THE NEXT 10 YEARS

The diverse and complex roles of NF-κB subunits in cancer

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Abstract | It is only recently that the full importance of nuclear factor-κB (NF-κB) signalling to cancer development has been understood. Although much attention has focused on the upstream pathways leading to NF-κB activation, it is now becoming clear that the inhibitor of NF-κB kinases (IKKs), which regulate NF-κB activation, have many independent functions in tissue homeostasis and normal immune function that could compromise the clinical utility of IKK inhibitors. Therefore, if the NF-κB pathway is to be properly exploited as a target for both anticancer and anti-inflammatory drugs, it is appropriate to reconsider the complex roles of the individual NF-κB subunits.

Since the realization more than 20 years ago that nuclear factor-κB (NF-κB) subunits have homology to the viral oncogene v-Rel, a role for these proteins in tumorigenesis has been accepted. Although research initially focused on the important role of NF-κB as a regulator of the immune response, the full importance of NF-κB signalling to cancer has been understood only quite recently, concomitant with an appreciation for the crucial role of the inflammatory response in cancer development[1,2]. Much attention has subsequently focused on the upstream pathways leading to NF-κB activation, resulting in the development of drugs that inhibit the function of the inhibitor of NF-κB kinases (IKKs), which are required for NF-κB activation in response to the majority of known inducers of NF-κB activity[3,4]. However, it is now becoming clear that IKKs have many independent functions[5,6]. Consequently, drugs targeting IKKs are not specific inhibitors of NF-κB transcriptional activity and will also have many off-target effects. Therefore, if the NF-κB pathway is to be properly exploited as a target for both anticancer and anti-inflammatory drugs, it is appropriate to reconsider the roles of the individual NF-κB subunits. This Review discusses the functions of the NF-κB subunits in tumorigenesis and in the response to current cancer therapies, together with potential routes through which this knowledge can be exploited for future drug development.

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The NF-κB signalling pathway

There are five proteins that make up the mammalian NF-κB subunit family, which all share a related DNA-binding and dimerization domain, termed the REL homology domain (RHD) (FIG. 1). The carboxyl termini of RELA (also known as transcription factor p65), RELB and REL (also known as c-Rel) all contain transactivation domains, which are capable of mediating interactions with basal transcription factors and cofactors, such as TATA binding protein (TBP), TFIIB, E1A binding protein 300KD (EP300; also known as p300) and CREB binding protein (CBP)[7,8]. The other two family members, NF-κB1 (also known as p105) and NF-κB2 (also known as p100) encode longer precursor proteins that can be processed, either during translation or through phosphorylation-induced partial proteolysis, to the active DNA-binding forms p50 and p52, respectively[9].

NF-κB activation constitutes a rapidly inducible first line of defence against infection and stress. As such, NF-κB complexes exist in a pre-synthesized form, poised for activation, whereupon they can determine the cellular and organismal response to danger. To keep NF-κB in this inactive state, there exists a family of inhibitor of NF-κB (IκB) proteins consisting of IκBα, IκBβ and IκBε[9]. Typically, IκBs bind to NF-κB complexes, inhibiting their DNA binding while keeping them in a predominantly cytoplasmic form, before exposure to an inducing stimulus. This results in the phosphorylation of the IκBs by the IκB kinase (IKK) complex, promoting their ubiquitylation and proteasome-mediated degradation with consequent NF-κB nuclear localization[10]. p100 and p105 also contain, in their C termini, the ankyrin repeat motifs that are found in the IκBs, which can mediate interaction with NF-κB subunits and which can themselves function as IκB proteins. IκB proteins have other functions in addition to this simple cytoplasmic retention and inhibition model. For example, IκBα can localize to the nucleus, bind to and remove NF-κB complexes

At a glance

- The nuclear factor-κB (NF-κB)–inhibitor of NF-κB kinase (IKK) pathway can promote the growth and survival of many solid and haematological malignancies and therefore has the potential to provide numerous targets for novel anticancer therapies.
- Most attention has focused on the development of IKKβ inhibitors, but it is now clear that IKKβ has many NF-κB-independent functions and its inhibition could result in undesired effects.
- Although it is apparent that NF-κB subunits have important roles in tumorigenesis and the response to cancer therapy, their individual contributions have not been clearly defined.
- The NF-κB response is highly pleiotropic and the consequences of its activation can be context dependent. NF-κB is not always tumour promoting and it can exhibit tumour suppressor-like activities.
- Crosstalk with tumour-suppressor proteins, such as p53, provides an important mechanism for regulating NF-κB activity and function in cancer. Tumour suppressors can inhibit the tumour-promoting activities of NF-κB subunits while facilitating their ability to suppress cancer progression.
- Understanding the regulation and function of the NF-κB subunits in cancer provides opportunities for the development of new therapies and allows the better use of existing drugs that affect NF-κB–IKK activity.

from promoter DNA and, at select promoters, IκBβ can prevent this[11]. Moreover, p105 is associated with activation of the MAPK–ERK signalling pathway through binding to MAP3K8 (also known as TPL2)[12,13].

Numerous and diverse stimuli can induce NF-κB activity. Typical inducers of NF-κB include cytokines, such as tumour necrosis factor (TNF), interleukin-1 (IL-1), viral and bacterial products, such as lipopolysaccharide (LPS), which can induce Toll-like receptor (TLR) signalling, and cellular stress, such as DNA damage and hypoxia. Although receptor proximal events or intracellular stimuli use signal-specific adaptor proteins that frequently involve ubiquitin-based scaffolds and signalling pathways[14], most of these inducers converge on IKK (FIG. 2).

NF-κB function and cancer

NF-κB can transcriptionally regulate a diverse array of genes. In normal circumstances, these genes comprehensively re-programme the pattern of gene expression of a cell to cope with a threat to the organism. NF-κB target genes encode proteins and microRNAs (miRNAs) that regulate a wide range of biological effects. These include cytokines, chemokines and their respective receptors, which are traditionally associated with the important role of NF-κB in the inflammatory response, together with genes regulating cell survival, proliferation, cell adhesion and the cellular microenviroment[15–21]. The outcome of NF-κB activation, in terms of the effects on gene expression, will vary, depending on the tissue or cell type. For example, although a number of the core target genes expressed may be similar, the NF-κB response to LPS in a macrophage will show differences from the NF-κB response to DNA damage in an epithelial cell. Although the NF-κB gene targets may be similar between normal and cancer cells, what will differ is the 'appropriateness' of their regulation. For example, tumour cell NF-κB targets may show sustained induction (or repression) of their expression, resulting from the loss of negative feedback control mechanisms. NF-κB activity in cancer cells can be thought of as a malignant reflection of its normal behaviour in protecting the organism from danger.

It is striking how closely the functional consequences of aberrant NF-κB activation correlate with the hallmarks of cancer[22] (FIG. 3). These consequences include an ability to promote cancer cell survival by inducing the expression of anti-apoptotic genes; induction of cell proliferation by inducing the expression of cyclins and proto-oncogenes; promotion of metastasis by regulating the expression of matrix metalloproteinase and cell adhesion genes; and stimulation of angiogenesis by regulating genes associated with the growth of new blood vessels[16–18,20]. Moreover, NF-κB can help to promote a metabolic switch in cancer cells from oxidative phosphorylation to glycolysis (the Warburg effect) by inducing the expression of glycolytic enzymes while also directly repressing mitochondrial gene expression[23,24]. The core ability of the IKK–NF-κB pathway to induce inflammation means that it is a crucial component in the link between chronic inflammatory conditions and cancer[25]. Indeed, there is a growing appreciation of the role that inflammation has in many types of cancer[1,26], underlying the important role of NF-κB, in conjunction with other proteins such as signal transduction and activator of transcription 3 (STAT3)[27,28], as a tumour-promoting transcription factor. In this context, the function of NF-κB as a tumour promoter is not limited to intrinsic effects within transformed tumour cells — NF-κB also influences the function of infiltrating lymphocytes and macrophages[25,29,30].

In the non-disease state, an NF-κB response is automatically self-limiting, through the induction of negative feedback loops. These include the transcription of *NFKBIA* (encoding IκBα) and *NFKBIE* (encoding IκBε), together with genes that encode proteins that negatively regulate the signalling pathways leading to IKK activation, such as *TNFAIP3* (which encodes A20 (REFS 31–33)) (FIG. 4). However, NF-κB activity becomes deregulated in cancer. This can occur either through mutations leading to intrinsically high levels of IKK–NF-κB signalling within the tumour cell or through continuous exposure to NF-κB activating stimuli, such as cytokine release by tumour-associated macrophages (TAMs)[16,25,34–37]. The list of tumour types displaying aberrant NF-κB activity is extensive and includes many solid tumours, as well as leukaemias and lymphomas[4]. As NF-κB is also induced by many common cancer chemotherapeutic drugs[16,20], NF-κB activity can potentially regulate the survival and malignancy of most, if not all, tumours.

Despite all of this knowledge, the influence of the different NF-κB subunits on cancer development remains unclear. Extrapolation from cell culture experiments suggests that the role of individual NF-κB subunits in different cancer types can vary. However, knowledge of the specific functional roles of individual subunits still remains limited. In part this derives from the difficulty of applying techniques used to evaluate NF-κB function in cell lines, such as reporter gene assays, RNA interference and chromatin immunoprecipitation (ChIP) analysis, to tumour samples. Moreover, the use of subunit mutations, which can be used to provide insight into the function

MicroRNAs
These single-stranded RNAs are approximately 21 to 23 nucleotides in length and regulate gene expression by partial complementary base pairing to mRNAs and recruitment to the RNA-induced silencing complex to inhibit translation (and possibly increase degradation) of mRNA.

Warburg effect
Named after a discovery made by the German biochemist Otto Warburg in the 1920s that cancer cells predominantly use anaerobic glycolysis rather than oxidative phosphorylation, even when oxygen is abundant. As a result, pyruvate is converted to lactate instead of being oxidized by the mitochondria of cancer cells.

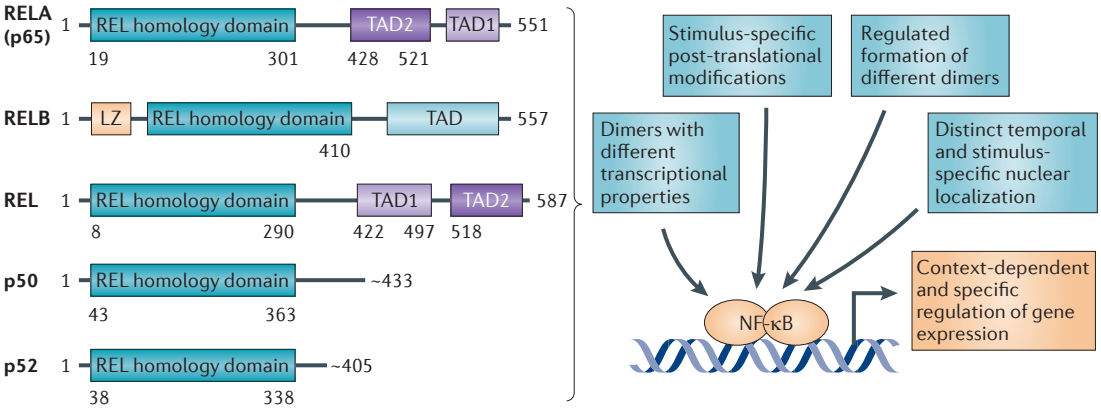

Figure 1 | **NF-κB subunits and the mechanisms that control context-specific gene regulation.** The term nuclear factor-κB (NF-κB) collectively describes the various homodimers and heterodimers that can be formed from the five mammalian NF-κB subunits. These all share an approximately 300 amino acid-long DNA binding and dimerization domain that is termed the REL homology domain (RHD). RELA, RELB and REL all contain carboxy-terminal transactivation domains (TADs), and RELB has an amino-terminal leucine zipper (LZ)-like motif. p52 and p50 are derived from proteolysis of their precursor proteins p100 and p105, respectively (not shown). Context-specific gene regulation by NF-κB is determined by a number of factors, including the selective activation of different dimers, as well as their different DNA binding and transactivation properties, together with post-translational modifications.

and the contribution of post-translational modifications (PTMs) (discussed below), are also not applicable to tumour analysis. Instead, the evaluation of NF-κB activity in tumours relies either on electrophoretic mobility shift analysis (EMSA) of DNA binding in protein extracts, or, more commonly, on immunofluorescent or immunohistochemical analysis of nuclear localization, which do not in themselves provide functional insights. Although PTM-specific antibodies can give some clues about activity, their accurate use is dependent on the quality of the tumour tissue used. Therefore, when nuclear NF-κB subunits are observed in tumours, there is frequently an assumption as to their activity, which can only be inferred by careful correlation with specific biomarkers, such as the expression levels of known target genes.

The lack of clarity of NF-κB subunit roles in tumours also derives from the types of mouse models being used. Most studies in this area have relied on IKK and NF-κB essential modifier (NEMO; also known as IKKγ)-knockout strains or mutants, or the expression of a mutated, degradation-resistant form of IκBα (the IκB super-repressor). All of these models result in the inhibition of multiple NF-κB complexes (see, for example, REFS 25,38–40). Consequently, this often means it is hard to disentangle NF-κB-independent IKK effects from those that are actually mediated by NF-κB subunits. Indeed, the tumour-promoting effects seen in these mouse models are likely to derive from a combination of both. Some studies have confirmed the important role of specific NF-κB subunits, such as the role of RELA in a mouse model of lung adenocarcinoma[41]. Nonetheless, this is an area that requires further investigation if the specific roles of NF-κB subunits are to be understood. Moreover, traditional gene knockouts may not provide the best route to achieve this: loss of an NF-κB subunit results in a rebalancing of the other subunits, which can frequently provide a partial compensatory effect[42–44]. Furthermore, because RELA can regulate the expression of the other NF-κB subunits[15], knock-on effects are possible. Therefore, the creation of specific subunit mutants that are designed to inhibit aspects of their function (or to mimic tumour-associated mutants), might provide a route to a greater understanding of the specific roles these proteins have in tumour cells. Currently, all the data point to their importance, but the precise transcriptional mechanisms involved, in contrast to upstream signalling, are largely vague and based on interpreting studies in cancer cell lines, which may not reflect the *in vivo* situation.

The nature of NF-κB deregulation in cancer. Genetic mutations that lead to aberrant nuclear NF-κB in tumours can generally take two forms. Oncogenes such as *HRAS* or *BCR–ABL1*, together with positive regulators, such as NF-κB inducing kinase (NIK), undergo gain-of-function mutations that lead to continuous signalling to the IKK complex. Alternatively, mutations that disrupt negative regulators such as CYLD or A20 lead to a failure to appropriately switch off NF-κB[16,31,36,45]. Direct activation of NF-κB complexes through the loss of the inhibitory proteins IκBα and IκBε have also been reported in Hodgkin's lymphoma, but this does not seem to be a common occurrence in other tumour types[34]. Although NEMO mutations that are associated with inherited diseases such as incontinentia pigmenti have been described, activating NEMO mutations in cancer have not been reported[34]. And, although some missense mutations of IKKα (also known as IKK1 and CHUK) and IKKβ (also known as IKK2) have been found[46], whether these are tumour promoting in their own right has not been investigated, despite the fact that constitutively active IKKβ can drive colorectal cancer in mice[47].

As v-Rel is a potent oncogene in B cells[48], and other proto-oncogenes that were identified as viral oncogenes, such as HRAS, are mutated in human cancers, it might be expected that the NF-κB subunits are subject to transforming mutations. Although *NFKB2* translocation,

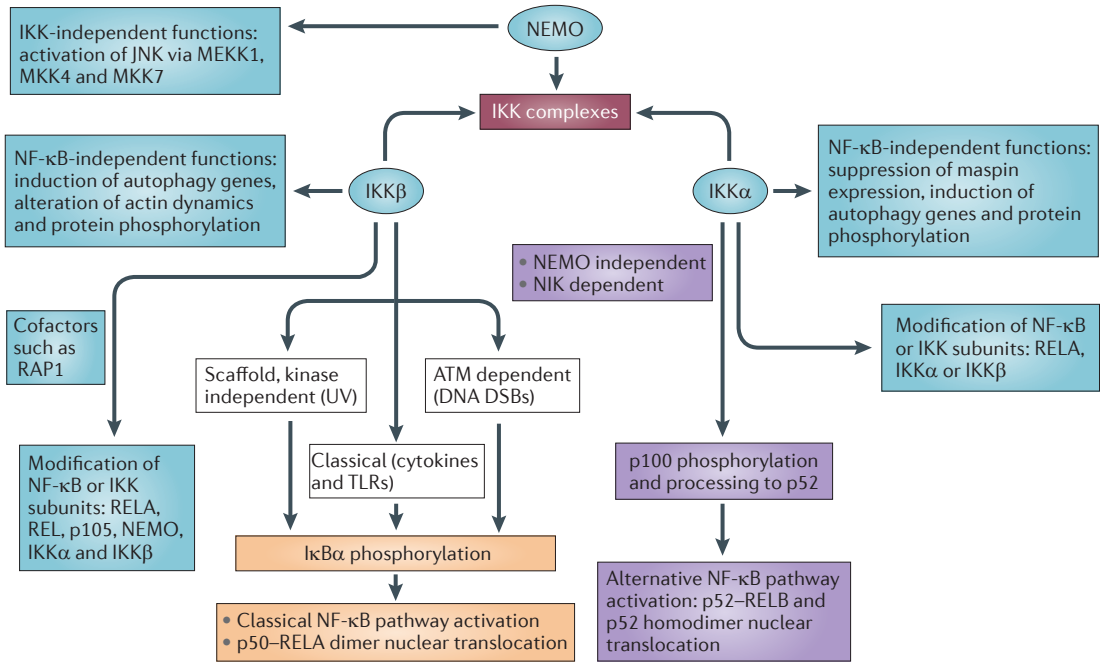

Figure 2 | **IKK signalling pathways.** The core components of the inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) complex consists of two catalytic subunits, IKKα and IKKβ, together with a regulatory subunit, NF-κB essential modifier (NEMO)[9]. Although highly homologous, IKKα and IKKβ have distinct functions, with IKKβ largely responsible for signal-induced phosphorylation and the subsequent degradation of inhibitor of NF-κB (IκBα), leading to the induction of the classical pathway of p50–RELA complexes[9] (shown in orange). By contrast, IKKα mediates the activation of the alternative (or non-canonical) pathway (shown in purple), involving the phosphorylation of p100, leading to its processing and the activation of p52-containing complexes[9]. There are also functionally distinct IKK complexes; for example, the adaptor protein RAP1 can be required for RELA phosphorylation by IKKβ at Ser536 (REF. 151), which is also regulated by the phosphatase WIP1 (REF. 152), and ELK1 is required for IKK activity after DNA damage, in a process also involving the association of the checkpoint kinase ataxia telangiectasia mutated (ATM)[109,111]. Based on the absence of a requirement for NEMO or IKKβ in this pathway, it is frequently referred to as an IKKα homodimer but little biochemical evidence exists that this is the case. In addition, both IKKα and IKKβ have nuclear functions[5] but the nature of these complexes has not been defined. IKKβ can also function in a kinase-independent capacity as a scaffold protein, facilitating CK2-mediated phosphorylation of IκBα following exposure to ultraviolet (UV) light[153]. This complexity in also reflected in the growing appreciation of the NF-κB-independent function of the IKKs[5], together with IKK-independent functions for NEMO[154,155]. For example, IKKβ can phosphorylate aurora A, nuclear receptor corepressor (NCOR3), FOXO3A, synaptosomyl-associated protein 23KD, TSC1, p53, 14–3- 3ε, docking protein 1, insulin receptor substrate 1, β-catenin, CYLD, CARMA1 and BCL10, and IKKα can phosphorylate CBP, NCOR3, NCOR2, protein inhibitor of activated STAT1, interferon regulatory factor 7, cyclin D1, β-catenin and histone H3, independently of NF-κB. These and other substrates can regulate a variety of cellular processes, including autophagy, actin dynamics, the cell cycle, survival and the DNA damage response. Whether these phosphorylation events are mediated by biochemically distinct IKK complexes is currently unclear. The phenotypes of IKK-mutant mice and the consequences of using IKK inhibitors will therefore be dependent on the totality of these effects. DSB, double-strand break; NIK, NF-κB inducing kinase; TLR, Toll-like receptor.

which leads to p100 truncation and constitutive p52 activity, and *REL* amplification have been reported in B cell and T cell lymphomas[34], these mutations are fairly rare (TABLE 1). Some missense mutations have also been reported, although again these are rare and their tumorigenic potential is mostly unexplored. This may simply result from a lack of investigation, although given the intensity of work in this area it is more likely that researchers, including this author, have looked but have not found such mutations. It remains possible that mutations in NF-κB subunits only occur frequently in late-stage, highly malignant, chemoresistant tumours, where the ability of NF-κB subunits to promote rather than inhibit cell death in response to chemotherapeutic drugs[49–51] will have been selected against. However, analyses of such samples are likely to be infrequent, as patients with advanced metastatic disease are less likely to undergo biopsy.

Another potential reason why NF-κB subunits might be rarely mutated lies in the difference between induction and activation of NF-κB. Induction of NF-κB can be described as the pathways that lead to the nuclear translocation of NF-κB subunits, and activation of NF-κB is the events that determine the transcriptional activity and the function of these subunits once they have relocated to the nucleus. To a great extent, the activity of NF-κB subunits is determined by numerous PTMs, including phosphorylation, acetylation, methylation and ubiquitylation[52–56]. The full effects of these PTMs, particularly for subunits other than RELA, are complex and still to be determined. Analysis of RELA suggests that these PTMs are frequently present at sub-stoichiometric levels and as a consequence,

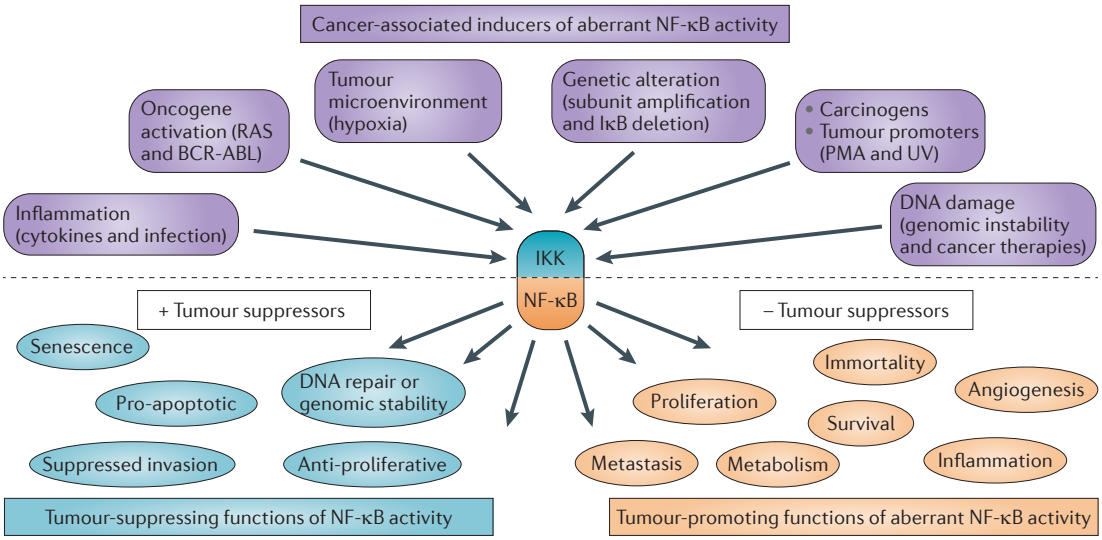

Figure 3 | **The diverse consequences of NF-κB activation.** Activation of inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) and NF-κB in cancer cells can occur through a wide variety of pathways. NF-κB-dependent gene expression can then either promote the growth and survival of cancer cells or contribute towards tumour suppressor mechanisms. A key determinant of which effects dominate is the tumour suppressor status of the cell: loss of key tumour suppressors such as p53 or PTEN can drive NF-κB towards oncogenic and tumour-promoting activity. PMA, phorbol 12-myristate 13-acetate; UV, ultraviolet.

activated NF-κB probably consists of many different, non-overlapping NF-κB subunit isoforms[57,58]. These isoforms fulfil distinct functions, such as gene-specific regulation, but they can also regulate subunit dimerization and proteolytic degradation[24,57–65]. Some modifications, such as RELA Ser468 and Ser536 phosphorylation, seem to fulfil multiple roles. For example, Ser468 modification is required for the COMMD1-mediated ubiquitylation and degradation of RELA[62,63], and Ser536 phosphorylation is required for the IKKα-mediated control of RELA degradation in macrophages[65]. By contrast, these modifications also control RELA transactivation of different target genes following TNF stimulation and can promote either lower (for example, *Icam1*, *Vcam1* and *Csf2*) or higher (for example, *Saa3*, *Mmp3* and *Mmp13*) levels of expression[57]. Moreover, at least in some cell types such as mouse embryo fibroblasts (MEFs), these modifications result in differential subcellular localization; Ser536-phosphorylated RELA was mostly perinuclear distribution, which is consistent with this site regulating nuclear import[66], but Ser468-modified forms were predominantly nuclear with a speckled distribution[57]. Neither of these modifications affected TNF-inducible expression of the *Cxcl2* gene in MEFs, which has been shown to be highly dependent on phosphorylation at another site, Thr435 (REF. 58). Furthermore, in response to some DNA-damaging agents such as cisplatin, Thr505 phosphorylation of RELA is required for the repression of the anti-apoptotic gene *BCLXL* and the induction of the pro-apoptotic gene *NOXA*[59,67]. Conversely, Ser276 phosphorylation of RELA, in addition to having gene-specific effects[60], has a more general role as an activator of RELA transcriptional activity, and transgenic mice with *Rela* mutated at this site exhibit widespread transcriptional repression and die at various stages of embryonic development[68].

Therefore, the outcome of NF-κB activation can be thought of as the sum total of the isoform-specific functions of the subunits (FIG. 5). Consequently, it follows that single missense mutations of NF-κB subunits that mimic an activated form of the protein will only partially recapitulate total NF-κB activity, while also skewing the pool of NF-κB isoforms to a particular subtype. If it is the diverse nature and complexity of the NF-κB response that leads to its tumour-promoting activities, this would explain why such mutants are rare and have not been demonstrated to function in an oncogenic manner: no single mutation can adequately mimic activated NF-κB. For example, mutations in REL do not easily recapitulate the oncogenicity of v-Rel[48].

The varied exposure of cancer cells to multiple NF-κB-inducing stimuli, including inflammatory cytokines, hypoxia, DNA damage and other downstream effects of oncogenic signalling, means that the activity of NF-κB will not be uniform throughout a given tumour. Thus, distinct gene targets have the potential to be induced and repressed depending on the tumour context, probably as a consequence of different PTMs and differential regulation of heterologous transcription factors, as well as co-activators and repressors[49,51,59,69,70] (FIG. 6). Once again, mutated subunits may not be able to fulfil the diversity of NF-κB responses required for a tumour to integrate these different stimuli in order to grow and survive. This could explain why NF-κB activation in tumours seems to occur upstream of IKK, so that the functionally diverse nature of NF-κB-dependent transcription is retained.

NF-κB and tumour suppressors

NF-κB activity is integrated with multiple tumour suppressor pathways, and antagonism with tumour suppressors can partly contribute to the tumour-promoting activity

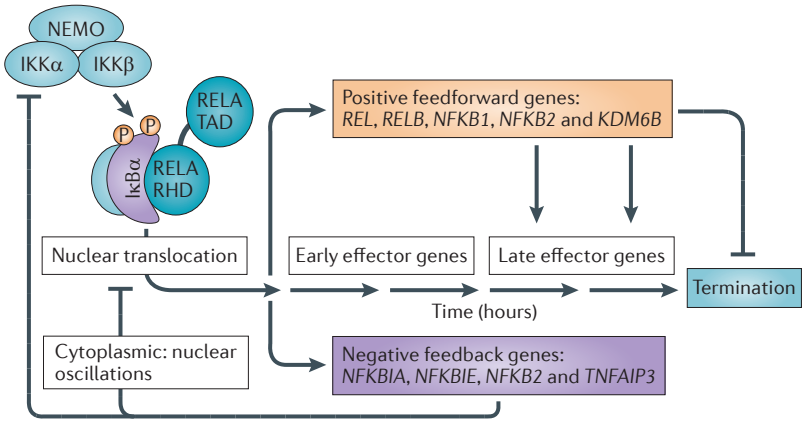

Figure 4 | **The NF-κB response involves both positive and negative feedback.** Nuclear factor-κB (NF-κB) translocation to the nucleus sets in motion a series of negative feedback and positive feedforward events as the products of NF-κB target genes can modulate inhibitor of NF-κB kinase (IKK)–NF-κB activity. Feedback events include the induction of the IκB proteins IκBα and IκBε, which can establish a series of RELA cytoplasmic and nuclear oscillations[32,33]. The induction of A20 (which is encoded by *TNFAIP3*), a ubiquitin-editing enzyme, negatively regulates IKK activity[31]. By contrast, NF-κB also induces the expression of the NF-κB subunits RELB, REL, NF-κB1 and NF-κB2 (REF. 15), which can result in changes to the composition of NF-κB complexes at later time points. The induction of co-activator proteins, such as the histone demethylase JMJD3 (REF. 156) can lead to the establishment of favourable chromatin configurations, allowing the activation of later NF-κB gene targets. NEMO, NF-κB essential modifier; P, phosphorylation; RHD, REL homology domain; TAD, transactivation domain.

of NF-κB subunits[6,71–73]. For example, NF-κB can inhibit p53 activity through competition for the p300 and CBP co-activator proteins[74–77] and can transcriptionally induce expression of MDM2 (REF. 78). However, known or putative tumour suppressors, such as p53, ARF, INK4A, LXXLL/leucine zipper-containing ARF binding protein (LZAP), PTEN, checkpoint protein with FHA and RING finger domains (CHFR) and transcription elongation factor A-like 7 (TCEAL7) can also regulate NF-κB[79–84]. The common theme is not inhibition of NF-κB but modulation of its activity. Either by direct interactions or by affecting subunit PTMs, tumour suppressors regulate NF-κB transcriptional activity and can, for example, suppress its ability to induce the expression of genes that are associated with tumour growth and survival. Moreover, p53 can prevent RELA mitochondrial localization and inhibition of oxidative phosphorylation[24], whereas RELA is required for p53 protein expression after glucose starvation, enabling p53 to promote oxidative phosphorylation through the expression of genes such as *SCO2* (REF. 85). This mechanism allows RELA to suppress oncogenic transformation by regulating energy metabolism[85]. Importantly, loss of p53 will result in a switch in RELA function, allowing it to suppress mitochondrial gene expression[24] and promote glycolysis[23].

The intimate relationship between NF-κB and the p53 tumour suppressor is further revealed by the identification of promoters that contain binding sites for both transcription factors, such as *SKP2*, *DR5* and *Cas4* (REFS 61,86,87), or where NF-κB is required indirectly for induction of a gene by p53 or its related family member p73 (REFS 59,88,89). These promoters function as important sites of integration between these pathways and allow them to function cooperatively, rather than antagonistically, to influence cell fate. Moreover, p53 and NF-κB subunits such as RELA and p52 can directly interact, meaning that the NF-κB subunits can function as transcriptional co-regulators for p53, and vice versa, independently of promoter-binding sites[61,90–92]. In addition, REL can interact with the p53 family member ΔNp63α, thereby antagonising the TA isoform of p73, leading to the survival of head and neck squamous cell carcinoma cells[93]. Under normal circumstances, such co-regulation between NF-κB and p53 family members is likely to be highly context dependent and function only in specific circumstances. Interestingly, a commonly occurring p53 polymorphism at codon 72, leading to either a proline or an arginine, was found to affect its ability to interact with RELA: the Pro72 p53 variant immunoprecipitates with RELA at a higher level and displays enhanced levels of expression of some NF-κB target genes, including *Casp4* (REF. 87). However, any effects on cancer that may result from this polymorphism and enhanced NF-κB crosstalk are unclear and may be subtle[87]. Importantly, cancer-associated mutants of p53 also display a markedly changed interaction with NF-κB. Although crosstalk between wild-type p53 and NF-κB is likely to be highly selective, mutant p53 seems to function in a deregulated manner, being recruited to NF-κB-regulated promoters, resulting in enhanced NF-κB transactivation; increased anti-apoptotic gene expression in a process that is likely to help drive NF-κB towards tumour promotion[94,95].

As a consequence of these pathways, NF-κB activity can be pro-apoptotic, can suppress metastasis or can inhibit proliferation[21] (FIG. 6). Other tumour-suppressing activities of NF-κB subunits include an ability to induce senescence and promote DNA repair[96–101] (FIG. 3). As tumour development is associated with the loss of tumour suppressors, a knock-on effect of this is that NF-κB is less tightly controlled and will be more likely to function in a tumour-promoting manner. Recent data from mouse models[40], analysis of clinical samples[102,103] and isogenically matched cell lines used in xenograft studies[50] support the concept of such a biphasic role for NF-κB in cancer. However, these studies are preliminary and it is unclear whether this represents a paradigm for NF-κB function in cancer or whether it is restricted to specific models or tumour types. For example, a recent analysis of the role of RELA in a mouse adenocarcinoma model found no evidence of a p53 modulatory role[41].

The mechanisms that underline the oncogenic functions of NF-κB are likely to require more than the loss of tumour suppressors and the activation of IKK. Transcription factors such as STAT3, which can function cooperatively with NF-κB, are likely to help to drive NF-κB-dependent tumorigenesis[27,28]. For example, SRC-mediated transformation requires NF-κB-dependent downregulation of the Let-7 miRNA, which leads to increased levels of IL-6 and activation of STAT3 (REF. 19). Moreover, parallel signalling pathways, such as deregulation of PI3K signalling, resulting in activation of AKT (also known as PKB) and suppression of GSK3β, activation of MAPK signalling and IKKε will also promote oncogenic NF-κB, either through direct modification or

Isogenically matched
Genetically identical.

through the induction of heterologous transcription factors[61,84,104–107]. These pathways combine to determine the role of NF-κB at different stages in the process of tumour development or in response to different oncogenic triggers, such as chronic inflammation or proto-oncogene activation. However, tumorigenesis itself is not the only route through which NF-κB can influence cancer patient survival: NF-κB activity is also an important determinant of the response to chemotherapy.

NF-κB subunits and cancer therapy

An important outcome of NF-κB activation and a core function of RELA is the ability to promote resistance to programmed cell death, primarily through the upregulation of anti-apoptotic proteins such as BCL-X_L and X-linked inhibitor of apoptosis (XIAP)[20]. Of all the NF-κB subunit-knockout mouse models, *Rela*$^{-/-}$ mice are the only ones that die *in utero* as a consequence of TNF-induced liver apoptosis[108]. Therefore, in addition to promoting tumorigenesis, aberrantly active NF-κB can also facilitate cancer cell chemoresistance by promoting resistance to apoptosis[20]. Even if NF-κB is not aberrantly active in tumours, DNA damage can induce IKK activity through an atypical pathway that involves the ataxia telangiectasia mutated (ATM) kinase and poly(ADP-ribose) polymerase 1 (PARP1)[109–111]. Consequently, NF-κB can promote resistance to cell death that is induced by many common cancer chemotherapeutic drugs[20]. Moreover, RELA can also promote DNA repair[98], and REL is required for claspin expression[112]. As claspin mediates CHK1 activation by the ataxia telangiectasia and Rad3 related (ATR) checkpoint kinase in response to replication stress, this pathway provides an additional route through which NF-κB can regulate the DNA damage response[112]. Together, these effects are likely to facilitate tumour escape from genotoxic therapy.

However, the ability of NF-κB subunits to exhibit duality of function — to be able to repress, as well as to induce, expression of the same target genes — means that an anti-apoptotic effect is not always the default mode following a DNA damage stimulus (FIG. 6). Through the repression of anti-apoptotic genes and the induction of pro-apoptotic genes, RELA can promote cell death[21,49,51,59,67,86,113,114]. Moreover, the p52 subunit can also contribute to pro-apoptotic effects after DNA damage[115]. Even within a single tumour cell line, the consequences of NF-κB activation can vary depending on the nature of the DNA damage-inducing stimulus. For example, drugs that promote replication stress can induce pro-apoptotic NF-κB, and DNA double-strand breaks have the opposite effect[67,113]. This is not a universal rule, as anthracyclins such as daunorubicin and doxorubicin can have differing effects depending on the cell line or laboratory investigating their effects[49,116–118]. These drugs are not 'clean': they induce multiple effects in cells, including DNA damage-independent stress, and it is likely that the differing signalling pathways that are induced result in these opposing outcomes. For example, one study suggested that it is the ability of some anthracyclins to intercalate with DNA that induced pro-apoptotic effects rather than inhibition of topoisomerase II[119].

Table 1 | **Compilation of genetic mutations associated with the NF-κB subunits in cancer**

Mutations	RELA	RELB	REL	NF-κB1	NF-κB2
Missense mutations	• Three of 827 samples*: T55S (lung), E498K (lung) and E127Q (ovary), and one Q132 nonsense mutation (lung) • One of 200 lymphoma samples E495D (multiple myeloma)[157]	One of 847 samples*: one silent mutation V372 (ovary)	• Two of 1,272 samples*: E38Q (ovary), V451I (breast) and one K135 silent change (ovary) • Two of 83 samples: S525P (B cell lymphoma)[158]	• One of 911 samples*: R335Q (ovary) • One L70 silent mutation (ovary) • One intronic mutation (breast)	Two of 522 samples*: V281L (lung), V519F (lung) and one E643 silent mutation (lung)
Amplifications and translocations	ND	ND	REL is frequently amplified in multiple B cell lymphomas[34]	ND	*NFKB2* translocation in <2% of B cell and T cell lymphomas
SNPs and other changes	Non-coding SNPs associated with predisposition to schizophrenia (no known cancer link)[159]	ND	Alternative spliced form of REL is associated with B cell lymphoma[34]	SNP in *NFKB1* promoter, resulting in reduced expression, is associated with predisposition to numerous cancer types[160,161]	ND
Biological effects	The RELA T55S and E127Q mutations are both exposed residues in the RHD that are unlikely to affect DNA binding or dimerization but that could disrupt specific protein–protein interactions. E498K mutation is in the TAD and therefore could disrupt transcriptional activity. However, the E495D mutation, originally referred to as E494 (REF. 157) was reported to affect DNA binding and dimerization	ND	The E38Q mutation is an exposed residue in the RHD and is unlikely to affect DNA binding, and the V451I mutation in TAD is not close to any obvious sites of PTMs. However, the S525P mutation does disrupt an IKK phosphorylation site, leading to enhanced *in vitro* transforming activity[158]	The R335Q mutation has the potential to disrupt the S338 putative phosphorylation motif, although the functional consequence of this in unclear	The V281L mutation is in the dimerization domain; so, although a conservative substitution, it could affect dimer affinity or specificity. The V519F mutation is in p100 not p52 and is not close to any obvious sites of PTM.The result of the translocation is a carboxy-terminal truncation of NF-κB2 and constitutive processing to p52 (REF. 34)

IKK, the inhibitor of NF-κB kinase; ND, not determined; NF-κB, nuclear factor-κB; PTM, post-translational modification; RHD, REL homology domain; SNP, single nucleotide polymorphism; TAD, transactivation domain. *Missense mutations were analysed using the Catalogue of Somatic Mutations in Cancer (COSMIC) database[162] (see Further information).

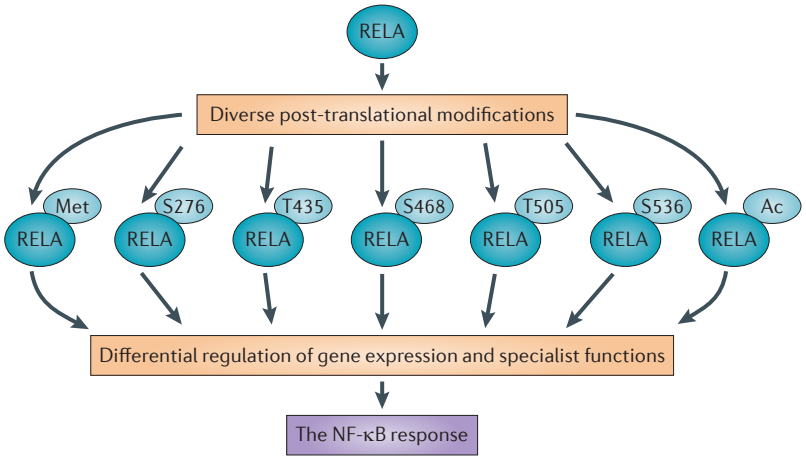

Figure 5 | **RELA isoforms.** Activated nuclear factor-κB (NF-κB) is composed of multiple subunit isoforms that differ in their post-translational modifications. A representative, although incomplete, selection for RELA is shown. This diversity is required for 'complete' NF-κB activity and may explain why missense mutations of NF-κB subunits are fairly rare: mutations that mimic a particular modified isoform of NF-κB will lead to under-representation of other isoforms and a failure to mount a proper NF-κB response.

RELA can also contribute towards a beneficial outcome from therapy by inducing chemotherapy-induced senescence-associated secretory phenotype (SASP)[100]. This process, through the release of cytokines, can help to stimulate immune cell surveillance and, in cooperation with RB- and p53-mediated senescence, can help to promote tumour cell killing[100].

These differential outcomes of NF-κB activation after treatment with chemotherapeutic drugs are probably mediated by PTMs. Differing effects of daunorubicin and doxorubicin seem to correlate with Ser536 phosphorylation of RELA[49,116,120,121] (FIG. 2). Moreover, phosphorylation of RELA at Thr505 by CHK1 in response to drugs such as cisplatin[67,122], provides an explanation, at least in part, for the pro-apoptotic effects of NF-κB following replication stress. A recent study of four ovarian cancer cell lines revealed that, although NF-κB facilitated cell death in the 'normal' cells, when chemoresistant isogenic lines were isolated, NF-κB function had switched to an anti-apoptotic, 'oncogenic' form[50]. Furthermore, in contrast to the usual dogma, high levels of nuclear RELA in epithelial cancer tissue corresponded to a good response to therapy in patients[50]. A similar scenario was also identified, using mouse models and patient data, in a subtype of germinal centre B cell-like (GCB) diffuse large B cell lymphoma (DLBCL), in which BCL2 is overexpressed independently of NF-κB activity. Here, as a consequence of its decoupling from anti-apoptotic effects, active NF-κB was found to enhance tumour killing and a beneficial outcome, at least in part through the promotion of therapy-induced senescence (TIS)[101]. This TIS study[101] suggested that the outcome of NF-κB inhibition, and whether it will have a positive or negative effect on patient survival, will depend on the route through which the tumour developed and the role of NF-κB in this process. These reports reveal the potential variability in the NF-κB response to anticancer drugs, although an important caveat is that, with the exception of more recent studies, the majority of these reports are currently restricted to studies in cell lines. Nonetheless, an important consideration when strategies to target NF-κB in patients are implemented is that its inhibition may not always promote a positive outcome in the tumour itself. In this regard, tumour stage, the nature of the oncogenic transformation and tumour suppressor status, as well as the type of therapy are likely to be important considerations. Early stage tumours, for which therapy is generally effective at killing the majority of tumour cells, might not be the best time to target NF-κB signalling. Rather, it is chemoresistant tumours, when NF-κB has probably switched to a default anti-apoptotic mode, for which its inhibition is most likely to be beneficial.

Targeting NF-κB subunits for cancer therapy

The rationale behind targeting NF-κB in cancer is straightforward: by inhibiting its activity, the anti-apoptotic and other tumour-promoting functions of NF-κB will be prevented, thus reducing malignancy and aiding tumour cell killing by current cancer therapies. For example, the basis of using bortezomib, a proteasome inhibitor that is used successfully for the treatment of a range of solid and haematological malignancies, is its ability to inhibit NF-κB induction by blocking IκB degradation[123]. Drugs such as bortezomib will clearly have effects other than inhibiting NF-κB and it is therefore desirable to specifically target this pathway. To date, most effort in this area has focused on the development of highly specific IKKβ inhibitors (reviewed in REFS 3,4,124,125; see also, for example, REFS 126–130). Although these can be effective *in vitro*, they will have other, NF-κB-independent effects and there is also a high risk of side effects[5,6] (FIG. 2). For example, NF-κB is a negative regulator of the processing and secretion of IL-1β and other cytokines, and so the enhanced levels of their expression that would be seen on global inhibition of IKKβ could lead to many complications[131]. In addition, NF-κB and IKK activity is required for epithelial homeostasis, and inhibition can lead to the development of severe and chronic inflammation[132].

However, opportunities exist to develop different strategies to exploit this pathway therapeutically through understanding the complexity of NF-κB signalling. By targeting the NF-κB subunits, there is the potential for more specific, modulatory effects on NF-κB signalling that could promote a beneficial outcome with reduced negative consequences. In particular, strategies to modulate rather than to totally inhibit NF-κB, such as the promotion of RELA pro-apoptotic responses and not anti-apoptotic pathways, have the potential to allow tumour cell killing without the substantial side effects of global IKK–NF-κB inhibition. There are several potential strategies to therapeutically target NF-κB subunits.

Direct targeting. Targeting transcription factor subunits is generally considered to be unfeasible, as they are not readily druggable. However, many natural products, such as sesquiterpene lactones, do seem to function as direct inhibitors of NF-κB subunit DNA binding through targeting a highly conserved cysteine residue (for example Cys38 in RELA) in the RHD[3]. The specificity of such

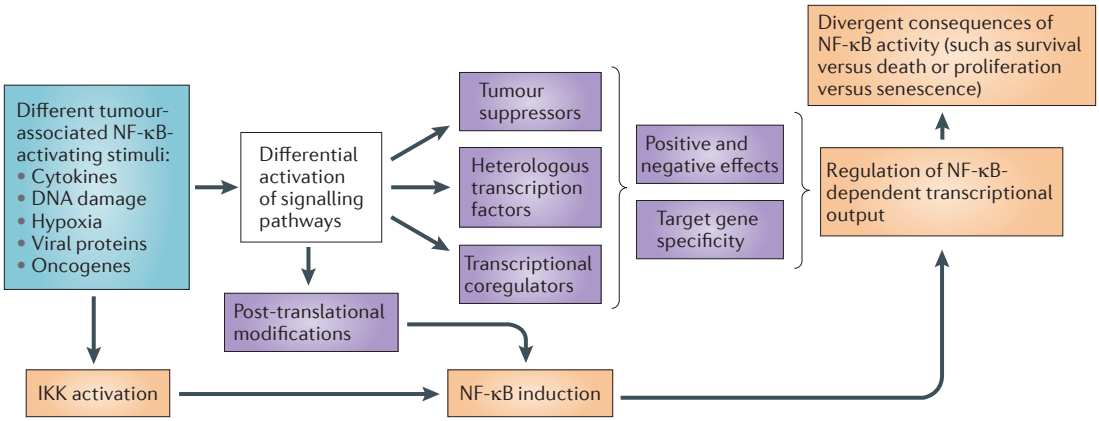

Figure 6 | **NF-κB signalling in tumour cells.** Tumour-associated nuclear factor-κB (NF-κB) can be exposed to multiple inducing stimuli, the divergent cellular effects of which can result in functionally distinct NF-κB responses. In addition to inhibitor of NF-κB kinase (IKK) activation, different tumour-associated stimuli will induce multiple parallel signalling pathways. These will affect the NF-κB response, either through direct modification of the subunits or through induction or repression of tumour suppressors, and heterologous transcription factors. Through the differential activation of these parallel pathways, many of which may be simultaneously induced in a tumour, the consequences of NF-κB activation can vary.

compounds will be an issue, as other proteins with reactive cysteines also have the potential to be targeted. Indeed, IKKβ also possesses a crucial cysteine in its activation loop (Cys179) that can be subject to chemical modification[3]. Biologically active, specific inhibitors of NF-κB subunits have been reported, although their mechanism of action has not been fully described[133].

Protein–protein interactions. NF-κB subunit function is mediated by a wide variety of protein–protein interactions. These facilitate subunit nuclear import, activation by PTMs and transcriptional regulation. In theory, NF-κB subunit activity could be selectively regulated by disrupting specific interactions with key regulators. Is this possible? A potential precedent exists with the p53 tumour suppressor, where compounds, such as nutlin 3, specifically disrupt the interaction between p53 and its negative regulator MDM2, leading to its accumulation in cancer cells that retain wild-type p53 (REF. 134). In this case, the ability to isolate specific inhibitors is facilitated by a highly defined site of interaction that involves a small, deep hydrophobic pocket in MDM2 (REF. 134). By analogy, if similar defined structural features can be identified in NF-κB subunits, or their interacting proteins, then they could be similarly targeted. However, although the structures of the NF-κB subunits in a variety of homodimer and heterodimer complexes have been solved, including association with some IκB proteins, these do not include the C-terminal transactivation domains of RELA, RELB and REL, either alone or bound to co-regulators[135]. Therefore, the opportunities for rational drug design to target such interactions are currently limited. The potential of such an approach is demonstrated by a peptide encompassing the Ser536 phosphorylation site of RELA, which can function as a specific NF-κB inhibitor[136] and which is biologically active *in vivo*[137]. This peptide probably has a dual mechanism of action, both blocking RELA Ser536 phosphorylation by functioning as a kinase decoy and also competing for RELA binding to transcriptional co-activator proteins. These proof-of-principle studies demonstrate that targeting NF-κB protein–protein interactions for therapeutic purposes is a promising route for drug development, although peptides and peptide mimetics themselves are not generally considered to be clinically useful.

Parallel signalling pathways. The functional outcome of NF-κB activation is, to a large part, determined by the integration of the signalling pathways that are simultaneously active in the cell[58]. This can occur through PTMs of the NF-κB subunits or through the activation of heterologous transcription factors that function cooperatively or antagonistically at shared target promoters[6,52,53,57,61]. Indeed, it is likely that many compounds currently being used or developed as anticancer therapies directly or indirectly target NF-κB subunits and inhibit or modulate NF-κB activity. For example, targeting of the PI3K pathway, which is commonly activated in cancer through the loss of the tumour suppressor PTEN, will result in AKT inhibition, and the resultant activation of GSK3β can potentially affect RELA[84,105,106,138], as well as p52 (REF. 61). For example, the phosphorylation of p52 by GSK3β disrupts p52 homodimer–BCL3 complexes that can function as important inducers of cyclin D1 expression[139–141]. Similarly, the p38 MAPK pathway has been shown to regulate NF-κB transactivation and to augment IKK activity. This can result in destructive colonic inflammation[142] that may predispose to colorectal cancer[25]. Reactivation of p53 using nutlin 3 has also been shown to inhibit NF-κB activity[143]. Targeting DNA repair and DNA damage-induced checkpoint kinases will also have knock-on effects on NF-κB activity. For example, ATM inhibitors will probably block NF-κB activation that is induced by many chemotherapeutic drugs[109,113], and CHK1 inhibitors will modulate RELA activity[59,67,122]. Moreover, PARP1 inhibitors[144] also inhibit NF-κB activity[145,146]. These examples demonstrate once again that a detailed understanding

of NF-κB activity will make an important contribution to the effective use of a wide range of cancer therapies. Moreover, there is great potential to move away from the post-development discovery of the beneficial side effects of these drugs and towards deliberately and specifically targeting the modulatory activities of NF-κB to promote tumour cell killing.

Transcriptional co-regulators. One consequence of targeting the NF-κB subunits through the mechanisms described above is to disrupt their interactions with transcriptional co-regulators, thereby altering NF-κB-dependent gene expression. Another strategy, however, is to directly target transcriptional co-regulators that NF-κB requires for its ability to induce tumour-promoting characteristics. This has recently been demonstrated with small molecules that inhibit bromodomain-containing transcriptional co-activators and thereby limit MYC activity in multiple myeloma[147,148]. Despite years of research, too little is known about the specific co-activator requirements of NF-κB subunits. However, the potential of this area has been revealed by recent studies. For example, the EZH2 histone methyl transferase interacts with RELA and RELB and it is a regulator of NF-κB-dependent gene expression in breast cancer, although at least some of its effects are independent of its catalytic activity[149]. REL has also been shown to interact with the histone demethylase AOF1, the activity of which is required for the 'pioneer' activity of REL in establishing a permissive chromatin structure at specific NF-κB-regulated promoters[150]. Although a role in cancer has not been established for this pathway, inhibiting such co-activator proteins might provide a route to indirectly targeting REL activity in B cell lymphomas, where the *REL* gene is frequently amplified[34] (TABLE 1).

Conclusion

Although it is well established that the NF-κB–IKK pathway is a fertile area for cancer drug discovery, the subunits themselves have not received much attention. This includes defining the molecular mechanisms through which they operate, as well as exploiting their activity therapeutically. There is much that we still do not know or understand, such as the PTMs that define their activity, as well as the subunit 'interactomes', both under normal circumstances and how these change in cancer. However, as the examples above demonstrate, a knowledge of both of these areas has the potential to inform current drug development strategies, as well as define new areas of activity. There is sometimes an assumption that we now understand NF-κB and know all we need to know to exploit this area therapeutically. In fact, this process has only just begun and there is much to learn about the complexity of NF-κB subunit biology. Moreover, we have only just scratched the surface of the potential clinical applications of targeting this pathway.

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