlling the function of the ubiquitin-editing enzyme A20. *Nat. Immunol.* **9**, 254–262
- 72 Lee, E. G., Boone, D. L., Chai, S. et al. (2000) Failure to regulate TNF-induced NF- κ B and cell death responses in A20-deficient mice. *Science* **289**, 2350–2354
- 73 Newton, K., Matsumoto, M. L., Wertz, I. E. et al. (2008) Ubiquitin chain editing revealed by polyubiquitin linkage-specific antibodies. *Cell* **134**, 668–678
- 74 Evans, P. C., Taylor, E. R., Coadwell, J., Heyninck, K., Beyaert, R. and Kilshaw, P. J. (2001) Isolation and characterization of two novel A20-like proteins. *Biochem. J.* **357**, 617–623
- 75 Tzimas, C., Michailidou, G., Arsenakis, M., Kieff, E., Mosialos, G. and Hatzivassiliou, E. G. (2006) Human ubiquitin specific protease 31 is a deubiquitinating enzyme implicated in activation of nuclear factor- κ B. *Cell. Signaling* **18**, 83–92
- 76 Zhou, H., Monack, D. M., Kayagaki, N. et al. (2005) Yersinia virulence factor YopJ acts as a deubiquitinase to inhibit NF- κ B activation. *J. Exp. Med.* **202**, 1327–1332
- 77 Tergaonkar, V., Li, Q. and Verma, I. (2006) Inhibitors of NF κ B activity: tools for treatment of human ailments. In *NF- κ B/Rel Transcription Factor Family* (Liou, H.-C., ed.), pp. 162–170, Springer, Berlin
- 78 Hoffmann, A., Levchenko, A., Scott, M. L. and Baltimore, D. (2002) The I κ B-NF- κ B signaling module: temporal control and selective gene activation. *Science* **298**, 1241–1245
- 79 Kearns, J. D., Basak, S., Werner, S. L., Huang, C. S. and Hoffmann, A. (2006) I κ B ϵ provides negative feedback to control NF- κ B oscillations, signaling dynamics, and inflammatory gene expression. *J. Cell Biol.* **173**, 659–664
- 80 Delhase, M., Hayakawa, M., Chen, Y. and Karin, M. (1999) Positive and negative regulation of I κ B kinase activity through IKK β subunit phosphorylation. *Science* **284**, 309–313
- 81 Prajapati, S., Verma, U., Yamamoto, Y., Kwak, Y. T. and Gaynor, R. B. (2004) Protein phosphatase 2C β association with the I κ B kinase complex is involved in regulating NF- κ B activity. *J. Biol. Chem.* **279**, 1739–1746
- 82 Li, H. Y., Liu, H., Wang, C. H. et al. (2008) Deactivation of the kinase IKK by CUEDC2 through recruitment of the phosphatase PPI. *Nat. Immunol.* **9**, 533–541
- 83 Li, S., Wang, L., Berman, M. A., Zhang, Y. and Dorf, M. E. (2006) RNAi screen in mouse astrocytes identifies phosphatases that regulate NF- κ B signaling. *Mol. Cell* **24**, 497–509
- 84 An, H., Hou, J., Zhou, J. et al. (2008) Phosphatase SHP-1 promotes TLR- and RIG-I-activated production of type I interferon by inhibiting the kinase IRAK1. *Nat. Immunol.* **9**, 542–550
- 85 Ananieva, O., Darragh, J., Johansen, C. et al. (2008) The kinases MSK1 and MSK2 act as negative regulators of Toll-like receptor signaling. *Nat. Immunol.* **9**, 1028–1036
- 86 Chen, L., Fischle, W., Verdin, E. and Greene, W. C. (2001) Duration of nuclear NF- κ B action regulated by reversible acetylation. *Science* **293**, 1653–1657

- 87 Garkavtsev, I., Kozin, S. V., Chernova, O. et al. (2004) The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. *Nature* **428**, 328–332
- 88 Sobic, D., Richardson, J. A., Yu, K., Ornitz, D. M. and Olson, E. N. (2003) Twist regulates cytokine gene expression through a negative feedback loop that represses NF- κ B activity. *Cell* **112**, 169–180
- 89 Courtois, G. and Smahi, A. (2006) NF- κ B-related genetic diseases. *Cell Death Differ.* **13**, 843–851
- 90 Smahi, A., Courtois, G., Vabres, P. et al. (2000) Genomic rearrangement in NEMO impairs NF- κ B activation and is a cause of incontinentia pigmenti. The International Incontinentia Pigmenti (IP) Consortium. *Nature* **405**, 466–472
- 91 Pasparakis, M., Courtois, G., Hafner, M. et al. (2002) TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. *Nature* **417**, 861–866
- 92 Bustamante, J., Boisson-Dupuis, S., Jouanguy, E. et al. (2008) Novel primary immunodeficiencies revealed by the investigation of paediatric infectious diseases. *Curr. Opin. Immunol.* **20**, 39–48
- 93 von Bernuth, H., Picard, C., Jin, Z. et al. (2008) Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* **321**, 691–696
- 94 Hanahan, D. and Weinberg, R. A. (2000) The hallmarks of cancer. *Cell* **100**, 57–70
- 95 Bissell, M. J. and Radisky, D. (2001) Putting tumours in context. *Nat. Rev. Cancer* **1**, 46–54
- 96 Basseres, D. S. and Baldwin, A. S. (2006) Nuclear factor- κ B and inhibitor of κ B kinase pathways in oncogenic initiation and progression. *Oncogene* **25**, 6817–6830
- 97 Karin, M., Cao, Y., Greten, F. R. and Li, Z. W. (2002) NF- κ B in cancer: from innocent bystander to major culprit. *Nat. Rev. Cancer* **2**, 301–310
- 98 Hussain, S. P., Hofseth, L. J. and Harris, C. C. (2003) Radical causes of cancer. *Nat. Rev. Cancer* **3**, 276–285
- 99 Tergaonkar, V. and Perkins, N. D. (2007) p53 and NF- κ B crosstalk: IKK α tips the balance. *Mol. Cell* **26**, 158–159
- 100 Elgert, K. D., Alleva, D. G. and Mullins, D. W. (1998) Tumor-induced immune dysfunction: the macrophage connection. *J. Leukocyte Biol.* **64**, 275–290
- 101 Lenz, G., Davis, R. E., Ngo, V. N. et al. (2008) Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science* **319**, 1676–1679
- 102 Annunziata, C. M., Davis, R. E., Demchenko, Y. et al. (2007) Frequent engagement of the classical and alternative NF- κ B pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* **12**, 115–130
- 103 Keats, J. J., Fonseca, R., Chesi, M. et al. (2007) Promiscuous mutations activate the noncanonical NF- κ B pathway in multiple myeloma. *Cancer Cell* **12**, 131–144
- 104 Ho, L., Davis, R. E., Conne, B. et al. (2005) MALT1 and the API2-MALT1 fusion act between CD40 and IKK and confer NF- κ B-dependent proliferative advantage and resistance against FAS-induced cell death in B cells. *Blood* **105**, 2891–2899
- 105 Karin, M. (2006) Nuclear factor- κ B in cancer development and progression. *Nature* **441**, 431–436
- 106 Lin, W. W. and Karin, M. (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. *J. Clin. Invest.* **117**, 1175–1183
- 107 Naugler, W. E. and Karin, M. (2008) The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. *Trends Mol. Med.* **14**, 109–119
- 108 El-Omar, E. M., Ng, M. T. and Hold, G. L. (2008) Polymorphisms in Toll-like receptor genes and risk of cancer. *Oncogene* **27**, 244–252
- 109 Bosch, F. X., Ribes, J., Diaz, M. and Cléries, R. (2004) Primary liver cancer: worldwide incidence and trends. *Gastroenterology* **127**, S5–S16
- 110 Naugler, W. E., Sakurai, T., Kim, S. et al. (2007) Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* **317**, 121–124
- 111 Greten, F. R., Eckmann, L., Greten, T. F. et al. (2004) IKK β links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118**, 285–296
- 112 Kalaitzidis, D. and Gilmore, T. D. (2005) Transcription factor cross-talk: the estrogen receptor and NF- κ B. *Trends Endocrinol. Metab.* **16**, 46–52
- 113 Imai, Y., Kuba, K., Neely, G. G. et al. (2008) Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell* **133**, 235–249
- 114 Cai, D., Frantz, J. D., Tawa, Jr, N. E. et al. (2004) IKK β /NF- κ B activation causes severe muscle wasting in mice. *Cell* **119**, 285–298
- 115 Arkan, M. C., Hevener, A. L., Greten, F. R. et al. (2005) IKK- β links inflammation to obesity-induced insulin resistance. *Nat. Med.* **11**, 191–198
- 116 Cai, D., Yuan, M., Frantz, D. F. et al. (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK- β and NF- κ B. *Nat. Med.* **11**, 183–190
- 117 Yuan, M., Konstantopoulos, N., Lee, J. et al. (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikk β . *Science* **293**, 1673–1677
- 118 Hirosumi, J., Tuncman, G., Chang, L. et al. (2002) A central role for JNK in obesity and insulin resistance. *Nature* **420**, 333–336
- 119 Solinas, G., Vilcu, C., Neels, J. G. et al. (2007) JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab.* **6**, 386–397
- 120 Bhakar, A. L., Tannis, L. L., Zeindler, C. et al. (2002) Constitutive nuclear factor- κ B activity is required for central neuron survival. *J. Neurosci.* **22**, 8466–8475
- 121 Lu, Z. Y., Yu, S. P., Wei, J. F. and Wei, L. (2006) Age-related neural degeneration in nuclear-factor κ B p50 knockout mice. *Neuroscience* **139**, 965–978
- 122 Gutierrez, H., Hale, V. A., Dolcet, X. and Davies, A. (2005) NF- κ B signalling regulates the growth of neural processes in the developing PNS and CNS. *Development* **132**, 1713–1726
- 123 Memet, S. (2006) NF- κ B functions in the nervous system: from development to disease. *Biochem. Pharmacol.* **72**, 1180–1195
- 124 Pizzi, M., Sarnico, I., Boroni, F. et al. (2005) NF- κ B factor c-Rel mediates neuroprotection elicited by mGlu5 receptor agonists against amyloid β -peptide toxicity. *Cell Death Differ.* **12**, 761–772
- 125 Barger, S. W., Horster, D., Furukawa, K., Goodman, Y., Kriegstein, J. and Mattson, M. P. (1995) Tumor necrosis factors α and β protect neurons against amyloid β -peptide toxicity: evidence for involvement of a κ B-binding factor and attenuation of peroxide and Ca²⁺ accumulation. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9328–9332
- 126 Barger, S. W. and Harmon, A. D. (1997) Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature* **388**, 878–881
- 127 Combs, C. K., Karlo, J. C., Kao, S. C. and Landreth, G. E. (2001) β -Amyloid stimulation of microglia and monocytes results in TNF α -dependent expression of inducible nitric oxide synthase and neuronal apoptosis. *J. Neurosci.* **21**, 1179–1188
- 128 Gilmore, T. D. and Herscovitch, M. (2006) Inhibitors of NF- κ B signaling: 785 and counting. *Oncogene* **25**, 6887–6899
- 129 Feldmann, M. and Maini, R. N. (2003) TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat. Med.* **9**, 1245–1250
- 130 Song, X. Y., Torphy, T. J., Griswold, D. E. and Shealy, D. (2002) Coming of age: anti-cytokine therapies. *Mol. Interv.* **2**, 36–46
- 131 Karin, M., Yamamoto, Y. and Wang, Q. M. (2004) The IKK NF- κ B system: a treasure trove for drug development. *Nat. Rev. Drug Discov.* **3**, 17–26
- 132 Dredge, K., Dalgleish, A. G. and Marriott, J. B. (2003) Thalidomide analogs as emerging anti-cancer drugs. *Anticancer Drugs* **14**, 331–335

- 133 Keifer, J. A., Guttridge, D. C., Ashburner, B. P. and Baldwin, Jr, A. S. (2001) Inhibition of NF- κ B activity by thalidomide through suppression of I κ B kinase activity. *J. Biol. Chem.* **276**, 22382–22387
- 134 Lam, L. T., Davis, R. E., Pierce, J. et al. (2005) Small molecule inhibitors of I κ B kinase are selectively toxic for subgroups of diffuse large B-cell lymphoma defined by gene expression profiling. *Clin. Cancer Res.* **11**, 28–40
- 135 May, M. J., D'Acquisto, F., Madge, L. A., Glockner, J., Pober, J. S. and Ghosh, S. (2000) Selective inhibition of NF- κ B activation by a peptide that blocks the interaction of NEMO with the I κ B kinase complex. *Science* **289**, 1550–1554
- 136 May, M. J., Marienfeld, R. B. and Ghosh, S. (2002) Characterization of the I κ B-kinase NEMO binding domain. *J. Biol. Chem.* **277**, 45992–46000
- 137 Jimi, E., Aoki, K., Saito, H. et al. (2004) Selective inhibition of NF- κ B blocks osteoclastogenesis and prevents inflammatory bone destruction *in vivo*. *Nat. Med.* **10**, 617–624
- 138 Lenz, H. J. (2003) Clinical update: proteasome inhibitors in solid tumors. *Cancer Treat. Rev.* **29** (Suppl. 1), 41–48
- 139 Tergaonkar, V. (2006) NF κ B pathway: a good signaling paradigm and therapeutic target. *Int. J. Biochem. Cell. Biol.* **38**, 1647–1653
- 140 Kim, H. J., Hawke, N. and Baldwin, A. S. (2006) NF- κ B and IKK as therapeutic targets in cancer. *Cell Death Differ.* **13**, 738–747
- 141 Lawrence, T., Willoughby, D. A. and Gilroy, D. W. (2002) Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat. Rev. Immunol.* **2**, 787–795
- 142 Biswas, S. K. and Tergaonkar, V. (2007) Myeloid differentiation factor 88-independent Toll-like receptor pathway: sustaining inflammation or promoting tolerance? *Int. J. Biochem. Cell. Biol.* **39**, 1582–1592
- 143 Wessells, J., Baer, M., Young, H. A. et al. (2004) BCL-3 and NF- κ B p50 attenuate lipopolysaccharide-induced inflammatory responses in macrophages. *J. Biol. Chem.* **279**, 49995–50003
- 144 Peggs, K. S., Segal, N. H. and Allison, J. P. (2007) Targeting immunosuppressive cancer therapies: accentuate the positive, eliminate the negative. *Cancer Cell* **12**, 192–199

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