Nuclear factor-κB in cancer development and progression

Michael Karin[1]

Nuclear factor-κB (NF-κB) transcription factors and the signalling pathways that activate them are central coordinators of innate and adaptive immune responses. More recently, it has become clear that NF-κB signalling also has a critical role in cancer development and progression. NF-κB provides a mechanistic link between inflammation and cancer, and is a major factor controlling the ability of both pre-neoplastic and malignant cells to resist apoptosis-based tumour-surveillance mechanisms. NF-κB might also regulate tumour angiogenesis and invasiveness, and the signalling pathways that mediate its activation provide attractive targets for new chemopreventive and chemotherapeutic approaches.

A link between inflammation and cancer was suspected well before the discovery of NF-κB or other transcription factors. Although Virchow suggested in the nineteenth century that chronic inflammation might give rise to malignancy[1], the link between inflammation and cancer was not widely understood until more recent times[1,2]. This 're-discovery' can be attributed, in part, to epidemiological studies that identified chronic infections and inflammation as major risk factors for various types of cancer. Collectively, underlying infections and inflammation are linked to 15–20% of all cancer deaths[3]. For instance, chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are major risk factors for hepatocellular carcinoma (HCC), whereas infections with *Helicobacter pylori* are associated with most gastric cancers[4]. Chronic inflammatory bowel diseases (IBDs), such as ulcerative colitis (UC), are thought to increase the risk of colorectal cancer by approximately 1% per year[5], and chronic airway irritation and inflammation caused by airborne particles and tobacco smoke are likely to be important promoters of lung carcinogenesis[6]. Although epidemiological studies are an excellent source of new working hypotheses, they only underline correlations and do not establish causal relationships or mechanistic links. Thus, the evidence listed above and elsewhere[2,7,8] begs the question of how chronic inflammation influences tumour development and progression.

In trying to provide an answer to this question, one needs to consider the cellular processes that contribute to the emergence of neoplasia and its malignant progression. These processes were elegantly summarized by Hanahan and Weinberg as self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, tissue invasion and metastasis, and sustained angiogenesis[9]. As recently discussed, inflammation and NF-κB can affect most of these processes[7,10] (Fig. 1). To minimize the reiteration of recent reviews on inflammation and cancer, this article focuses on the role of a single transcription factor, NF-κB, in linking these pathophysiological processes. Although a role for NF-κB in cancer development and progression has been suspected[11,12], this hypothesis has rested mainly on circumstantial evidence, such as the occurrence of constitutively active NF-κB in many types of cancer. Only recently has solid genetic and biochemical evidence for a causative role of NF-κB in malignant conversion and progression been obtained. As discussed below, depending on the cell type in which it acts, NF-κB can either promote or inhibit carcinogenesis. Such information, available from various mouse models of cancer in which NF-κB activation has been blocked by genetic means, is crucial for the clinical development of NF-κB inhibitors as cancer therapeutics.

How infection and inflammation affect cancer development

Cancer is a chronic disease that is caused by defective genome-surveillance and signal-transduction mechanisms[9]. If infection and inflammation enhance tumour development, they must do so through signal-transduction mechanisms that influence factors involved in either malignant conversion or cancer surveillance. In general, unless they carry their own oncogenes, toxins or growth factors, infectious organisms affect the host through pattern-recognition receptors (PRRs), most commonly those that belong to the Toll-like receptor (TLR) family[13,14]. PRR engagement activates numerous signal-transduction pathways that target several transcription factors, which control the expression of genes encoding cytokines, chemokines and enzymes that regulate innate and adaptive immune responses[14,15]. In turn, some of these polypeptides activate receptors that further propagate and amplify the inflammatory response, which is a particular manifestation of a much broader innate immune response (Fig. 2). Although engagement of TLRs and receptors for proinflammatory cytokines, such as tumour-necrosis factor-α (TNF-α) and interleukin-1 (IL-1), leads to activation of many important signalling pathways, it is well accepted that the central role in inflammation and innate immunity is played by the NF-κB group of transcription factors[16]. The details of NF-κB regulation are outlined in Box 1.

It is noteworthy that many of the receptors and cytokines mentioned above have also been linked to carcinogenic processes. Genetic polymorphisms in a *TLR* gene cluster are associated with high risk for prostate cancer[17], whereas polymorphisms in the *IL1B* promoter that enhance IL-1β production are associated with increased risk of gastric cancer[18]. Another proinflammatory cytokine involved in cancer is TNF-α. Despite being named for its ability to induce tumour necrosis, which is an activity that is mostly mediated through increased vascular permeability and subsequent vascular collapse, there is ample evidence that TNF-α acts as a tumour promoter in several models of experimental cancer[19–23].

Of all the different signalling pathways activated by inflammation and infection, NF-κB might be the most important component of the

[1]Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology, University of California San Diego School of Medicine, 9500 Gilman Drive, La Jolla, California 92093, USA.

tumour-promoting machinery. This suggestion is based on the realization that NF-κB is a major activator of anti-apoptotic gene expression[24–27]. These findings were first made in the context of TNF-α signalling (Fig. 3). Despite the presence of death domains (DDs) in the intracellular portion of its major receptor TNFR1, TNF-α does not trigger apoptosis unless it is combined with inhibitors of RNA or protein synthesis. The requirement for such inhibitors can be alleviated through inactivation of NF-κB by either deletion of its RelA subunit or expression of a degradation-resistant form of inhibitor of NF-κB (IκB) — the so-called IκB-superrepressor (IκB-SR). A similar effect is seen upon deletion of *Ikbkb* (IκB kinase (IKK) β-subunit) or *Ikbkg* (IKK γ-subunit)[28]. Such interventions were found to inhibit the expression of several critical anti-apoptotic proteins, including the specific inhibitor of caspase 8 activation c-FLIP[29], the caspase inhibitors cIAP1 and cIAP2, and the anti-apoptotic member of the B-cell leukaemia/lymphoma 2 (Bcl2) family Bcl-X_L (ref. 30). As it was already established that genes encoding anti-apoptotic proteins — for instance Bcl2 — function as oncogenes[31], it was reasonable to predict that NF-κB activation in chronic infection and inflammation could also promote tumour development[11]. Although this hypothesis was consistent with the presence of activated NF-κB in many cancers, it required validation in appropriate animal models. Because the deletion of individual NF-κB subunit genes, with the exception of RelA, resulted in partially redundant phenotypes, two complementary approaches were undertaken to inhibit most forms of NF-κB in the mouse, and to examine its role in tumorigenesis. To avoid the embryonic lethality associated with the loss of a substantial amount of NF-κB activity, both approaches were based on the cell type-specific inhibition of NF-κB, in one case by expressing IκB-SR from a cell-specific promoter[32], and in the other case through conditional inactivation of the *Ikbkb* gene[33]. Both methods resulted in substantial inhibition of the classical NF-κB signalling pathway, with no direct effect on the alternative pathway (Box 1). The results of these mouse studies are described below.

NF-κB in inflammation-linked cancers

Colitis-associated cancer (CAC) is a colorectal disease that arises in patients suffering from the chronic IBD UC. In mice, CAC can be modelled by injection of the procarcinogen azoxymethane (AOM), which undergoes metabolic activation in intestinal epithelial cells (enterocytes). AOM exposure alone causes cancer with low incidence, but this can be augmented by three rounds of exposure to dextran-sulphate sodium salt (DSS), which is an irritant that causes colonic inflammation

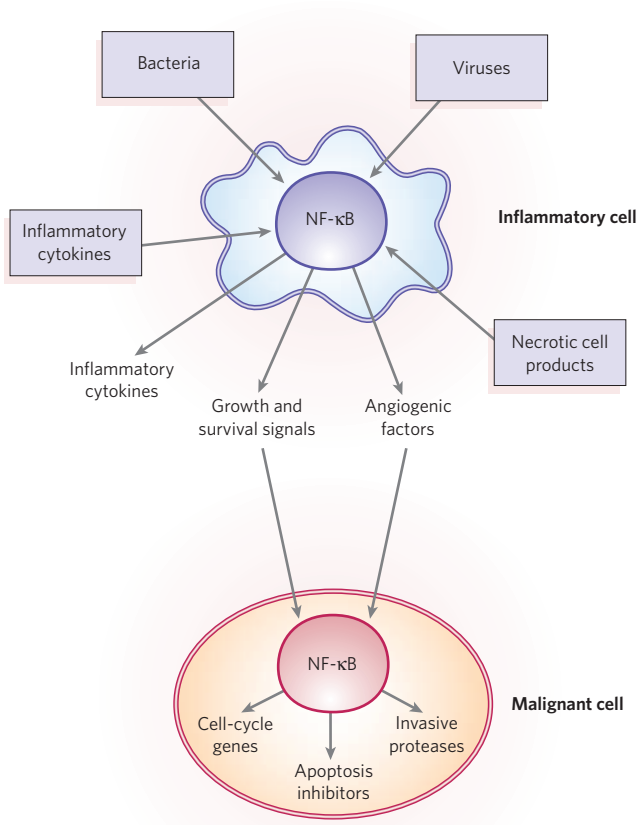

Figure 1 | NF-κB activation, and the interaction between inflammatory and malignant cells, can promote malignant conversion and progression. Activation of nuclear factor-κB (NF-κB) in inflammatory cells in response to infectious agents, inflammatory cytokines and proteins, and danger signals released by necrotic cells lead to the production of secreted factors that enhance the growth, survival and vascularization of carcinoma cells. Activation of NF-κB in the latter results in elevated expression of cell-cycle genes (such as cyclin D1), inhibitors of apoptosis (such as B-cell leukaemia/lymphoma-X_L) and proteases that promote the invasive phenotype (such as matrix metalloproteinase-9).

Box 1 | Nuclear factor-κB transcription factors and their regulation

Nuclear factor-κB (NF-κB) transcription factors are assembled through the dimerization of five subunits: RelA (p65), c-Rel, RelB, p50/NF-κB1 and p52/NF-κB2 (ref. 59). The first clue linking NF-κB to cancer was the recognition that c-*rel*, which is the cellular homologue of the v-*rel* oncogene, encodes an NF-κB subunit, and that all of these proteins share the same DNA binding domain — the Rel homology domain[60]. Not surprisingly, overexpression of normal Rel proteins promotes oncogenic transformation.

Before cell stimulation, most NF-κB dimers are retained in the cytoplasm by binding to specific inhibitors — the inhibitors of NF-κBs (IκBs). Cell stimulation activates the IκB kinase (IKK) complex, which is composed of two catalytic subunits (IKK-α and IKK-β) and a regulatory subunit (IKK-γ/NEMO)[61]. Activated IKK phosphorylates NF-κB-bound IκB proteins, and targets them for polyubiquitination and rapid degradation by creating a binding site for the $SCF^{\beta TrCP}$ ubiquitin ligase complex[62]. Freed NF-κB dimers translocate to the nucleus where they coordinate the transcriptional activation of several hundred target genes, many of which also depend on other transcription factors[63–65].

Although it is of fundamental importance, this pathway, which is called the classical NF-κB signalling pathway, is only one of two major pathways that activate NF-κB transcription factors. The second pathway, the alternative pathway, results in specific activation of p52:RelB heterodimers and is not required for activation of the more ubiquitous p50:RelA dimers[66]. Unlike the classical pathway, which is dependent on IKK-γ and to a large extent on the activity of IKK-β, the alternative pathway is based on IKK-α homodimers, the preferred substrate of which is the precursor of p52 — p100/NF-κB2 (ref. 67). This protein binds RelB through its amino-terminal Rel homology domain, and keeps itself and its partner in the cytoplasm through its carboxy-terminal IκB-like domain. Activation of IKK-α dimers results in degradation of the latter and nuclear entry of p52:RelB dimers.

Although this pathway is not directly involved in innate immunity and inflammation, it is required for the generation of secondary lymphoid organs, and for B-cell maturation and survival[66]. Its relevance to cancer is underscored by chromosomal translocations associated with B-cell lymphoma that remove the region encoding the IκB-like inhibitory domain of p100 (ref. 68). In addition, the alternative pathway might be involved in mammary carcinogenesis[69]. Mutations that affect other NF-κB subunits were identified in other types of cancer, especially those of lymphoid origin[12].

Another pathway that can lead to NF-κB activation is independent of IKK and, instead, is based on activation of casein kinase 2 (CK2), which induces IκBα degradation through the phosphorylation of carboxy-terminal sites[70]. This pathway has only a minor role in physiological NF-κB activation, although it might contribute to skin carcinogenesis because it is activated by ultraviolet radiation.

(colitis) by eroding the mucosal barrier, thereby exposing lamina propria macrophages to normal enteric bacteria[34]. These macrophages are activated to produce a range of inflammatory mediators. Selective inactivation of the *Ikbkb* gene within enterocytes resulted in an 80% decrease in CAC tumour multiplicity[35]. As tumour size was not affected, it can be concluded that IKK-β-dependent NF-κB in enterocytes contributes to tumour initiation or early tumour promotion, rather than tumour growth and progression. Indeed, analysis of enterocyte IKK-β-deleted mice shortly after exposure to AOM plus DSS revealed increased apoptosis of enterocytes, including pre-neoplastic cells in which AOM led to mutational activation of the β-catenin pathway[35]. Enhanced apoptosis is probably caused by defective induction of Bcl-X_L. However, when IKK-β was deleted in myeloid cells (for example, mature macrophages, dendritic cells and neutrophils), tumour multiplicity was reduced by only 50%, although tumour size was also reduced[35]. Indeed, deletion of IKK-β in myeloid cells, but not in enterocytes, diminished the proliferation of AOM-exposed enterocytes. The myeloid-specific mutation, however, had no effect on apoptosis of AOM-exposed enterocytes. These results led to the conclusion that IKK-β-driven NF-κB contributes to the development of CAC through two distinct cell-type-specific mechanisms: in enterocytes it activates anti-apoptotic genes and thereby suppresses the apoptotic elimination of pre-neoplastic cells, whereas in myeloid cells it promotes the production of cytokines that act as growth factors for pre-malignant enterocytes. One of these growth factors was subsequently identified as IL-6, which is encoded by an NF-κB target gene[36]. The inhibition of IL-6 signalling with antagonistic anti-IL-6 receptor antibodies inhibited tumour growth with little effect on tumour multiplicity, thereby resembling IKK-β ablation in myeloid cells. Curiously, in the early stages of the carcinogenic protocol, IL-6 is produced by lamina propria myeloid cells[35], whereas at the end of the CAC protocol it is mainly expressed by tumour-infiltrating T cells[36]. As with other chronic inflammatory responses[15], the colonic inflammation induced by DSS seems to be initiated through rapid activation of tissue macrophages but is sustained through prolonged activation of pro-inflammatory T cells.

Another interesting model of inflammation-driven cancer is the multidrug resistance 2 (*Mdr2*)-knockout mouse, in which the absence of the MDR2 transporter leads to accumulation of bile acids and phospholipids within hepatocytes, resulting in low-grade liver inflammation, which eventually gives rise to HCC at 8–10 months of age[37]. Unlike the CAC model described above, the initiating event that leads to cancer development in *Mdr2*$^{-/-}$ mice is not known, because inflammation probably only accounts for tumour promotion. Nonetheless, as in the CAC model, inhibition of NF-κB through expression of IκB-SR under the control of a promoter that is highly active in hepatocytes blocked tumour development[21]. Exactly how bile acid and phospholipid accumulation lead to NF-κB activation in this model is not clear, but it seems to depend on the production of TNF-α by non-parenchymal cells (that is, Kupffer and endothelial cells). Indeed, inhibition of TNF-α signalling prevented activation of NF-κB in hepatocytes and early tumours, and, just like the inhibition of NF-κB itself, increased the number of apoptotic hepatocytes and reduced tumour multiplicity[21]. Thus, in this model too, an important pro-tumorigenic function of NF-κB is the suppression of apoptosis of pre-malignant or early neoplastic cells (Fig. 1). The role of NF-κB in inflammatory cells has not been investigated in this model but is presumably important for the production of TNF-α and other cytokines.

Additional inflammation-driven cancers include lymphomas of mucosal-associated lymphoid tissue (MALT), which develop in the context of prolonged lymphoid proliferation caused by chronic microbial infections, such as *H. pylori* gastritis[38]. Interestingly, MALT lymphoma is associated with elevated expression of proteins that are involved in IKK and NF-κB activation in response to the occupancy of antigen receptors, namely Bcl10 (ref. 39) and MALT1 (refs 40, 41). Presumably, MALT lymphoma is initiated by repeated antigenic stimulation of B cells and, up to a certain point, can be reversed by antibiotic treatment. Later on, however, chromosomal translocations, which are more frequent in activated B cells, place the *Bcl10* and *MALT* genes under the control of heterologous promoters and cause their overexpression, most commonly as fusion proteins (for example, IAP2–MALT1), thereby leading to persistent activation of NF-κB. This suppresses B-cell apoptosis and contributes to uncontrolled B-cell proliferation. At this point, the tumours no longer regress upon elimination of the antigenic challenge. Although Bcl10-deficient and MALT1-deficient mice have been generated, and used to confirm the role of these molecules in NF-κB activation[39,41], an appropriate mouse model of MALT lymphoma is yet to be described. Nonetheless, there is little doubt that MALT lymphoma is caused by the constitutive activation of NF-κB.

NF-κB and chemically induced liver and skin cancers

Although NF-κB has emerged as a critical promoter of inflammation-linked cancers, there is also strong evidence that it has the opposite effect in models of chemically induced skin and liver cancers. The first evidence for such an effect came from a two-stage skin carcinogenesis model based on 7,12-dimethylbenz(a)anthracene (DMBA) as a tumour initiator and the phorbol ester TPA as a tumour promoter. Inhibition of NF-κB in keratinocytes greatly enhanced the multiplicity of squamous cell carcinomas (SCCs) caused by exposure to DMBA plus TPA[42,43]. Similarly, inhibition of NF-κB in primary human keratinocytes promoted their Ras-mediated transformation[44], indicating that the anti-tumorigenic effect is not limited to the two-stage model. The tumour-suppressing activity of NF-κB was explained by either inhibition of cell-cycle progression[43,44] or downmodulation of Jun kinase (JNK) activity[45]. JNK is a member of the MAP kinase (MAPK) family that stimulates activator protein-1 (AP-1) transcription factors[46]. It is well established that NF-κB activation in response to TNFR1 engagement (but not other

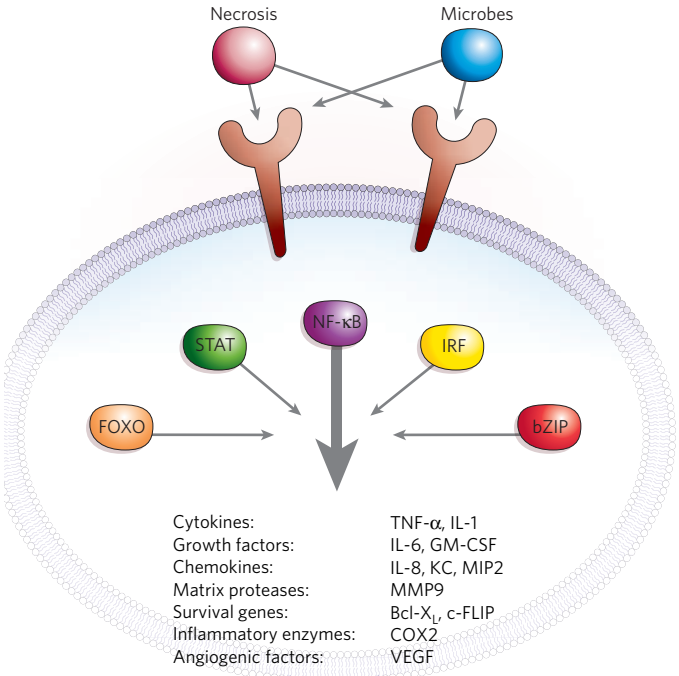

Figure 2 | Microbial pathogens and tissue necrosis lead to activation of NF-κB and other transcription factors in cells that express pattern-recognition receptors. Activation of NF-κB and other transcription factors involved in the innate immune/inflammatory response can upregulate the expression of many genes, the products of which promote and support the malignant phenotype. Bcl-X_L, B-cell leukaemia/lymphoma-X_L; bZIP, basic leucine zipper protein; c-FLIP, c-FLICE-inhibitory protein; COX-2, cyclooxygenase-2; FOXO, forkhead transcription factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; IRF, interferon response factor; KC, Cxcl1 chemokine; MIP2, macrophage inflammatory protein-2; MMP9, matrix metalloproteinase-9; STAT, signal transducer and activator of transcription; TNF-α, tumour-necrosis factor-α; VEGF, vascular endothelial growth factor.

stimuli) promotes termination of JNK activation through a mechanism that depends on induction of anti-oxidant proteins[47,48] (Fig. 3). Indeed, excessive SCC formation in mice lacking keratinocyte NF-κB activity is suppressed by inactivation of the *TNFR1* gene[20], and TNF-α has long been known to promote skin carcinogenesis through its effect on AP-1 activity[19]. Thus, by inducing termination of JNK activity, NF-κB acts as a negative regulator of TNF-α-induced AP-1 activity, thereby suppressing the tumour-promoting activity of TNF-α. The exact source of TNF-α during skin carcinogenesis remains to be identified.

The negative interplay between NF-κB and JNK was also found to have a critical role in the development of chemically induced HCC in response to administration of the pro-carcinogen diethylnitrosamine (DEN). Unlike the carcinogens used in CAC or two-stage skin carcinogenesis, DEN does not require any assistance from inflammation-inducing tumour promoters if it is given to 2-week-old male mice. In contrast to the *Mdr2*$^{-/-}$ HCC model, but similar to two-stage skin carcinogenesis, hepatocyte-specific IKK-β ablation greatly augmented HCC multiplicity and size in DEN-treated mice[49]. Curiously, DEN administration induces rapid TNF-α production and activation of TNFR1 (T. Sakurai and M.K., unpublished observations); thus, TNFR1 signalling might also be important in this cancer model. Decreased NF-κB activity and elevated JNK activity promote TNF-α-induced cell death[47,50] (Fig. 3); accordingly, ablation of hepatocyte IKK-β results in higher DEN-induced JNK activity and more cell death[49]. However, owing to the strong regenerative capacity of the liver, elevated hepatocyte death enhances compensatory proliferation. Prolonged JNK activation in the absence of NF-κB depends on the accumulation of reactive oxygen species[47] (Fig. 3). Correspondingly, feeding hepatocyte-IKK-β-deficient mice with the potent anti-oxidant butylated hydroxyanisole (BHA) prevented DEN-induced prolonged JNK activation and excessive hepatocyte death, resulting in strong inhibition of compensatory proliferation[49]. Furthermore, feeding BHA before DEN administration[49], or ablation of the major death-promoting and growth-promoting JNK isoform JNK1 (T. Sakurai and M.K., unpublished observations), prevented the increase in hepatocarcinogenesis seen in hepatocyte-IKK-β-deficient mice. Notably, the increase in hepatocyte proliferation in the absence of IKK-β is not due solely to direct effects of NF-κB or JNK1 on the hepatocyte cell-cycle machinery. Much of the compensatory proliferation that is observed depends on the production of factors such as TNF-α, IL-6 and hepatocyte growth factor (HGF) by non-parenchymal cells. Correspondingly, ablation of IKK-β in liver myeloid cells, which are known as Kupffer cells, prevented the induction of these cytokines in response to DEN administration, thereby resulting in a marked decrease in HCC load[49]. The production of IL-6 by Kupffer cells might depend on their activation by proteins that are released by necrotic hepatocytes, which are potent macrophage activators at least *in vitro*[49,51]. The induction of TNF-α production by DEN is another likely contributor to this carcinogenesis model, as found in the two-stage skin carcinogenesis model[19,20], and could be the reason why inhibition of NF-κB potentiates carcinogenesis in both of these models.

Mechanisms of NF-κB action in cancer

Although NF-κB can either promote or oppose tumour development, several general mechanisms of action emerge from the work discussed above. First and foremost, NF-κB is an activator of anti-apoptotic genes[30]. Both in CAC and cholestatic liver cancer, the activation of NF-κB within pre-neoplastic or progressing cancer cells is the rate-limiting event, which ensures their survival and prevents elimination by pro-apoptotic tumour-surveillance mechanisms. However, as a result of different tissue kinetics, inhibition of cell death — as seen in hepatocytes exposed to the cytotoxic chemical DEN — prevents compensatory proliferation, thereby attenuating tumour development. Compensatory proliferation is critical for DEN-induced hepatocarcinogenesis because the target population for this carcinogen comprises non-proliferating differentiated hepatocytes[49]. In contrast to the liver, the colonic epithelium is subject to constant renewal, and compensatory proliferation is not a rate-limiting event in CAC.

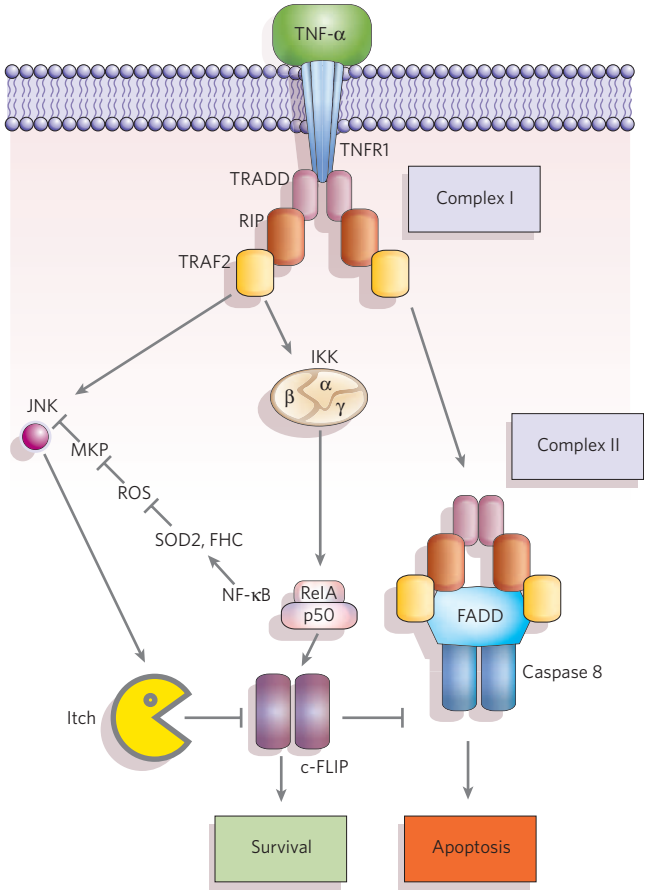

Figure 3 | TNFR1 signalling controls cell survival and death by through an interplay between NF-κB and JNK. Activation of tumour-necrosis factor receptor 1 (TNFR1) by binding of TNF-α results in rapid assembly of complex I, which is composed of TNF receptor 1-associated protein (TRADD), receptor-interacting protein 1 (RIP1) and TNFR-associated factor 2/5 (TRAF2/5). This complex leads to activation of inhibitor of NF-κB kinase (IKK) and the Jun kinase (JNK) cascade. Activation of IKK leads to nuclear translocation of NF-κB and upregulation of several anti-apoptotic genes, including the gene encoding c-FLICE-inhibitory protein (c-FLIP), which is a specific inhibitor of caspase 8 activation. NF-κB also upregulates the expression of genes encoding the antioxidant proteins superoxide dismutase (SOD2) and ferritin heavy chain (FHC), which block the accumulation of reactive oxygen species (ROS) and prevent the inhibition of MAP kinase phosphatases (MKPs). Overall, this promotes the rapid termination of JNK activity and, generally, signalling through complex I enhances cell survival. After some time, complex I dissociates and the soluble complex II is formed, which, in addition to TRADD, RIP1 and TRAF2/5, includes the adaptor protein Fas-associated death domain protein (FADD). The latter can recruit and activate caspase 8, and thereby trigger apoptosis, but this does not occur if c-FLIP levels are high. Defective activation of NF-κB prevents *de novo* synthesis of c-FLIP, resulting in accumulation of ROS and inhibition of MKPs, thereby promoting prolonged JNK activation. This leads to extended phosphorylation and activation of the ubiquitin ligase Itch, which promotes c-FLIP degradation. Once the level of c-FLIP falls below a certain threshold, caspase 8 is activated and the cell undergoes apoptosis.

Importantly, even in those cases in which NF-κB activation in epithelial cells negatively affects tumour development, its activation in inflammatory cells has the opposite effect. In the four experimental cancer models mentioned above, tumour promotion and progression largely depend on production of the proinflammatory cytokines TNF-α and IL-6, which serve as growth factors for pre-malignant cells and already formed tumours. Interference with either TNF-α or IL-6 signalling blocks tumour growth and progression in the *Mdr2*$^{-/-}$ and CAC models[21,36]. Even in SCC, the development of which is enhanced by inhibition of NF-κB, interference with TNF-α signalling blocks tumour

development[20]. Other NF-κB target genes encode chemokines such as the mouse orthologues of human IL-8: MIP2, KC and Gro1. Interference with Gro1 signalling inhibits the development of colorectal cancer in mice[52], whereas IL-8 (CXCL8) inhibition blocks the growth of a Ras-transformed human xenograft in these animals[53]. Inhibition of IL-8 produced by malignant cells prevents tumour angiogenesis by blocking inflammatory cell recruitment. Additional evidence for a role of NF-κB in tumour angiogenesis comes from studies on the *ING4* tumour-suppressor gene, the expression of which is reduced in gliomas. ING4 directly interacts with RelA and inhibits expression of angiogenesis-promoting genes, such as *IL8* and cyclooxygenase 2 (*COX2*), by interfering with NF-κB-dependent transcription[54].

In addition to effects on cell survival, and the production of growth and angiogenesis factors, NF-κB might directly stimulate cell-cycle progression through transcriptional activation of cell-cycle genes, especially cyclin D1 (ref. 55). Interestingly, in mammary epithelial cells[56] and ErbB2-transformed mammary carcinomas (Y. Cao and M.K., unpublished observations), expression of cyclin D1 depends on IKK-α; however, this function of IKK-α is mediated through the classical, and not the alternative, pathway. NF-κB is also involved in TNF-α-mediated induction of cyclin D1 and other cell-cycle genes in mouse colorectal carcinoma[57]. Yet, in epidermal keratinocytes, NF-κB is a negative regulator of cell proliferation[44]. This function of NF-κB was suggested to be mediated through suppression of JNK activation[45]. Importantly, JNK activation is also an important contributor to chemically induced HCC[49]. Thus, in addition to being an important regulator of cell survival in the context of TNF-α signalling (Fig. 3), the NF-κB–JNK interplay seems to be a critical regulator of cancer development and progression, especially in cases where tissue injury triggers compensatory proliferation. Thus, the role of epithelial cell NF-κB in tumorigenesis is highly dependent on its overall effect on tissue kinetics.

NF-κB as a target for cancer prevention and therapy

Given the drastic and invariable effects of inflammatory cell NF-κB on tumour development and progression, this factor and the signalling pathways involved in its activation are attractive targets for cancer prevention and therapy. Although epithelial-specific inhibition of NF-κB might increase cancers of the liver and skin, it should be recognized that clinically relevant inhibitors of this pathway are unlikely to act in a cell-type-specific manner, and, as long as NF-κB is inhibited in inflammatory cells, the net effect should be less cancer and slower tumour development. However, NF-κB in inflammatory cells serves an important immune function, and its absence can result in severe immunodeficiency. Thus, prolonged and substantial inhibition of NF-κB might not be practical in cancer prevention. More effective preventive measures might be those that are directed at the initial causes of persistent NF-κB activation: microbial and viral infections or chronic inflammatory disorders. However, the prolonged use of any anti-inflammatory drug, regardless of its impact on NF-κB, is likely to have adverse side effects that cannot be tolerated in cancer prevention.

Given these considerations, NF-κB inhibitors are more likely to be of use in cancer therapy, in which they can be administered intermittently for shorter durations, thereby avoiding immunosuppression associated with long-term inhibition. Although, in some cases, NF-κB inhibition contributes to tumour development, the most likely outcome of its inhibition in existing tumours is increased cancer cell apoptosis. Nonetheless, in most cases, the mere inhibition of NF-κB is insufficient for a pronounced apoptotic response unless combined with apoptosis-inducing drugs or radiation. Thus, NF-κB inhibitors are most likely to be used as adjuvants along with other cancer therapies. As recently reviewed[58], several IKK and NF-κB inhibitors are under development, as well as a number of natural products that can inhibit NF-κB activation when used at high doses but can also affect several other targets. There are no published accounts, as yet, on the use of specific NF-κB inhibitors in cancer therapy, but given the great interest in the role of NF-κB in cancer development and progression, and the current emphasis on clinical translation of basic research findings, this situation is likely to change in the not so distant future. Progress in this area will, of course, depend not only on the development of potent and specific, orally available IKK and NF-κB inhibitors, but also on the direct demonstration of an involvement of the specific pathways discussed above in various human malignancies. ■

1. Balkwill, F. & Mantovani, A. Inflammation and cancer: back to Virchow? *Lancet* **357,** 539–545 (2001).
2. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420,** 860–867 (2002).
3. Kuper, H., Adami, H. O. & Trichopoulos, D. Infections as a major preventable cause of human cancer. *J. Intern. Med.* **248,** 171–183 (2000).
4. Roder, D. M. The epidemiology of gastric cancer. *Gastric Cancer* **5 (Suppl. 1),** 5–11 (2002).
5. Ekbom, A. Risk of cancer in ulcerative colitis. *J. Gastrointest. Surg.* **2,** 312–313 (1998).
6. Dhala, A., Pinsker, K. & Prezant, D. J. Respiratory health consequences of environmental tobacco smoke. *Med. Clin. North Am.* **88,** 1535–1552, xi (2004).
7. Greten, F. R. & Karin, M. NF-κB: linking inflammation and immunity to cancer development and progression. *Nature Rev. Immunol.* **5,** 749–759 (2005).
8. Balkwill, F., Charles, K. A. & Mantovani, A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* **7,** 211–217 (2005).
9. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100,** 57–70 (2000).
10. Li, Q., Withoff, S. & Verma, I. M. Inflammation-associated cancer: NF-κB is the lynchpin. *Trends Immunol.* **26,** 318–325 (2005).
11. Karin, M., Cao, Y., Greten, F. R. & Li, Z. W. NF-κB in cancer: from innocent bystander to major culprit. *Nature Rev. Cancer* **2,** 301–310 (2002).
12. Gilmore, T. D. The Re1/NF-κB/IκB signal transduction pathway and cancer. *Cancer Treat. Res.* **115,** 241–265 (2003).
13. Medzhitov, R. Toll-like receptors and innate immunity. *Nature Rev. Immunol.* **1,** 135–145 (2001).
14. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **124,** 783–801 (2006).
15. Nizet, V., Lawrence, T. & Karin, M. Innate immunity gone awry: mechanisms linking microbial infections to chronic inflammatory disorders and cancer. *Cell* **124,** 823–835 (2006).
16. Li, Q. & Verma, I. M. NF-κB regulation in the immune system. *Nature Rev. Immunol.* **2,** 725–734 (2002).
17. Sun, J. *et al.* Sequence variants in Toll-like receptor gene cluster (*TLR6–TLR1–TLR10*) and prostate cancer risk. *J. Natl Cancer Inst.* **97,** 525–532 (2005).
18. El-Omar, E. M. *et al.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* **404,** 398–402 (2000).
19. Arnott, C. H. *et al.* Tumour-necrosis factor-α mediates tumour promotion via a PKCα- and AP-1-dependent pathway. *Oncogene* **21,** 4728–4738 (2002).
20. Lind, M. H. *et al.* Tumor necrosis factor receptor 1-mediated signaling is required for skin cancer development induced by NF-κB inhibition. *Proc. Natl Acad. Sci. USA* **101,** 4972–4977 (2004).
21. Pikarsky, E. *et al.* NF-κB functions as a tumour promoter in inflammation-associated cancer. *Nature* **431,** 461–466 (2004).
22. Balkwill, F. Tumor necrosis factor or tumor promoting factor? *Cytokine Growth Factor Rev.* **13,** 135–141 (2002).
23. Oshima, M., Oshima, H., Matsunaga, A. & Taketo, M. M. Hyperplastic gastric tumors with spasmolytic polypeptide-expressing metaplasia caused by tumor necrosis factor-α-dependent inflammation in cyclooxygenase-2/microsomal prostaglandin E synthase-1 transgenic mice. *Cancer Res.* **65,** 9147–9151 (2005).
24. Liu, Z.-G., Hu, H., Goeddel, D. V. & Karin, M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis, while NF-κB activation prevents cell death. *Cell* **87,** 565–576 (1996).
25. Beg, A. A. & Baltimore, D. An essential role for NF-κB in preventing TNF-α induced cell death. *Science* **274,** 782–784 (1996).
26. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R. & Verma, I. M. Suppression of TNFα-induced apoptosis by NF-κB. *Science* **274,** 787–789 (1996).
27. Wang, C.-Y., Mayo, M. W. & Baldwin, A. S. Jr. TNF-α and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. *Science* **274,** 784–787 (1996).
28. Makris, C. *et al.* Female mice heterozygous for IKKγ/NEMO deficiencies develop a dermatopathy similar to the human X-linked disorder incontinentia pigmenti. *Mol. Cell* **5,** 969–979 (2000).
29. Micheau, O., Lens, S., Gaide, O., Alevizopoulos, K. & Tschopp, J. NF-κB signals induce the expression of c-FLIP. *Mol. Cell. Biol.* **21,** 5299–5305 (2001).
30. Karin, M. & Lin, A. NF-κB at the crossroads of life and death. *Nature Immunol.* **3,** 221–227 (2002).
31. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116,** 205–219 (2004).
32. Lavon, I. *et al.* High susceptibility to bacterial infection, but no liver dysfunction, in mice compromised for hepatocyte NF-κB activation. *Nature Med.* **6,** 573–577 (2000).
33. Chen, L. W. *et al.* The two faces of IKK and NF-κB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia–reperfusion. *Nature Med.* **9,** 575–581 (2003).
34. Okayasu, I., Ohkusa, T., Kajiura, K., Kanno, J. & Sakamoto, S. Promotion of colorectal neoplasia in experimental murine ulcerative colitis. *Gut* **39,** 87–92 (1996).
35. Greten, F. R. *et al.* IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118,** 285–296 (2004).
36. Becker, C. *et al.* TGF-β suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity* **21,** 491–501 (2004).
37. Mauad, T. H. *et al.* Mice with homozygous disruption of the *mdr2* P-glycoprotein gene. A novel animal model for studies of nonsuppurative inflammatory cholangitis and hepatocarcinogenesis. *Am. J. Pathol.* **145,** 1237–1245 (1994).
38. Zucca, E., Bertoni, F., Roggero, E. & Cavalli, F. The gastric marginal zone B-cell lymphoma of MALT type. *Blood* **96,** 410–419 (2000).

39. Ruland, J. *et al.* Bcl10 is a positive regulator of antigen receptor-induced activation of NF-κB and neural tube closure. *Cell* **104,** 33–42 (2001).
40. Lucas, P. C. *et al.* Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-κB signaling pathway. *J. Biol. Chem.* **276,** 19012–19019 (2001).
41. Thome, M. CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nature Rev. Immunol.* **4,** 348–359 (2004).
42. van Hogerlinden, M., Rozell, B. L., Ahrlund-Richter, L. & Toftgard, R. Squamous cell carcinomas and increased apoptosis in skin with inhibited Rel/nuclear factor-κB signaling. *Cancer Res.* **59,** 3299–3303 (1999).
43. van Hogerlinden, M., Auer, G. & Toftgard, R. Inhibition of Rel/nuclear factor-κB signaling in skin results in defective DNA damage-induced cell cycle arrest and Ha-ras- and p53-independent tumor development. *Oncogene* **21,** 4969–4977 (2002).
44. Dajee, M. *et al.* NF-κB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. *Nature* **421,** 639–643 (2003).
45. Zhang, J. Y., Green, C. L., Tao, S. & Khavari, P. A. NF-κB RelA opposes epidermal proliferation driven by TNFR1 and JNK. *Genes Dev.* **18,** 17–22 (2004).
46. Karin, M. The regulation of AP-1 activity by mitogen-activated protein kinases. *J. Biol. Chem.* **270,** 16483–16486 (1995).
47. Kamata, H. *et al.* Reactive oxygen species promote TNFα-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* **120,** 649–661 (2005).
48. Pham, C. G. *et al.* Ferritin heavy chain upregulation by NF-κB inhibits TNFα-induced apoptosis by suppressing reactive oxygen species. *Cell* **119,** 529–542 (2004).
49. Maeda, S., Kamata, H., Luo, J. L., Leffert, H. & Karin, M. IKKβ couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* **121,** 977–990 (2005).
50. Chang, L. *et al.* The E3 ubiquitin ligase itch couples JNK activation to TNFα-induced cell death by inducing c-FLIP(L) turnover. *Cell* **124,** 601–613 (2006).
51. Park, J. S. *et al.* Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J. Biol. Chem.* **279,** 7370–7377 (2004).
52. Holla, V. R. *et al.* Prostaglandin E2 regulates the complement inhibitor CD55/decay-accelerating factor in colorectal cancer. *J. Biol. Chem.* **280,** 476–483 (2005).
53. Sparmann, A. & Bar-Sagi, D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* **6,** 447–458 (2004).
54. Garkavtsev, I. *et al.* The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. *Nature* **428,** 328–332 (2004).
55. Joyce, D. *et al.* NF-κB and cell-cycle regulation: the cyclin connection. *Cytokine Growth Factor Rev.* **12,** 73–90 (2001).
56. Cao, Y. *et al.* IKKα provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. *Cell* **107,** 763–775 (2001).
57. Luo, J. L., Maeda, S., Hsu, L. C., Yagita, H. & Karin, M. Inhibition of NF-κB in cancer cells converts inflammation- induced tumor growth mediated by TNFα to TRAIL-mediated tumor regression. *Cancer Cell* **6,** 297–305 (2004).
58. Karin, M., Yamamoto, Y. & Wang, Q. M. The IKK NF-κB system: a treasure trove for drug development. *Nature Rev. Drug Discov.* **3,** 17–26 (2004).
59. Ghosh, S. & Karin, M. Missing pieces in the NF-κB puzzle. *Cell* **109** (suppl.)**,** S81–S96 (2002).
60. Gilmore, T. D. The Rel/NF-κB signal transduction pathway: introduction. *Oncogene* **18,** 6842–6844 (1999).
61. Rothwarf, D. M. & Karin, M. The NF-κB activation pathway: a paradigm in information transfer from membrane to nucleus. *Sci. STKE* **1999,** RE1 (1999).
62. Karin, M. & Ben-Neriah, Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. *Annu. Rev. Immunol.* **18,** 621–663 (2000).
63. Werner, S. L., Barken, D. & Hoffmann, A. Stimulus specificity of gene expression programs determined by temporal control of IKK activity. *Science* **309,** 1857–1861 (2005).
64. Park, J. M. *et al.* Signaling pathways and genes that inhibit pathogen-induced macrophage apoptosis — CREB and NF-κB as key regulators. *Immunity* **23,** 319–329 (2005).
65. Covert, M. W., Leung, T. H., Gaston, J. E. & Baltimore, D. Achieving stability of lipopolysaccharide-induced NF-κB activation. *Science* **309,** 1854–1857 (2005).
66. Bonizzi, G. & Karin, M. The two NF-κB activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* **25,** 280–288 (2004).
67. Senftleben, U. *et al.* Activation by IKKα of a second, evolutionary conserved, NF-κB signaling pathway. *Science* **293,** 1495–1499 (2001).
68. Neri, A. *et al.* B-cell lymphoma-associated chromosomal translocation involves candidate oncogene *lyt-10*, homologous to NF-κB p50. *Cell* **67,** 1075–1087 (1991).
69. Demicco, E. G. *et al.* RelB/p52 NF-κB complexes rescue an early delay in mammary gland development in transgenic mice with targeted superrepressor IκB-α expression and promote carcinogenesis of the mammary gland. *Mol. Cell. Biol.* **25,** 10136–10147 (2005).
70. Kato, T. Jr., Delhase, M., Hoffmann, A. & Karin, M. CK2 Is a c-terminal IκB kinase responsible for NF-κB activation during the UV response. *Mol. Cell* **12,** 829–839 (2003).

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The author declares no competing financial interests. Correspondence should be addressed to M.K. (karinoffice@ucsd.edu).